Bioprocess Biosyst Eng (2005) 27: 319–327
DOI 10.1007/s00449-005-0410-8

ORIGINAL PAPERS

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Nonlinear control for algae growth models in the chemostat

Received: 15 November 2004 / Accepted: 24 November 2005 / Published online: 18 June 2005

Abstract This paper deals with output feedback control of phytoplanktonic algae growth models in the chemostat. The considered class of model is of variable yield type, meaning that the ratio between the environmental nutrient absorption rate and the cells' growth rate varies, which is different from classical bioprocesses assumptions. On the basis of weak qualitative hypotheses on the analytical expressions of the involved biological phenomena (which guarantee robustness of the procedure toward modeling uncertainties) we propose a nonlinear controller and prove its ability to globally stabilize such processes. Finally, we illustrate our approach with numerical simulations and show its benefits for biological laboratory experiments, especially for ensuring persistence of the culture facing classical experimental problems.

Keywords Algae growth · Chemostat · Variable yield model · Nonlinear control

Introduction

The first models describing micro-organisms growth in continuous controlled laboratory devices, so-called chemostats, were proposed by Monod [14]. This class of models are based on the assumption that the micro-organisms growth rate is proportional to their consumption rate of some extracellular limiting nutrient. Thus, these models are often referred to as constant yield models [18]. Constant yield models predictions remain good compared to experimental data for micro-organisms like bacteria, but some important differences appear as unicellular photosynthetic algae are considered. This phenomenon was first described by Droop [4]. He, therefore, proposed a new approach dedicated to phytoplanktonic algae growth, reconsidering the "constant yield" hypothesis. Droop assumed that unicellular algae growth on a limiting nutrient is a two-step phenomenon: first, uptake of the nutrient in the cell and then, use of the intracellular nutrient to support cell's growth. As a result, the ratio between cells growth rate and nutrient consumption rate is no more constant. Hence, these kind of models are called variable yield models [18].

In previous works, we exhibited new nonlinear feedback controls for constant yield models, coming from the more general theoretical framework proposed in [9, 10]. A crucial point of these works is that the control procedure requires only qualitative hypotheses on the micro-organisms' growth rates, what is important for the robustness toward modeling uncertainties that are common in biological models [11, 12]. Here, we propose to extend these results to variable yield models.

This paper is organized as follows: first, we state the general variable yield model for unicellular algae growth in chemostats and we make some qualitative hypotheses about the algae's substrate uptake and growth rates. Then, we propose the nonlinear controller and prove the global asymptotic stability of the closed loop resulting system. Finally, some numerical simulations, assuming various experimental operating conditions or failures, illustrate our approach.

The variable yield model

A chemostat is a laboratory apparatus consisting of a vessel enclosing the liquid culture medium. The micro-organism population grows in this medium consuming a nutrient (i.e., substrate). A liquid flow (F) passes through the vessel; the inflow feeds the chemostat with the substrate at concentration s_{in}, while the outflow is composed by the same compounds than inside the chemostat. The volume of the culture medium V remains constant since the inflow and the outflow are equal. Let us define the dilution rate $D = F/V$.

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The model

The model variables are the extracellular limiting nutrient concentration (denoted s), the intracellular nutrient per unit of biomass (called cell quota and denoted q) and the biomass concentration (denoted x). Have a look at the nomenclature in Appendix A for the variables' units. All the concentrations are supposed to be homogeneous. The function $\rho(.)$, depending on the substrate concentration s, is the substrate uptake rate while the function $\mu(.)$, depending on the cell quota q, is the growth rate of the algae. According to [4, 18], we obtain the following variable yield model:

$$\begin{cases} \dot{s} = D(s_{\text{in}} - s) - \rho(s)x \\ \dot{q} = \rho(s) - \mu(q)q \\ \dot{x} = \mu(q)x - Dx \end{cases} \tag{1}$$

It is well known in biology that the most crucial problem in modeling the considered phenomenon is to propose some realistic analytical expressions for the biological functions $\rho(.)$ and $\mu(.)$. As in [16], in order to bypass these modeling difficulties, we only suppose qualitative hypotheses about $\rho(.)$ and $\mu(.)$.

Hypothesis 1 (H1) $\rho(0) = 0$ *and* $\rho(s)$ *is* $\mathcal{C}^1$, *increasing and bounded*; $\mu(q)$ *is* $\mathcal{C}^1$, *non-negative, increasing and bounded*; *there exists* $q_m > 0$ *such that* $\mu(q_m) = 0$

These hypotheses mean that: if substrate s is available then the cell uptakes it. More the substrate available, the higher the uptake rate. Boundedness of both $\rho(.)$ and $\mu(.)$ comes from biological evidences. The parameter q_m stands for the minimum cell quota: when q drops below q_m, there is insufficient internal nutrient for the cell to grow, and when more internal quota q is available, the cell grows more.

Hence, we do not consider neither the possibility of inhibition of substrate that is modeled by nonmonotone functions (e.g., Haldane model). However, this phenomenon may also be addressed with a quite similar procedure [11, 12].

Throughout the paper, we only consider initial conditions for the state variables belonging to the open biological meaningful cone $\Omega = \{s > 0,\ q > q_m,\ x > 0\}$. Observe that the closure of Ω is invariant by system 1. In order to exhibit a better form of system Eq. 1, we use the change of coordinates $z = s + qx$. z represents the total amount of intracellular and extracellular nutrient in the chemostat. Then, we obtain the following system, which is easier to deal with, especially due to the autonomous and almost linear z dynamics:

$$\begin{cases} \dot{z} = D(s_{\text{in}} - z) \\ \dot{s} = D(s_{\text{in}} - s) - \rho(s)x \\ \dot{x} = \mu\left(\dfrac{z - s}{x}\right)x - Dx \end{cases} \tag{2}$$

Behavior of the open loop model

The asymptotic behavior of system Eq. 1 has been thoroughly studied in [2, 7, 18]; results are of two different types depending on the value of D compared to s_{in}: either there exists a positive equilibrium point and each forward positive orbit initiated in Ω goes toward it, either not and every forward orbit goes to the washout point corresponding to the disappearance of the algae from the chemostat ($x = 0$).

It is clear that washout of the culture must not happen. Here, via the control of the model, we aim at preventing the disappearance of the positive equilibrium, i.e., at preventing biomass washout. In other words, we intend to impose the convergence of the state toward a positive equilibrium point. Specifically, we want to drive and keep biomass concentration toward a chosen positive value. Moreover, uncertainty in feeding substrate concentration s_{in} may sometimes destabilize the system leading to biomass washout. Another point important to be addressed is the possible algae stress (unpredicted fall of cells growth rate) that may as well lead to biomass washout. Then, it is crucial to guarantee that the biomass goes toward its chosen positive value, independently from s_{in} variations and/or potential algae stress.

Nonlinear control design

Statement of the control framework

Applied control of biological systems generally differs from the classical framework of control where it is usually assumed that the model is perfectly known [15]. To control biological systems, we have to take into account that the model may be only qualitatively known and that the outputs may be some unknown nonlinear functions of the state variables. Moreover, inputs are considered unconstrained in classical control theory, whereas they usually fulfill some constraints (e.g., positivity) in biological systems.

Due to the high variability of biological phenomena, we consider here a qualitatively known model, qualitative outputs and constrained input. Therefore, we cannot apply classical linearisation techniques requiring a detailed analytical expression of the model (see e.g., [5]).

However, we still have to define the manipulated variable (input), and the online measured variable (output). In chemostat-like systems, it is well known that the (non-negative) dilution rate D is easy to manipulate, thus, we use it as the (constrained) input of the system. Now, we define the output; here, we suppose that our chemostat is instrumented with sensors that can measure, either the uptaked carbon or the produced oxygen due to the algae photosynthesis. Note that both quantities are proportional to the cells growth. Hence, we assume that the output $y = \mu(q)x$, namely the cells population growth velocity, is available online from the

plant. Let us summarize these assumptions in the following hypothesis.

Hypothesis 2 (H2) $D \geq 0$ *is the constrained input of system* 1; $y = \mu(q)x$ *is an output of system Eq. 1*

Nonlinear control design

Now, we state and prove our main result, using the notation ξ for the state vector.

Proposition 1 Under assumptions (H1) and (H2), the nonlinear output feedback control law:

$$D(.) = \gamma y = \gamma \mu(q)x \quad \text{with } \gamma > \frac{q_m}{s_{\text{in}}} \tag{3}$$

globally stabilizes system Eq. 1 *toward the single positive equilibrium* $\xi^{\star}$, *determined by the value of* γ.

By "positive" equilibrium point, we refer to a point whose elements are all positive. For instance, the washout point that corresponds to the disappearance of algae from the chemostat ($x = 0$) is an equilibrium point of Eq. 1, but not a positive one. Moreover, note that with expression Eq. 3, the input $D(.)$ remains non-negative and therefore, fulfills its positivity constraint (see (H2)).

The control law Eq. 3 leads to the following closed loop system:

$$\begin{cases} \dot{z} = D(.)(s_{\text{in}} - z) \\ \dot{x} = D(.)\left(\frac{1}{\gamma} - x\right) \\ \dot{s} = D(.)(s_{\text{in}} - s) - \rho(s)x \end{cases} \tag{4}$$

We first want to show that for the closed loop system Eq. 4, both variables z and x converge (asymptotically) to s_{in} and $1/\gamma$respectively. Let us integrate the equations $\dot{z}$ and $\dot{x}$ of system Eq. 4, we have:

$$\begin{cases} z(t) = s_{\text{in}} + (z(0) - s_{\text{in}})\mathrm{e}^{-\int_0^t D(\tau)d\tau} \\ x(t) = \dfrac{1}{\gamma} + \left(x(0) - \dfrac{1}{\gamma}\right)\mathrm{e}^{-\int_0^t D(\tau)d\tau} \end{cases} \tag{5}$$

Thus, we have to prove that the quantity $\int_0^t D(\tau)\mathrm{d}\tau$ diverges toward infinity as time tends to infinity to show the convergence of z and x toward s_{in} and $1/\gamma$.

Therefore, before proving Proposition 1, we show the following:

Lemma 1 Under hypotheses (H1) and (H2) and with the control procedure Eq. 3 applied to model Eq. 1, we have:

$$\lim_{t \to +\infty} \int_0^t D(\tau)\mathrm{d}\tau = +\infty.$$

Proof Since $D(.)$ is non-negative, it is straightforward that: $\mathrm{e}^{-\int_0^t D(\tau)d\tau} \in [0, 1]$. Then, we have:

$$\forall t \geq 0 \begin{cases} \max(s_{\text{in}}, z(0)) \geq z(t) \geq \min(s_{\text{in}}, z(0)) > 0 \\ \max\left(\frac{1}{\gamma}, x(0)\right) \geq x(t) \geq \min\left(\frac{1}{\gamma}, x(0)\right) > 0 \end{cases} \tag{6}$$

Let us suppose that $\lim_{t \to +\infty} \int_0^t D(\tau)d\tau$ is bounded. Thus, since $D(.) \geq 0$, a necessary condition is that:

$$\lim_{t \to +\infty} D(t) = 0$$

From Eqs. 6 and 3, since γ is positive and x lower bounded by a positive constant, it implies at least that:

$$\lim_{t \to +\infty} \mu(q(t)) = 0 \Rightarrow \lim_{t \to +\infty} q(t) = q_m$$

Since $q(t)$ is a time-Lipschitz function ($\dot{q}$ is bounded), it is uniformly continuous in time. Then, using Barbalat's lemma (see Appendix B), we show that:

$$\lim_{t \to +\infty} \dot{q} = 0$$

that leads to (see Eq. 1): $\lim_{t \to +\infty} \rho(s(t)) = 0$ and thus:

$$\lim_{t \to +\infty} s(t) = 0$$

Observe that these points, corresponding to $q = q_m$ and $s = 0$, are equilibria of the system Eq. 4 for all values of the variable x. Since x is positively lower bounded, they are defined, for all $x \geq \min(1/\gamma, x(0))$, by:

$$\xi_u = (s = 0, q = q_m, x)^{\mathrm{T}}$$

Now, we want to show that these equilibria are not reachable from initial conditions belonging to the cone Ω. To achieve this purpose, let us compute the Jacobian matrix at these equilibrium points, in the $(s, q, x)^{\mathrm{T}}$ variables. With $\rho'(0) = (\partial\rho/\partial s)_{s=0}$ and $\mu'(q_m) = (\partial\mu/\partial q)_{q=q_m}$ we have:

$$\mathcal{J}(\xi_u) = \begin{pmatrix} -\rho'(0)x & \gamma\mu'(q_m)xs_{\text{in}} & 0 \\ \rho'(0) & -\mu'(q_m)q_m & 0 \\ 0 & \gamma\mu'(q_m)x\left(\frac{1}{\gamma} - x\right) & 0 \end{pmatrix} \tag{7}$$

It is straightforward that one of the eigenvalues is zero, with the associated eigenvector $(0,0,1)^{\mathrm{T}}$, which corresponds to the fact that we have a continuum of equilibria along the x direction.

Now, let us wonder about the two other eigenvalues. These are the same eigenvalues as the matrix B:

$$B = \begin{pmatrix} -\rho'(0)x & \gamma\mu'(q_m)xs_{\text{in}} \\ \rho'(0) & -\mu'(q_m)q_m \end{pmatrix} \tag{8}$$

Remember that since (H1) holds, $\rho'(0)$ and $\mu'(q_m)$ are positive, then the trace of matrix B is obviously negative. Now, we compute the determinant, we have:

$$\det B = \rho'(0)x\mu'(q_m)(q_m - \gamma s_{\text{in}})$$

This determinant is negative since $\gamma > q_m/s_{\text{in}}$, then there exists a positive real eigenvalue and unfortunately a negative real one that generates a stable manifold of the point ξ_u. Now, we focus only on the stable manifold, since the equilibrium point ξ_u can only be reached from this set.

Hence, we want to show that the stable eigenvector, at ξ_u, does not point from the cone Ω toward the point

ξ_u, which will prove that ξ_u cannot be locally reached from Ω.

Note that the matrix B is off-diagonal positive and irreducible. Then, we apply a corollary of the Perron-Frobenius theorem (see Appendix C), showing that the positive eigenvectors are only associated with the eigenvalue of largest real part (here the positive one). Then, the stable eigenvector of matrix B is not positive (not all its elements are positive).

From matrix B, since none of its components is zero, straightforward calculus show that the stable eigenvector has no zero component and then, both its components have different signs. Remind that these two components are the first two of the stable eigenvector of $\mathcal{J}(\xi_u)$. Considering the opposite sign of the first two components of the stable eigenvector, it is clear that this vector does not point toward the positive cone $\mathbb{R}^3_{+,*}$ and thus not to Ω (a translation of $\mathbb{R}^3_{+,*}$).

Therefore, the stable eigenvector does not point from Ω to ξ_u. Then, ξ_u cannot be locally reached from Ω. From the invariance of the closure of Ω by system Eq. 4, the stable manifold of ξ_u can not be reached from Ω and no trajectory initiated in Ω converges toward ξ_u.

Remember that the convergence toward ξ_u is a necessary condition for the boundedness of $\lim_{t\to\infty}\int_0^t D(\tau)\mathrm{d}\tau$, hence, it cannot be bounded, and since $D(.)\geq 0$, we have:

$$\lim_{t\to+\infty}\int_0^t D(\tau)\mathrm{d}\tau = +\infty$$

□

Now, using Lemma 1, we can prove Proposition 1.

Proof Note that Lemma 1 together with Eq. 5 implies that:

$$\begin{cases}\lim_{t\to+\infty} z(t) = s_{\mathrm{in}}\\ \lim_{t\to+\infty} x(t) = \frac{1}{\gamma}\end{cases} \tag{9}$$

Then all forward trajectories of system Eq. 4 converge toward the set $\mathcal{E} = \{\xi \in \Omega, z = s_{\mathrm{in}}, x = 1/\gamma, s_{\mathrm{in}}\}$.

Now, let us consider the "reduced" system Eq. 4, in s, under the constraint $\xi \in \mathcal{E}$., we have:

$$\dot{s} = (s_{\mathrm{in}} - s)\mu(\gamma(s_{\mathrm{in}} - s)) - \frac{\rho(s)}{\gamma} \tag{10}$$

which, using a time scale change, is equivalent to (see, e.g., [3]):

$$\dot{s} = \gamma(s_{\mathrm{in}} - s)\mu(\gamma(s_{\mathrm{in}} - s)) - \rho(s) \tag{11}$$

From the invariance of the cone Ω and since $\mu(.)$ is an increasing function, it is straightforward that:

$$g(\gamma, s) = \gamma(s_{\mathrm{in}} - s)\mu(\gamma(s_{\mathrm{in}} - s))$$

is a decreasing function of s. Furthermore, $g(\gamma, s)$ is an increasing function of γ. This situation corresponds to Fig. 1, which shows that there exists a single, positive, equilibrium s for Eq. 11 which is globally asymptotically stable. Note that $s^\star$ increases as γ increases.

Then, system Eq. 4 has a single, positive, equilibrium denoted $\xi^\star = (z^\star = s_{in}, x^\star = 1/\gamma, s^\star)^{\mathrm{T}}$. Note that the choice of the gain γ allows to choose either the value $s^\star$ or the value $x^\star$.

Now, let us come back to system Eq. 4 and consider the $\dot{s}$ equation, injecting the solutions $z(t)$ and $x(t)$ initiated at $z(0)$ and $x(0)$, respectively. Then, for each couple of initial conditions $(z(0), x(0))$, we obtain the following nonautonomous system:

$$\dot{s} = D(s, z(t), x(t))(s_{\mathrm{in}} - s) - \rho(s)x(t) \tag{12}$$

Note that Lemma 1 implies that for each couple of initial conditions $(z(0), x(0))$, the non-autonomous system Eq. 12 is "asymptotically autonomous" (see Appendix D) with limit Eq. 10. Applying a theorem on asymptotically autonomous systems from [13, 19] (see Appendix D), we conclude that for each couple of initial conditions $(z(0), x(0))$, each forward trajectory of system Eq. 12 converges toward the globally asymptotically stable equilibrium point $s^\star$ of the limit autonomous system Eq. 10. Thus, for each initial state vector $\xi(0) \in \Omega$, the forward orbit of system Eq. 4 converges asymptotically toward the point $\xi^\star = (z^\star = s_{\mathrm{in}}, x^\star = 1/\gamma, s^\star)^{\mathrm{T}}$, which is therefore globally attractive on Ω.

Now, let us compute the Jacobian matrix of the closed loop system Eq. 4 around the equilibrium point $\xi^\star$ in the $(z, x, s)^{\mathrm{T}}$ coordinates. Remark that this matrix is lower triangular, then, we only consider the diagonal terms (• stands for any possible term). We have:

$$\mathcal{J}^\star = \begin{pmatrix} -D(\xi^\star) & 0 & 0\\ 0 & -D(\xi^\star) & 0\\ \bullet & \bullet & -D(\xi^\star) - \frac{\rho'(s^\star)}{\gamma}\end{pmatrix}$$

Since $\rho(.)$ is an increasing function, γ is positive and $D(\xi^\star) = \mu(\gamma(s_{in} - s^\star))$ is positive, it is straightforward

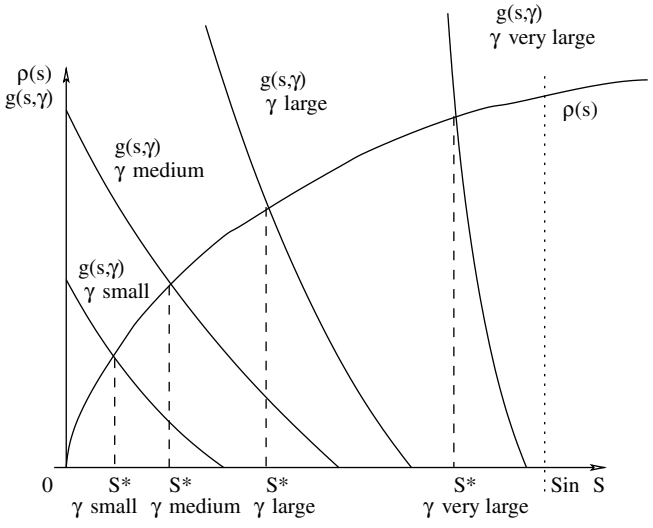

Fig. 1 Existence, unicity and stability of $s^\star$ for system Eq. 11 from the intersection of $\rho(s)$ and $g(s,\gamma)$

that $\xi^\star$ is locally stable for system Eq. 4. Since $\xi^\star$ is globally attractive too, we conclude that $\xi^\star$ is a positive, globally asymptotically stable equilibrium point for the closed loop system 4. □

Remark 1 Remember that the demonstration is not based on any analytical expression for the "biological" functions $\mu(.)$ and $\rho(.)$ which is particularly important regarding the difficulty of modeling and identification of these functions.

Remark 2 It is important to note that the asymptotic behavior of biomass concentration x does not depend on parameter s_{in}. Then, even for a time varying parameter $s_{\text{in}}(t)$, biomass concentration x will asymptotically converge toward $1/\gamma$, provided that for all time $\gamma > q_m/(s_{\text{in}}(t))$.

Remark 3 Observe that the condition $\gamma > q_m/s_{\text{in}}$ imposes, for a fixed feeding substrate concentration s_{in}, an upper limit on the reachable biomass concentration. This limit is independent from the analytical modeling of the growth rate $\mu(.)$, and moreover, the uptake rate of extracellular substrate $\rho(.)$ does not affect this limit at all, which is only determined by the minimum cell quota q_m.

Numerical simulations

We consider as an example the growth of *Dunaliella tertiolecta*, a green micro-algae. Then, according to [1], the uptake and growth rates are (for all the following simulations):

$$\rho(s) = \frac{\rho_m s}{k+s} \quad \text{and} \quad \mu(q) = \max\left(0, \mu_m\left(1 - \frac{q_m}{q}\right)\right)$$

Parameters values and units are to be found in the nomenclature (Appendix A).

Simple noisy simulation

We first show in Fig. 2 a simple noisy numerical simulation of the controlled process. The parameter s_{in} is assumed to be equal to 20\simg.L^{-1}. In addition, we corrupt the output $y = \rho(q)x$ with a relative white noise of 30% amplitude.

The obtained results agree with the predicted theoretical behavior of the controlled plant (see Proposition 1). From the biological point of view, the control law Eq. 3 drives the state variables toward the desired equilibrium determined by the value of the feedback gain γ; indeed, since $\gamma = 0.1.10^{-6}$L.c^{-1}, biomass concentration reaches asymptotically $x^\star = \gamma^{-1} = 10.10^6 \sim c.L^{-1}$. Furthermore, note that despite the high level of noise on the output (30%) perturbations are almost completely filtered and do not really affect the state variables.

Controlled process facing varying s_{in}

To illustrate the fact that time variations of influent substrate concentration s_{in} do not change the biomass

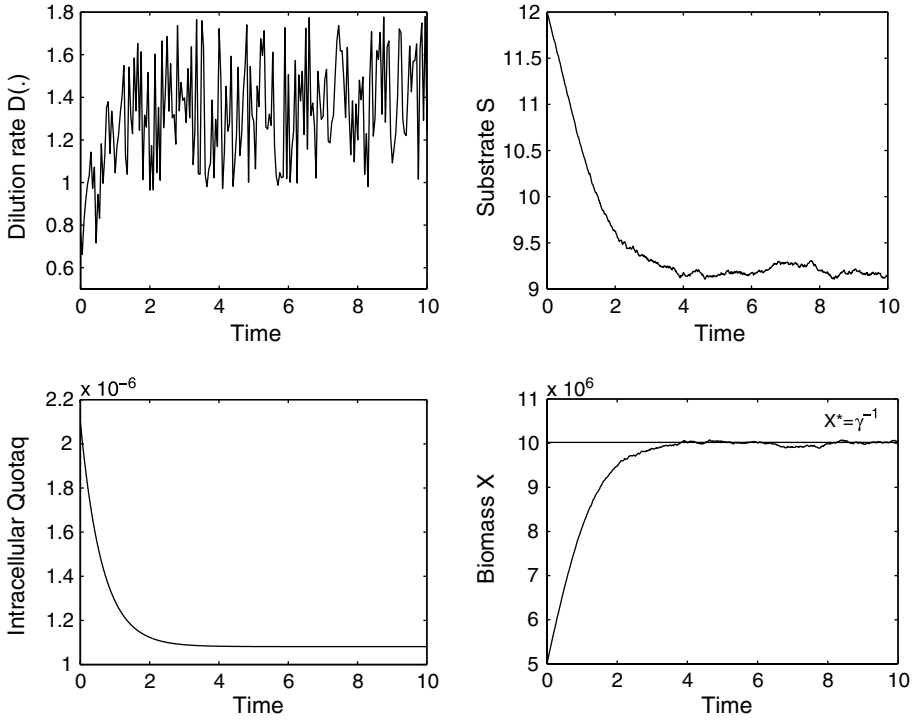

Fig. 2 Simulation of the closed loop system; constant s_{in}; 30% relative white noise on y

concentration x behavior, we consider a piecewise constant time-dependent s_{in} that moves from 40 $\mu g.L^{-1}$ to 2.5 $\mu g.L^{-1}$ and back to 40 $\mu g.L^{-1}$ each half day. Results are shown in Fig. 3. We check that, despite fast and large s_{in} variations, the behavior of the variable x remains almost the same than with fixed s_{in}: it converges toward its equilibrium $x^{\star} = 1/\gamma$.

From the controllers point of view, note that the only required knowledge for control is the output y and the feedback gain γ. It ensures (provided that $\gamma > q_m/\ [\min_t(s_{in}(t))]$) a very simple behavior for biomass concentration x that goes asymptotically toward $1/\gamma$ like a first order, independently from $s_{in}(t)$, even for quick and/or large variations. Of course, since model Eq. 1 is not controllable in the usual sense [15], some other state variables may change in time as x remains at equilibrium $x^{\star}$ (here, s does vary; so does q but its variations remain so small that they do not appear on the graph).

Controlled process facing periodic algae stress

We show in Fig.5 a comparison between open and closed loop chemostats facing a periodic "algae stress" that corresponds to a fall of the algae growth rate. This problem is frequently encountered while carrying out chemostat experiments: the medium feeding the vessel (particularly with substrate concentration s_{in} but with a blend of nutrient required for algae growth too) has to be regularly changed (usually each week). Even if one tries to use medias which are very similar, there always remain little differences in the composition, the pH or the temperature between the new medium and the previously used one. These differences may cause what is referred to as algae stress.

We choose to model this phenomenon assuming that the cells growth rate is time dependent such that:

$$\mu(.) = \Delta(t)\mu(q)$$

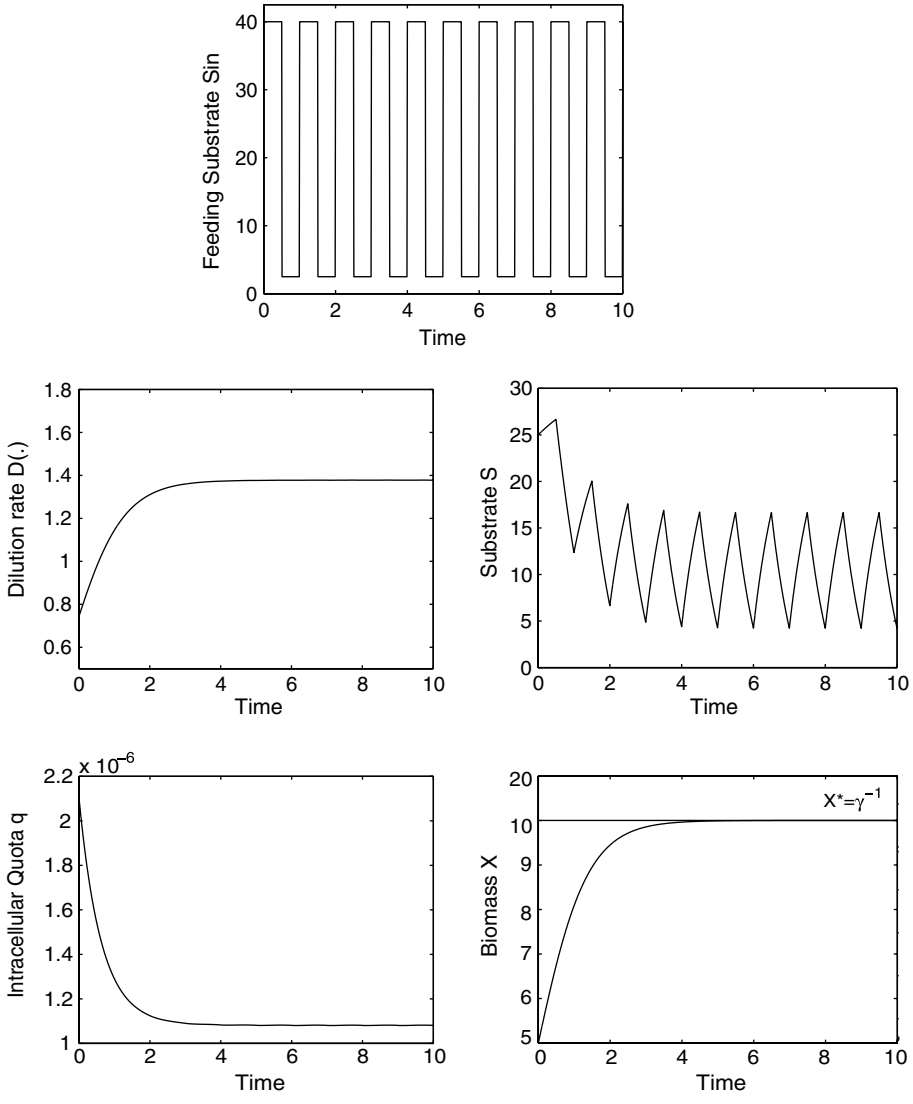

Fig. 3 Simulation of the closed loop system; varying $s_{in}(t)$

$\mu(q)$ being as previously defined and $\Delta(t)$ following the periodic graph of period 7 days depicted in Fig. 4. The first 5.5 days, the algae population is in good conditions for growth; at day 5.5, the feeding medium is changed; the differences between this medium and the previous one lead to a sudden fall (from 100% to 10%) of the algae growth rate amplitude that lasts for half a day; this features algae stress; during the last day of the period, the algae population growth goes back to normal as the algae adapt themselves to the new medium. $\Delta(t)$ is defined modulo 7 days.

For the open loop process simulation, we choose the dilution $D = D^\star$, the required value so that biomass concentration x would reach $x^\star = 10.10^6 \text{c.L}^{-1}$ if the algae were not stressed, while for the closed loop process the dilution $D(.)$ follows law Eq. 3. Results are presented in Fig.5.

It is worth noting that the open loop strategy seems dangerous for the culture. Note that in less than 3 weeks (while experiments usually lasts for 2 or 3 months), the algae population is almost completely removed from the chemostat, which is of no more use from this time. This leads to the restart of the experiment. On the contrary, the controlled chemostat drives the algae concentration to its desired value $x^\star$. Despite the algae stress, biomass concentration dynamics still follows the predicted behavior. Indeed, this interesting property (biomass concentration x can only go closer to $x^\star$ as time goes forward) holds due to Eq. 5 and remains true as long as biomass population growth stays positive, even if it is time varying [see 10].

Conclusions

In this contribution, we have proposed a nonlinear output feedback controller able to globally stabilize variable yield growth models in the chemostat. The hypotheses assumed on the model are of qualitative and of structural type, therefore, our approach is suitable for a wide class of variable yield models for micro-organisms growth in continuous bioreactors. Some simulations for *Dunaliella tertiolecta* growth with realistic parameters together with realistic experimental scenarios have been performed and have shown the relevance of our approach. Indeed, in each of the considered case (noisy output, time-varying s_{in} or periodic algae stress) the controller prevents the culture from washout and drives biomass concentration to a chosen steady-state value.

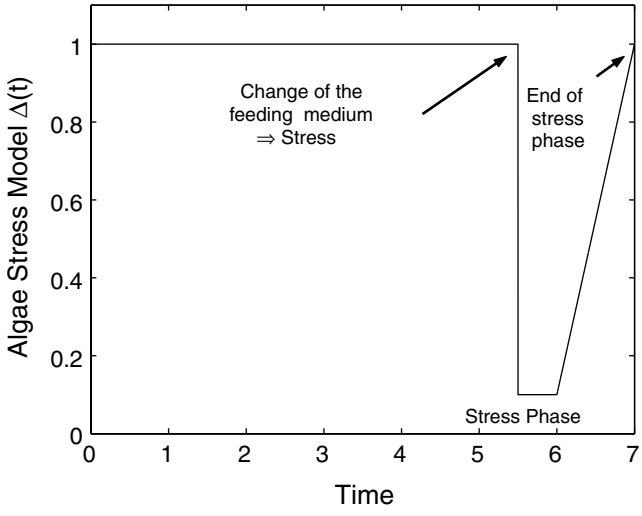

Fig. 4 $\Delta(t)$ model for algae stress simulation for the 7 days period

Acknowledgements We would like to thank the "Action Inter EPST Bioinformatique" for its financial support (SEMPO II Project).

A Nomenclature

Parameter values and units are according to [1] (for all the simulations):

B Barbalat's lemma

Lemma 2 (Barbalat, [6]) *Let $\phi : \mathbb{R} \to \mathbb{R}$ be a uniformly continuous function on $[0, \infty)$. Suppose that $\lim_{t\to+\infty} \int_0^t \phi(t)\mathrm{d}t$ exists and is finite. Then:*

$$\lim_{t\to+\infty} \phi(t) = 0$$

C Corollary of the Perron-Frobenius theorem

Definition 1, (Metzler matrix, [8]) *A is a Metzler matrix iff all its off-diagonal elements are non-negative.*

Corollary 1, (Perron-Frobenius, [17]) *Let A be an irreducible Metzler matrix. Then, λ_M, the eigenvalue of A of largest real part is real, and the elements of its associated eigenvector v_M are positive. Moreover, any eigenvector of A with non-negative elements belongs to* span $\{v_M\}$.

Remark 4 Actually, Smith proves more in his corollary (see [17]), but the remaining results are of no use for our purpose.

D Asymptotically autonomous systems

Definition 2 [13, 19] *Consider the systems*:

$$\dot{x} = f(t, x) \tag{13}$$

$$\dot{y} = g(y) \tag{14}$$

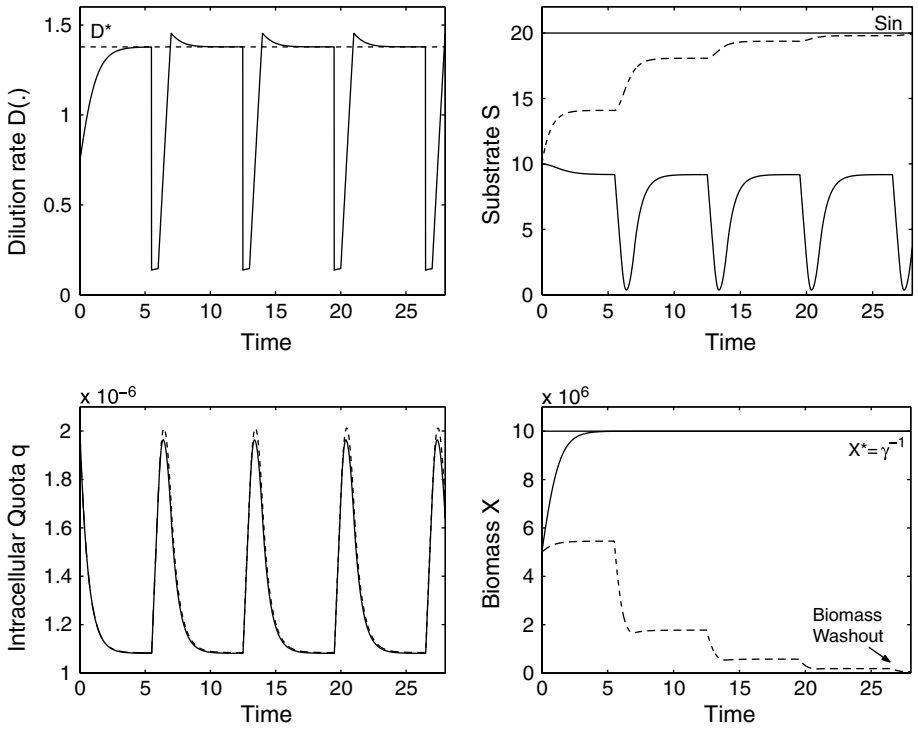

Fig. 5 Simulation of closed loop and open loop processes facing periodic algae stress; controlled process (—); open loop process with $D = D^{\star}$ (– –)

with $f(x,t)$ and $g(x)$ continuous in x and t and locally Lipschitz in x on an open set $\theta \subset \mathbb{R}^n$. System Eq. 13 is asymptotically autonomous with limit system Eq. 14 if for all compact $K \subset \theta$:

$$\lim_{t \to +\infty} f(t,x) = g(x), \quad \forall x \in K$$

Theorem 1 [13, 19] Consider the asymptotically autonomous system Eq. 13 with limit system Eq. 14. Let e be a locally asymptotically stable equilibrium of Eq. 14 and ω the ω -limit set of a bounded solution $x(t,x_0)$ of Eq. 13. If ω contains a point y_0 such that the forward trajectory $y\ (t,y_0)$ of Eq. 14 converges to e, then:

$$\lim_{t \to +\infty} x(t) = e$$

Table 1 Nomenclature

Symbol	Name	Unit	Value
c	Number of cells	–	–
µg	10^{-6} g of nitrogen	–	–
L	Liters	–	–
d	Days	–	–
x	Biomass concentration	$c.L^{-1}$	–
s	Substrate concentration	$\mu g.L^{-1}$	–
q	Intracellular quota	$\mu g.c^{-1}$	–
ρ_m	Maximum uptake rate	$\mu g.(c.d)^{-1}$	$1.5.10^{-6}$
k	1/2 saturation (uptake)	$\mu g.L^{-1}$	0.06
μ_m	Maximum growth rate	d^{-1}	1.6
q_m	Minimum quota	$\mu g.c^{-1}$	$0.15.10^{-6}$
γ	Feedback gain	$L.c^{-1}$	10^{-7}

Remark 5 Observe that in our case, each forward trajectory of the limit system Eq. 11 initiated in $\mathcal{E}$. converges toward $s^{\star}$, and each trajectory of the asymptotically autonomous system Eq. 12 converges to $\mathcal{E}$. Then, each trajectory of the asymptotically autonomous system Eq. 12 converges to $s^{\star}$.

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