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# Observer-Based Robust Control of a Continuous Bioreactor with Heterogeneous Community

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**Abstract:** This work addresses the problem of robust control of the continuous bioreactor – namely the chemostat – using an observer-based approach. We design a control law aiming to regulate the growth of a certain species and to stabilize the output – a growth-associated product of interest – in a prescribed level. In addition, to avoid the single-species dogma (which takes only one species into account for control design), we add to the microbial community unknown species competing for the limiting substrate. Finally, this control architecture is able to maintain the productivity, despite this competing heterogeneity (or biodiversity) of the community.

*Keywords:* robust control, observer-based control, bioreactor, biochemical process

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## 1. INTRODUCTION

The bioreactor is a well-known device in biotechnological industries and laboratories nowadays. This device allows experiments involving living micro-organisms (such as bacteria, yeast and micro-algae), to be carried out under controlled conditions. Many different applications take place in a bioreactor, such as production of pharmaceuticals, biofuels (like ethanol and hydrogen), fermentation, wastewater treatment and also the cultivation of cells.

Due to their highly nonlinear aspects, different models of bioreactors have been extensively used as a benchmark for control and observer design. Many results have been published, for instance, on feedback control (De Leenheer and Smith, 2003), adaptive control (Mailleret et al., 2004), robust control (Robledo, 2006) and optimal control (Moreno, 1999). Also, since monitoring of such processes is often difficult (due to lack of sensors or long sampling) (Bogaerts and Wouwer, 2003), observation has also been extensively studied (see the review given in Ali et al. (2015)).

Throughout the years, many application-oriented results arose from this vast literature, such as species separation (Bayen and Mairet, 2014) and selection (Masci et al., 2008). However, many of these results are based on the *single-species dogma* and do not address competition between species or the effect of a heterogeneous community on the productivity.

Out of this dogma, in Mairet and Bernard (2017) the authors proposed a new perspective for robustness analysis of bioprocess control: evaluating the sensitivity of a control

law against *biodiversity*. This means that, despite the presence of other competing species, the control problem (such as stabilization, production, substrate depletion, etc.) is still solved under some conditions.

Heterogeneity of the microbial community can be due to many factors: whether deterministic (such as pH, temperature, salinity) or stochastic (colonization, mutation) (Zhou et al., 2013). In this sense, many challenges may arise in a heterogeneous microbial community, such as inhibition, mutualism and competition. The latter is an important result in biological modeling: the *competitive exclusion principle* (Smith and Waltman, 1995) states that the competition of  $N$  species for a single limiting substrate will result in the survival of at most one species.

In the present work, the objective is to extend previous results on robust control of competitive environments (Reis de Souza et al., 2019) to the context of biodiversity, by taking it (in the form of uncertainty) into account in the design. In this sense, we apply an observer-based sliding-mode control scheme, aiming to stabilize the concentration of a species of interest. The main feature is guaranteed robustness against possible “bad species” in the microbial community (i.e., appearance of competitors) that might lead to a productivity depreciation or even shutdown of the bioreactor (e.g., the producer species is outcompeted or the substrate is depleted).

The organization of the paper is as follows: Section 2 introduces the problem statement and main assumptions, Section 3 discusses the design of the observer-based control scheme, Section 4 illustrates the methodology with numerical examples and Section 5 concludes and discusses future directions.

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## 2. PROBLEM STATEMENT

Consider the following non-linear system, which describes microbial growth inside a chemostat with a single limiting substrate (time-dependence was omitted for readability):

$$\begin{aligned} \frac{dS}{dt} &= (S_{in} - S)D - k\mu(S)x - d \\ \frac{dx}{dt} &= (\mu(S) - D)x \\ S(0) &> 0, x(0) > 0, d > 0 \end{aligned} \quad (1)$$

where  $S$  and  $x$  are, respectively, the concentration of the substrate and the microbe species. The variables  $S_{in}$  (nutrient inflow concentration, in  $g/l$ ) and  $D$  (dilution rate, in  $1/h$ ) are non-negative control inputs. Function  $\mu(S)$  (in  $1/h$ ) is the *specific growth rate*, describing the nutrient uptake kinetics, and  $k > 0$  is the constant yield coefficient. The perturbation  $d$  will be defined in the following.

*Assumption 1.* The specific growth rate  $\mu(S)$  is supposed to be a monotonic function such that  $\mu(0) = 0$ ,  $\mu(S) \leq \mu_{\max}$  and  $\mu'(S) \geq 0$  for all  $S \geq 0$ , where  $\mu'(S) = \frac{d\mu(S)}{dS}$  and  $\mu_{\max} > 0$  is a known parameter.

Such an assumption is fulfilled, for instance, by the Monod Law, given by

$$\mu(S) = \frac{\mu_{\max}S}{b + S} \quad (2)$$

where  $\mu_{\max}, b > 0$  are constants.

We consider a perturbation  $d$  on the dynamics of  $S$ , which might describe, for instance, unknown species competing for the substrate. Clearly, this term relates to the consumption of  $N$  unknown species such as  $d = \sum_{i=1}^N k_i \mu_i(S) x_i$ , where  $k_i$ ,  $\mu_i$  and  $x_i$  have the same meaning for these additional unmodeled species as the one in (1). In such a case, the model parametric uncertainty of any species can be described by the triplet  $\Theta_i = [\mu_{\max_i}, b_i, k_i]$  under assumption that all growth rates have a form as in (2).

*Assumption 2.* An upper estimate  $\bar{d}$  of  $d$ , i.e.,  $d \leq \bar{d}$  for all  $t \geq 0$ , is supposed to be known.

The estimate  $\bar{d}$  plays an important role when designing a state observer: it is directly related to correction term that compensates the consumption of substrate by the unknown species.

Furthermore, we consider the (measured) output to be a “growth-associated” formation of product - i.e., some compound that is produced proportionally to the growth of  $x$ , such as gases or proteins - being described by

$$p = \gamma\mu(S)x$$

where  $\gamma > 0$  is a known constant and is determined by the sensor used.

The objective of this work is to propose an observer-based control architecture, aiming to stabilize the product of interest  $p$  at a prescribed productivity index  $p^*$ , despite the biodiversity of the community. This problem is motivated by evidence of mutations/fluctuations in biological processes, which implies that “bad species” might appear in the course of operation of a bioreactor.

*Motivating example:* Let us consider a control law similar as the one presented in (Reis de Souza et al., 2019) for  $D$

(using information on bounds of  $\mu(S)$ ), which has been applied for (finite-time) robust stabilization of  $p$  at a desired level  $p^* = 100$ , related to some productivity index.

Considering two scenarios: one with a single species  $x_1$ , described by  $\Theta_1 = [3.2, 5, 1]$ , and another with an additional species  $x_d$  appearing during the course of operation of the bioreactor, described by  $\Theta_d = [4, 8, 1]$ . Assuming  $S_{in} = 65$ , Fig. 1 shows the effect of a competitor not taken into account: although in the single-species scenario the stabilization is successfully attained, substrate is depleted when species  $x_d$  is considered, obviously leading to  $p(t) = 0$ . Clearly, the second scenario is highly undesirable, since it means no production on the bioreactor.

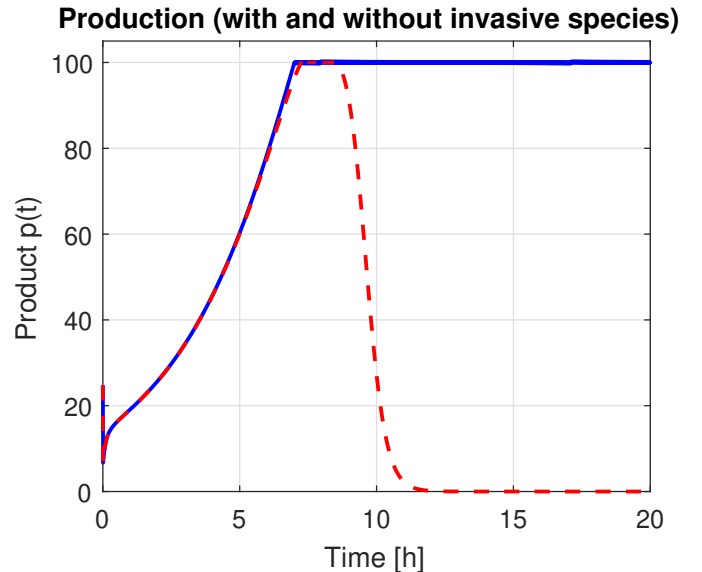


Fig. 1. The effect of competition on the stabilization of  $p(t)$ . Solid line: without competition. Dashed line: with competition.

In this case, since the employed control law is proportional to  $\mu_1(S)$ , substrate depletion also leads to  $D = 0$ , shutting down the bioreactor. Hence, the aim in this work is to develop a control architecture, manipulating both  $D$  and  $S_{in}$ , that improves the robustness of such a production process against this undesired behavior caused by heterogeneity in the microbial community.

## 3. OBSERVER-BASED CONTROL DESIGN

Before starting the development of the control architecture, it is worth stressing the nuances of the problem. As one can easily see, the quantity of interest  $p$  depends on both  $x$  and  $S$  and, naturally, its regulation requires control over both variables. Clearly,  $x$  must not be outcompeted by any other species while  $S$  must not be depleted from the bioreactor (which would happen in case  $S_{in}$  is not sufficiently high).

In this section, we will discuss the design of both controls and observer for the proposed problem.

### 3.1 Controller Design

For simplicity in this first section, all state variables are considered to be available for the design. The use of

estimates coming from an observer will be tackled in the subsequent subsection.

Regarding the stabilization problem, let us define a decision variable  $\sigma = p - p^*$ , where  $p^* > 0$  is the prescribed set-point in which  $p$  is to be regulated in accordance with some productivity index. Then, the dynamics of  $\sigma$  is given by

$$\begin{aligned}\gamma^{-1}\dot{\sigma} &= \mu'(S)\dot{S}x + \mu(S)\dot{x} \\ &= x \left( D(\mu'(S)(S_{in} - S) - \mu(S)) \right. \\ &\quad \left. - \mu'(S) \left( \frac{k}{\gamma}(\sigma + p^*) + d \right) + \mu^2(S) \right)\end{aligned}$$

Clearly, one can see that these dynamics can be (generically) written as

$$\dot{\sigma} = x(a(S, p, d) + b(S, S_{in})D) \quad (3)$$

where  $x \geq 0$ , suggesting that  $\sigma$  can be controllable through  $D$  and a proper selection of  $S_{in}$  (indeed, it is enough to guarantee that  $b(S, S_{in})$  is greater from zero).

As a first step, let us analyze the dynamics of the substrate  $S$ . Since the quantity of interest  $p$  depends on  $S$ , substrate depletion, i.e.  $S = 0$ , implies the shutdown of the bioreactor. In order to avoid such a phenomenon, define the following control law:

$$S_{in} = S + \frac{kp + \bar{d}}{\gamma D} \quad (4)$$

*Lemma 1.* Let Assumption 2 be satisfied. Consider model (1) and control law (4). Provided that  $D > 0$  for all  $t > 0$  and  $S(0) > 0$ , we have  $S \geq S(0)$  for all  $t \geq 0$ .

*Proof.* Since control (4) is positive and well-defined for all  $S > 0$  and  $D > 0$ , substituting (4) in the dynamics (1) we obtain  $\dot{S} = \bar{d} - d \geq 0$  hence, as  $\bar{d} - d \geq 0$  by definition, this proves the claim.  $\square$

The previous result can be clearly seen if the reader realizes that  $S_{in}$  is directly proportional to the (upper bounded) consumption and inversely proportional to the dilution rate.

Now, let us focus our attention on the dilution rate  $D$ . It is clear from model (1) that the dilution rate can be used to stabilize a single species, and many results were performed in this sense in the literature (see the references cited above). In this work, we will design  $D$  in order to stabilize the surface  $\sigma = 0$ , taking into account the previous selection of  $S_{in}$ .

To this end, let us consider the following control law for the dilution rate:

$$D = \begin{cases} (1 + \rho) \frac{\mu'(S)\bar{d} + \mu^2(S)}{\mu(S)}, & \text{if } \sigma \geq 0 \\ (1 - \rho)\mu(S), & \text{if } \sigma < 0 \end{cases} \quad (5)$$

where  $\rho \in (0, 1)$  is a tuning parameter.

This brings us to the main result of this section:

*Theorem 1.* Let assumptions 1 and 2 be satisfied. Consider model (1) and the decision variable  $\sigma$  with  $p^* > 0$ . Then, applying control laws (5) and (4), the output  $\sigma$  is stabilized in a finite-time.

*Proof. (Sketch)* Stability of output  $\sigma$  is shown by considering  $V = \frac{1}{2}\sigma^2$  as a Lyapunov function candidate and showing that  $\dot{V} \leq -\eta|\sigma|$ , for some  $\eta > 0$  dependent on initial conditions.  $\square$

### 3.2 Robust Observer Design

As discussed in the previous section, the implementation of controllers (4) and (5) requires perfect knowledge of  $S$ . However, since this variable is usually not available, an observer is then needed so this part of the dynamics can be estimated.

In this section, we will consider the design of a robust observer for system (1), using the aforementioned output  $p$ . First, for simplicity in the following, let us introduce the correction term by output injection as  $y_e = p - \gamma\mu(\hat{S})\hat{x}$ , where  $\hat{S}$  and  $\hat{x}$  are the estimates of  $S$  and  $x$ , respectively, designed below. This correction term can be written as  $y_e = \gamma(e_\mu x + e_x \mu(\hat{S}))$ , where  $e_\mu = \mu(S) - \mu(\hat{S})$  and  $e_x = x - \hat{x}$ .

Now, let us consider the following observer:

$$\begin{cases} \dot{\hat{S}} &= D(S_{in} - \hat{S}) - \frac{kp}{\gamma} + L_1 \text{sign}(y_e) \\ \dot{\hat{x}} &= \frac{p}{\gamma} - D\hat{x} \end{cases} \quad (6)$$

*Remark 1.* Although control laws (5)–(4) do not require feedback on  $x$ , its estimation is still needed since the correction term by output injection requires such an information.

In this light, by introducing  $e_s = S - \hat{S}$ , the dynamics of the estimation error is given by

$$\begin{cases} \dot{e}_s &= -De_s - d - L_1 \text{sign}(y_e) \\ \dot{e}_x &= -De_x \end{cases} \quad (7)$$

This brings us to the following result:

*Lemma 2.* Let assumptions 1 and 2 be satisfied. Consider model (1) and observer (6). Provided that  $D \geq D_{\min} > 0$ ,  $x \geq x_{\min} > 0$  and  $L_1 > \bar{d}$ , then the origin of the estimation error dynamics (7) is asymptotically stable.

*Proof. (Sketch)* First, it is readily seen from (7) that, if  $D > 0$ , the dynamics of  $e_x$  is globally asymptotically stable. The stability analysis of the estimation error  $e_s$  is proved by showing that  $V_s = \frac{1}{2}e_s^2$  is a local *input-to-state* (ISS) Lyapunov function.  $\square$

### 3.3 Issues with Observer-Based Control

For linear systems, observer-based control is an easy task thanks to the *separation principle*, which states that stability of the feedback control and observer are independent, thus their design can be performed separately. For most nonlinear systems, such as the one considered in this work, this principle is in general not easy to be verified.

In order to implement control laws (5)–(4) using estimates coming from observer (6), we need to investigate the impact of the estimation error (7) in the control architecture.

Recalling properties of (7), we have that the solution of  $e_s$  satisfies  $|e_s| \leq E(t)$ , where  $E(t) = Ke^{-D_{\min}t}$  for some  $K > 0$ .

*Remark 2.* The constant  $K$  above is related to the unknown initial estimation errors  $|e_s(0)|$  and  $|e_x(0)|$ . However, since the operation of a bioreactor is physically constrained, it is reasonable to admit estimated bounds for the initial conditions, such as  $[\underline{S}(0), \bar{S}(0)]$  and  $[\underline{x}(0), \bar{x}(0)]$ . Then, the specification of  $E$  simply relies on considering these estimated bounds in the initialization of the observer (6).

Then, let us analyze the impact of the estimation error over (4). Recalling that  $\hat{S} = S - e_s$ , we introduce the following modification of (4):

$$S_{in} = (S - e_s) + \frac{kp + \bar{d}}{D} + E \quad (8)$$

and, consequently, it renders the dynamics of  $S$  as

$$\dot{S} = D(E - e_s) + \bar{d} - d$$

and  $\dot{S} \geq 0$  for all  $t \geq 0$  again.

With this in mind, let us recall the candidate Lyapunov function  $V = \frac{1}{2}\sigma^2$ , as defined previously. Differentiating it with respect to time and evoking (8), we have that

$$\dot{V} \leq \gamma x \sigma (\mu'(S)(\bar{d} - d + D(E - e_s)) + \mu^2(S) - \mu(S)D)$$

which, rearranging terms so  $D$  is made explicit, can be written as

$$\begin{aligned} \dot{V} \leq & \gamma x \sigma \left( \mu'(S)(\bar{d} - d) + \mu^2(S) \right. \\ & \left. + D \left( \mu'(S)(E - e_s) - \mu(S) \right) \right) \end{aligned} \quad (9)$$

A closer look at this last expression suggests that (5) is no longer suitable. Hence, a modification on such a control law is needed. As it will play a key role in the following, let us consider the following time-varying interval

$$S \triangleq [\max\{\hat{S} - E, \underline{S}(0)\}, \hat{S} + E] \quad (10)$$

In this behalf, we need an extra assumption:

*Assumption 3.* Let  $2 \max_{r \in \mathcal{S}} \mu'(r)E < \min_{r \in \mathcal{S}} \mu(r)$  hold for all  $S > 0$  and  $t > 0$ .

Such an assumption is naturally satisfied if we operate in the domain of ‘‘saturation’’ of  $\mu(S)$ , when  $\mu'(S)$  is small and  $\mu(S)$  is close to the maximum. Another motivation for this assumption is that  $E$  can be assigned sufficiently small if initial uncertainty in the values  $S(0)$  and  $x(0)$  is moderate.

Then, similarly as in the previous work (Reis de Souza et al., 2019), we design a robust version of the dilution rate by taking into account the worst-case error (induced by the estimated variables). In this sense, we propose the following switching law for the dilution rate:

$$D = \begin{cases} (1 + \rho)\alpha, & \text{if } \sigma \geq 0 \\ (1 - \rho)\beta, & \text{if } \sigma < 0 \end{cases} \quad (11)$$

where  $\rho \in (0, 1)$  and

$$\begin{aligned} \alpha &= \frac{\max_{r \in \mathcal{S}} \mu'(r)\bar{d} + \mu^2(r)}{\min_{r \in \mathcal{S}} \mu(r) - 2 \max_{r \in \mathcal{S}} \mu'(r)E} \\ \beta &= \min_{r \in \mathcal{S}} \mu(r) \end{aligned}$$

*Remark 3.* The computation of  $\alpha$  and  $\beta$  requires no optimization procedure. Profiting on the monotonicity of  $\mu(r)$ , all maximum (resp. minimum) on these functions can be computed by evaluating  $\mu$  at the upper (resp. lower) boundary of (10), i.e.:

$$\begin{aligned} \alpha &= \frac{\mu'(\underline{r})\bar{d} + \mu^2(\bar{r})}{\mu(\underline{r}) - 2\mu'(\underline{r})E} \\ \beta &= \mu(\underline{r}) \end{aligned}$$

where, for readability,  $\underline{r} = \max\{\hat{S} - E, \underline{S}(0)\}$  and  $\bar{r} = \hat{S} + E$ .

This brings us to the main result of this subsection:

*Theorem 2.* Let assumptions 1-3 be satisfied. Consider model (1), observer (6) and the decision variable  $\sigma$  with  $p^* > 0$ . Then, applying control laws (8) and (11), the output  $\sigma$  is stabilized in a finite-time.

*Proof. (Sketch)* First, since the convergence of the observer has been proved previously, it is easy to notice that  $|S - \hat{S}| \leq E$ , hence we can still state that  $S$  is strictly greater from zero. Then, stability of the output  $\sigma$  is then proven by considering (9), showing that  $\dot{V} < 0$ .  $\square$

The fact that we are able to stabilize the output  $\sigma$  in a finite-time even with an asymptotically converging observer is not surprising, and it follows a special structure (3) of the regulated dynamics.

#### 4. NUMERICAL EXAMPLE

This section illustrates the usefulness of this methodology through a numerical example. The continuous bioreactor is considered as given by model (1), simulated with initial conditions  $S(0) = 3$ ,  $x_1(0) = 5$ . The measured output,  $p(t)$ , is supposed to be corrupted by measurement noise with a variance of 0.1.

The objective is to stabilize  $p(t)$ , produced by a species described by  $\Theta_1 = [3.2, 5, 1]$  and with  $\gamma = 1$  for simplicity, at  $p^* = 100$ . However, we simulate an invasion caused by a second species, described by  $\Theta_d = [4, 8, 1]$ , at  $t_i = 1$ h and  $x_d(t_i) = 5$  g/L. This means that this species will compete for substrate and do not produce the compound of interest.

For control and observer design, we select  $\rho = 0.01$  and  $\bar{d} = 1$ . Furthermore, the observer (6) is initialized with initial conditions  $\hat{S}(0) = 10$  and  $\hat{x}(0) = 10$  and a gain  $L_1 = 30$ . Thus, we have all information needed to implement control laws (8)–(11) and all simulation results are shown in the following.

The evolution of the states is shown in Fig. 3, where the effect of the invasion and the respective response of the controller can be easily noticeable. Indeed, before the invasion, the substrate concentration was  $S \approx 25$  g/L, in which the invasive species has more affinity to the substrate (i.e.,  $\mu_d(S) > \mu_1(S)$ , see Fig. 2). Since this leads to a decrease in the production of  $p(t)$  (as seen in

Fig. 5), the control architecture responds and regulates the substrate to  $S \approx 8 \text{ g/L}$ , leading to a more advantageous scenario to the species of interest after a small transient.

Accordingly, Fig. 4 shows the convergence of the observer to the true values of  $x_1$  and  $S$  after a small transient phase. The effect of noise is noticeable in  $\hat{S}$  and not in  $\hat{x}$ , since no output injection is needed for convergence of the latter. Furthermore, by comparison of Fig. 3 and 4, we notice that the effect of the convergence of  $\hat{S}$  in the control is very low. Finally, Fig. 5 shows the successful stabilization of output  $p$  at the productivity index  $p^*$ , in a finite-time.

In Fig. 6, we can see that the resulting control inputs show larger variations, which are due to the switching nature of control law (5). The sliding-phase is also affected by the measurement noise, being directly related to the output feedback on the decision variable  $\sigma$  and can be alleviated in practice by filtering. Furthermore, practical implementations of such a controller (which are future directions of research) can rely results on consistent discretization (such as Polyakov et al. (2019)).

Another interesting fact to be highlighted in Fig. 6 is that, although there is an additional species, concentration  $S_{in}$  is practically equal to the one selected for the motivating example, given in Section 2. This means that the proposed control law does not induce a trivial solution (i.e., having a large constant value for  $S_{in}$  to overcompensate the consumption of the invasive species – which might be unpractical or unprofitable).

As it can be seen on these results, the proposed control had no explicit knowledge on the “bad species”, but successfully attained the stabilization problem. By overcoming uncertainty (introduced by unmodelled dynamics) and also a noise-corrupted measured output, this numerical experiment suggests that observed-based control is suitable for these applications.

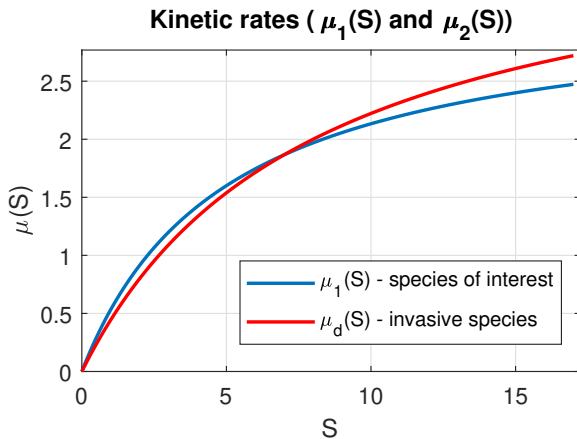


Fig. 2. Comparison of kinetic rates  $\mu_1(S)$  and  $\mu_d(S)$

## 5. CONCLUSIONS AND PERSPECTIVES

In this paper, we investigated the problem of stabilizing the output of a continuous bioreactor, which represents a productivity index, despite of the biodiversity of the microbial culture. The control architecture is designed in a nonlinear observer-based paradigm, aiming to prevent the

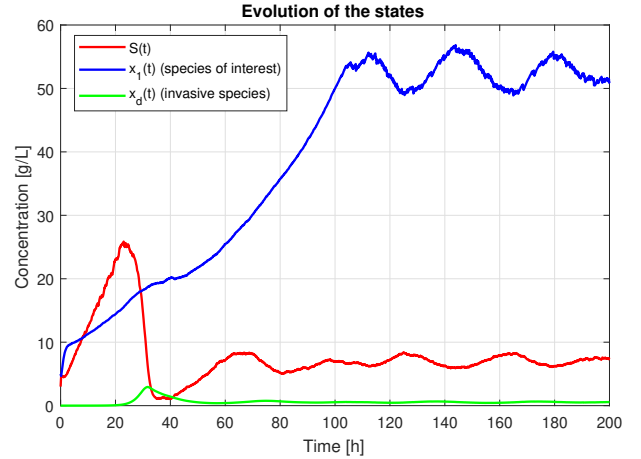


Fig. 3. Evolution of the states

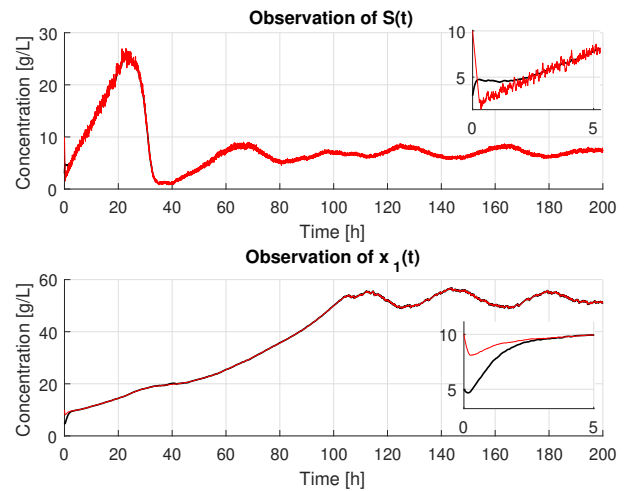


Fig. 4. Above: convergence of  $\hat{S}$ . Below: convergence of  $\hat{x}$ . The small boxes show the initial error due to the initialization of the observer.

species of interest (i.e., the producer) to be out-competed while also preventing shutdown by substrate depletion.

The requirement of perfect knowledge on  $\mu(S)$  on the design of both controller and observer is a harsh assumption that might restrain practical applications, since the parameter of this function are often uncertain. This issue is to be studied in future research.

Exploiting biodiversity in a positive manner, i.e., having (and automatically selecting the best) microbes that actually help the production among a plural community is an intended future direction of research. This would require overcoming challenges on observation and control.

Also, other two interesting directions emerge on this framework: considering inhibition (meaning that the specific growth rate is not monotone) and also considering a certain dynamics for the product of interest (i.e., if it participates on the reaction and thus is affected by the dilution rate/substrate concentration).

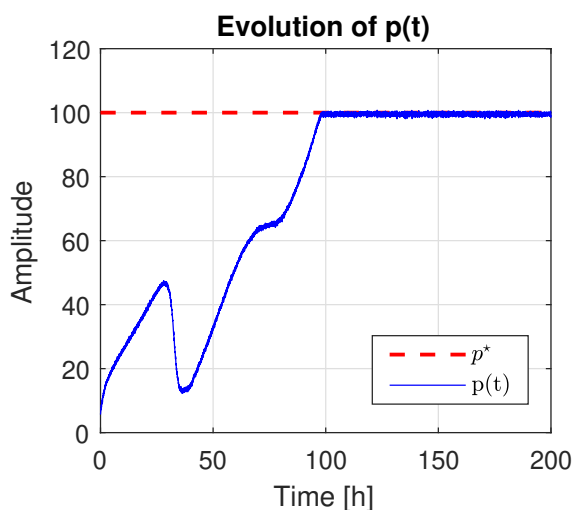


Fig. 5. Stabilization of output  $p$  in a finite-time ( $t \approx 100$ )

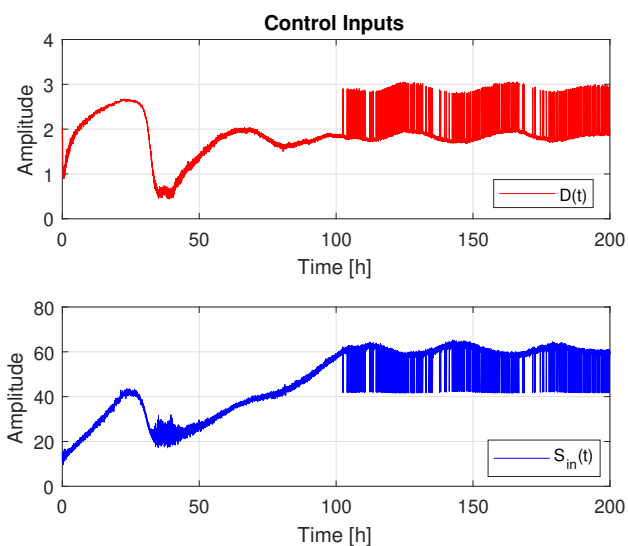


Fig. 6. Control inputs: dilution rate (above) and substrate inflow concentration (below). The effect of noise (propagated by the output feedback) upon the sliding regime is noticeable.

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