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Additional information is available at the end of the chapter

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1. Introduction

Microbial growths and their use for environmental purposes, such as bio-degradations, are widely studied in the industry and research centres. Several models of microbial growth and bio-degradation kinetic have been proposed and analysed in the literature. The Monod's model is one of the most popular ones that describes the dynamics of the growth of a biomass of concentration X on a single substrate of concentration S in batch culture [15, 18]:

$$\dot{S} = -\frac{\mu(S)}{Y} X, \quad \dot{X} = \mu(S) X. \quad (1)$$

where the specific growth rate $\mu(\cdot)$ is:

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

with μ_{max} , K_s and Y are respectively the maximum specific growth rate, the affinity constant and the yield coefficient. Other models take explicitly into account a lag-phase before the growth, such as the Baranyi's [1–3] or the Buchanam's [6] ones. These models are well suited for the growth phase (i.e. as long as a substantial amount of substrate remains to be converted) but not after [18], because the accumulation of dead or non-viable cells is not taken into account. Part of the non-viable cells release substrate molecules, in quantities that are no longer negligible when most of the initial supply has been consumed. The on-line observation of the optical density of the biomass provides measurements of the total biomass, but not of the proportion among dead and viable cells. Some tools allow the distinction between viable and dead cells but do not detect non-viable non-dead ones [22].

In this work, we consider an extension of the model (1) considering both the accumulation of dead cells and the recycling of part of it into substrate, and tackle the question of parameters

and state reconstruction. To our knowledge, this kind of question has not been thoroughly studied in the literature. Models of continuous culture with nutrient recycling have already been studied [4, 5, 9, 12–14, 16, 20, 21, 24, 25] but surprisingly few works considers batch cultures. A possible explanation comes from the fact that only the first stage of the growth, for which cell mortality and nutrient recycling can be neglected, is interested for industrial applications. Nevertheless, in natural environment such as in soils, modelling the growth end is also important, especially for biological decontamination and soil bioremediation.

Moreover, we face a model for which the parameters are not identifiable at steady state. Then, one cannot apply straightforwardly the classical estimation techniques, that usually requires the global observability of the system. Estimation of parameters in growth models, such as the Baranyi's one, are already known to be difficult to tackle in their differential form [11]. In addition, we aim here at reconstructing on-line unmeasured state variables (amounts of viable and non-viable cells), as well as parameters. For this purpose, we propose the coupling of two non-linear observers in cascade with different time scales, providing a practical convergence of the estimation error. Design of cascade observers in biotechnology can be found for instance in [17, 23], but with the same time scale.

2. Derivation of the model

We first consider a mortality rate in the model (1):

$$\dot{X} = \mu(S)X - mX$$

where parameter $m > 0$ becomes not negligible when $\mu(S)$ takes small values. In addition, we consider an additional compartment X_d that represents the accumulation of dead cells:

$$\dot{X}_d = \delta mX,$$

where the parameter $\delta \in (0, 1)$ describes the part of non-viable cells that are not burst. We assume that the burst cells recycle part of the substrate that has been assimilated but not yet transformed. Then, the dynamics of the substrate concentration can be modified as follows:

$$\dot{S} = -\frac{\mu(S)}{Y}X + \lambda(1 - \delta)mX,$$

where $\lambda > 0$ is recycling conversion factor. It appears reasonable to assume that the factor λ is smaller than the growth one:

Assumption A1. $\frac{1}{Y} > \lambda$.

In the following we assume that the growth function $\mu(\cdot)$ and the yield coefficient Y of the classical Monod's model are already known. Typically, they can be identified by measuring the initial growth slope on a series of experiments with viable biomass and different initial concentrations, mortality being considered to be negligible during the exponential growth. We aim at identifying the three parameters m , δ and λ , and on-line reconstructing the variables

X and X_d , based on on-line observations of the substrate concentration S and the total biomass $B = X + X_d$.

Without any loss of generality, we shall assume that the growth function $\mu(\cdot)$ can be any function satisfying the following hypotheses.

Assumption A2. The function $\mu(\cdot)$ is a smooth increasing function with $\mu(0) = 0$.

For sake of simplicity, we normalise several quantities, defining

$$s = S, x = X/Y, x_d = X_d/Y, a = (1 - \delta)m \text{ and } k = \lambda Y.$$

Then, our model can be simply written as

$$\begin{cases} \dot{s} = -\mu(s)x + kax, \\ \dot{x} = \mu(s)x - mx, \\ \dot{x}_d = mx - ax, \end{cases} \quad (3)$$

along with the observation vector $y = \begin{pmatrix} s \\ x + x_d \end{pmatrix}$. Typically, we consider known initial conditions such that

$$s(0) = s_0 > 0, \quad x_d(0) = 0 \quad \text{and} \quad x(0) = x_0 > 0.$$

Our purpose is to reconstruct parameters m , a and k and state variable $x(\cdot)$ or $x_d(\cdot)$, under the constraints $m > a$ and $k < 1$, that are direct consequences of the definition of a and Assumption A1. Moreover, we shall assume that a priori bounds on the parameters are known i.e.

$$(m, a, k) \in [m^-, m^+] \times [a^-, a^+] \times [k^-, k^+]. \quad (4)$$

3. Properties of the model

Proposition 1. The dynamics (3) leaves invariant the 3D-space $\mathcal{D} = \mathbb{R}_+^3$ and the set

$$\Omega = \left\{ (s, x, x_d) \in \mathcal{D} \mid s + x + \frac{(m - ka)}{(m - a)} x_d = s_0 + x_0 \right\}.$$

Proof. The invariance of \mathbb{R}_+^3 is guaranteed by the following properties:

$$\begin{aligned} s = 0 &\Rightarrow \dot{s} = kax \geq 0, \\ x = 0 &\Rightarrow \dot{x} = 0, \\ x_d = 0 &\Rightarrow \dot{x}_d = (m - a)x \geq 0. \end{aligned}$$

Consider the quantity $M = s + x + \frac{(m - ka)}{(m - a)} x_d$. One can easily check from equations (3) that one has $\dot{M} = 0$, leading to the invariance of the set Ω . ■

Let \bar{s} be the number $\bar{s} = \mu^{-1}(m)$ or $+\infty$.

Proposition 2. *The trajectories of dynamics (3) converge asymptotically toward an equilibrium point*

$$E^* = \left(s^*, 0, \frac{m-a}{m-ka}(s_0 + x_0 - s^*) \right)$$

with $s^* \leq \min(s_0 + x_0, \bar{s})$.

Proof. The invariance of the set Ω given in Proposition 1 shows that all the state variables remain bounded. From equation $\dot{x}_d = (m-a)x$ with $m > a$, and the fact that x_d is bounded, one deduces that $x(\cdot)$ has to converge toward 0, and $x_d(\cdot)$ is non increasing and converges toward x_d^* such that $x_d^* \in [0, (s_0 + x_0)(m-a)/(m-ka)]$. Then, from the invariant defined by the set Ω , $s(\cdot)$ has also to converges to some $s^* \leq s_0 + x_0$. If s^* is such that $s^* > \bar{s}$, then from equation $\dot{x} = (\mu(s) - m)x$, one immediately see that $x(\cdot)$ cannot converge toward 0. ■

4. Observability of the model

We recall that our aim is to estimate on-line both parameters and unmeasured variables x, x_d , based on the measurements. One can immediately see from equations (3) that parameters (m, a, k) cannot be reconstructed observing the system at steady state. Nevertheless, considering the derivative μ' of μ with respect to s and deriving the outputs:

$$\begin{cases} \dot{y}_1 = (-\mu(y_1) + ka)x, \\ \dot{y}_2 = (\mu(y_1) - a)x, \\ \ddot{y}_1 = (\mu(y_1) - m)\dot{y}_1 - \mu'(y_1)x\dot{y}_1, \\ \ddot{y}_2 = (\mu(y_1) - m)\dot{y}_2 + \mu'(y_1)x\dot{y}_1. \end{cases}$$

one obtains explicit expression of the parameters and unmeasured state variable as functions of the outputs and its derivatives, away from steady state:

$$\begin{cases} m = \mu(y_1) - \frac{\ddot{y}_1 + \ddot{y}_2}{\dot{y}_1 + \dot{y}_2}, \\ x = \frac{\ddot{y}_2 - (\mu(y_1) - m)\dot{y}_2}{\mu'(y_1)\dot{y}_1}, \\ x_d = y_2 - x, \\ a = \mu(y_1) - \frac{\dot{y}_2}{x}, \\ k = \frac{\mu(y_1)}{a} + \frac{\dot{y}_1}{ax}, \end{cases} \quad (5)$$

from which one deduces the observability of the system.

5. Design of a practical observer

Playing with the structure of the dynamics, we are able to write the model as a particular cascade of two sub-models. We first present a practical observer for the reconstruction of the parameters a and k using the observation y_1 only, but with a change of time that depends on y_1 and y_2 . We then present a second observer for the reconstruction of the parameter m

and the state variables x and x_d , using both observations y_1 and y_2 and the knowledge of the parameters a and k . Finally, we consider the coupling of the two observers, the second one using the estimations of a and k provided by the first one. More precisely, our model is of the form

$$\dot{Z} = F(Z, P) \quad , \quad y = H(Z)$$

where F is our vector field with the state, parameters and observation vectors Z , P and y of dimension respectively 3, 3 and 2. We found a partition

$$Z = \begin{pmatrix} Z_1 \\ Z_2 \end{pmatrix}, P = \begin{pmatrix} P_1 \\ P_2 \end{pmatrix} \text{ s.t. } \begin{cases} \dim Z_1 = 1, \dim P_1 = 2 \\ \dim Z_2 = 2, \dim P_2 = 1 \end{cases}$$

$$y = \begin{pmatrix} y_1 \\ y_2 \end{pmatrix} = \begin{pmatrix} H_1(Z_1) \\ H_2(Z_2) \end{pmatrix}$$

and the dynamics is decoupled as follows

$$\begin{aligned} \dot{Z}_1 &= \frac{1}{\frac{d\phi(y)}{dt}} F_1(Z_1, P_1) \\ \dot{Z}_2 &= F_2(Z_2, y_1, P_1, P_2) \end{aligned}$$

with $d\phi(y)/dt > 0$. Moreover, the following characteristics are fulfilled:

- i. (Z_1, P_1) is observable for the dynamics (F_1, H_1) i.e. without the term $d\phi(y)/dt$,
- ii. (Z_2, P_2) is observable for the dynamics (F_2, H_2) when P_1 is known. Then, the consideration of two observers $\hat{F}_1(\cdot)$ and $\hat{F}_2(P_1, \cdot)$ for the pairs (Z_1, P_1) and (Z_2, P_2) respectively, leads to the construction of a cascade observer

$$\begin{aligned} \frac{d}{d\tau} \begin{pmatrix} \hat{Z}_1 \\ \hat{P}_1 \end{pmatrix} &= \hat{F}_1(\hat{Z}_1, \hat{P}_1, y_1), \\ \frac{d}{dt} \begin{pmatrix} \hat{Z}_2 \\ \hat{P}_2 \end{pmatrix} &= \hat{F}_2(\hat{P}_1, \hat{Z}_2, \hat{P}_2, y_2) \end{aligned}$$

with $\tau(t) = \phi(y(t)) - \phi(y(0))$, that we make explicit below. Notice that the coupling of two observers is made by \hat{P}_1 , and that the term $d\phi(y)/dt$ prevents to have an asymptotic convergence when $\lim_{t \rightarrow +\infty} \tau(t) < +\infty$.

Definition 1. An estimator $\hat{Z}_\gamma(\cdot)$ of a vector $Z(\cdot)$, where $\gamma \in \Gamma$ is a parameter, is said to have a practical exponential convergence if there exists positive constants K_1, K_2 such that for any $\epsilon > 0$ and $\theta > 0$, the inequality

$$\|\hat{Z}_\gamma(t) - Z(t)\| \leq \epsilon + K_1 e^{-K_2 \theta t}, \quad \forall t \geq 0$$

is fulfilled for some $\gamma \in \Gamma$.

In the following we shall denote by $\text{sat}(l, u, \iota)$ the saturation operator $\max(l, \min(u, \iota))$.

5.1. A first practical observer for k and a

Let us consider the new variable

$$\tau(t) = y_1(0) - y_1(t) + y_2(0) - y_2(t)$$

that is measured on-line. From Proposition 1, one deduces that $\tau(\cdot)$ is bounded. One can also easily check the property

$$\frac{d\tau}{dt} = (1-k)ax(t) > 0, \quad \forall t \geq 0.$$

Consequently, $\tau(\cdot)$ is an increasing function up to

$$\bar{\tau} = \lim_{t \rightarrow +\infty} \tau(t) < +\infty \quad (6)$$

and $\tau(\cdot)$ defines a diffeomorphism from $[0, +\infty)$ to $[0, \bar{\tau})$. Then, one can check that the dynamics of the variable s in time τ is decoupled from the dynamics of the other state variables:

$$\frac{ds}{d\tau} = \alpha - \beta\mu(s)$$

where α and β are parameters defined as combinations of the unknown parameters a and k :

$$\alpha = \frac{k}{1-k},$$

$$\beta = \frac{1}{a(1-k)}$$

and from (4) one has $(\alpha, \beta) \in [\alpha^-, \alpha^+] \times [\beta^-, \beta^+]$. For the identification of the parameters α , β , we propose below to build an observer. Other techniques, such as least squares methods, could have been chosen. An observer presents the advantage of exhibiting an innovation vector that gives a real-time information on the convergence of the estimation.

Considering the state vector $\xi = \left[s \quad \frac{ds}{d\tau} \quad \frac{d^2s}{d\tau^2} \right]^T$, one obtains the dynamics

$$\frac{d\xi}{d\tau} = A\xi + \begin{pmatrix} 0 \\ 0 \\ \varphi(y_1, \xi) \end{pmatrix} \quad \text{with } y_1 = C\xi,$$

$$\varphi(y_1, \xi) = \frac{\xi_3^2}{\xi_2} + \xi_2 \xi_3 \frac{\mu''(y_1)}{\mu'(y_1)},$$

and the pair (A, C) in the Brunovsky's canonical form:

$$A = \begin{pmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \end{pmatrix} \quad \text{and } C = (1 \ 0 \ 0). \quad (7)$$

The unknown parameters α and β can then be made explicit as functions of the observation y_1 and the state vector ξ :

$$\alpha = l_\alpha(y_1, \xi) = \xi_2 - \frac{\xi_3 \mu(y_1)}{\xi_2 \mu'(y_1)},$$

$$\beta = l_\beta(y_1, \xi) = -\frac{\xi_3}{\xi_2 \mu'(y_1)}.$$

One can notice that functions $\varphi(y_1, \cdot)$, $l_\alpha(y_1, \cdot)$ and $l_\beta(y_1, \cdot)$ are not well defined on \mathbb{R}^3 , but along the trajectories of (3) one has $\xi_3/\xi_2 = -\beta\mu'(y_1)$ and $\xi_2 = \alpha - \beta\mu(y_1)$, that are bounded. Moreover Assumption A2 guarantees that $\mu'(y_1)$ is always strictly positive. We can consider (globally) Lipschitz extensions of these functions away from the trajectories of the system, as follows:

$$\tilde{\varphi}(y_1, \xi) = \xi_3 \left(h_1(y_1, \xi) + \frac{\mu''(y_1)}{\mu'(y_1)} h_2(y_1, \xi) \right),$$

$$\tilde{l}_\alpha(y_1, \xi) = \xi_2 - h_1(y_1, \xi) \frac{\mu(y_1)}{\mu'(y_1)},$$

$$\tilde{l}_\beta(y_1, \xi) = -\frac{h_1(y_1, \xi)}{\mu'(y_1)}$$

with

$$h_1(y_1, \xi) = \text{sat} \left(-\beta^+ \mu'(y_1), -\beta^- \mu'(y_1), \frac{\xi_3}{\xi_2} \right),$$

$$h_2(y_1, \xi) = \text{sat} \left(\alpha^- - \beta^+ \mu(y_1), \alpha^+ - \beta^- \mu(y_1), \xi_2 \right).$$

Then one obtains a construction of a practical observer.

Proposition 3. *There exist numbers $b_1 > 0$ and $c_1 > 0$ such that the observer*

$$\frac{d\hat{\xi}}{d\tau} = A\hat{\xi} + \begin{pmatrix} 0 \\ 0 \\ \tilde{\varphi}(y_1, \hat{\xi}) \end{pmatrix} - \begin{pmatrix} 3\theta_1 \\ 3\theta_1^2 \\ \theta_1^3 \end{pmatrix} (\hat{\xi}_1 - y_1) \quad (8)$$

$$(\hat{\alpha}, \hat{\beta}) = (\tilde{l}_\alpha(y_1, \hat{\xi}), \tilde{l}_\beta(y_1, \hat{\xi}))$$

guarantees the convergence

$$\max(|\hat{\alpha}(\tau) - \alpha|, |\hat{\beta}(\tau) - \beta|) \leq b_1 e^{-c_1 \theta_1 \tau} \|\hat{\xi}(0) - \xi(0)\| \quad (9)$$

for any θ_1 large enough and $\tau \in [0, \bar{\tau}]$.

Proof. Consider a trajectory of dynamics (3) and let $O_1 = \{y_1(t)\}_{t \geq 0}$. From Proposition 1, one knows that the set O_1 is bounded.

Define $K_{\theta_1} = -(3\theta_1 \ 3\theta_1^2 \ \theta_1^3)^T$. One can check that $K_{\theta_1} = -P_{\theta_1}^{-1}C^T$, where P_{θ_1} is solution of the algebraic equation

$$\theta_1 P_{\theta_1} + A^T P_{\theta_1} + P_{\theta_1} A = C^T C.$$

Consider then the error vector $e = \hat{\xi} - \xi$. One has

$$\frac{de}{d\tau} = (A + K_{\theta_1}C)e + \begin{pmatrix} 0 \\ 0 \\ \tilde{\varphi}(y_1, \hat{\xi}) - \tilde{\varphi}(y_1, \xi) \end{pmatrix}$$

where $\tilde{\varphi}(y_1, \cdot)$ is (globally) Lipschitz on \mathbb{R}^3 uniformly in $y_1 \in O_1$. We then use the result in [10] that provides the existence of numbers $c_1 > 0$ and $q_1 > 0$ such that $\|e(\tau)\| \leq q_1 e^{-c_1 \theta_1 \tau} \|e(0)\|$ for θ_1 large enough. Finally, functions $\tilde{l}_\alpha(y_1, \cdot)$, $\tilde{l}_\beta(y_1, \cdot)$ being also (globally) Lipschitz on \mathbb{R}^3 uniformly in $y_1 \in O_1$, one obtains the inequality (9). ■

Corollary 1. Estimation of a and k with the same convergence properties than (9) are given by

$$\hat{k}(\tau), \hat{a}(\tau) = \text{sat} \left(k^-, k^+, \frac{\hat{a}(\tau)}{1 + \hat{a}(\tau)} \right), \text{sat} \left(a^-, a^+, \frac{1 + \hat{a}(\tau)}{\hat{\beta}(\tau)} \right)$$

Remark. The observer (8) provides only a practical convergence since $\tau(t)$ does not tend toward $+\infty$ when the time t get arbitrary large. For large values of initial x , it may happens that $\mu(t) > t$ for some times $t > 0$. Because the present observer requires the observation y_1 until time τ , it has to be integrated up to time $\min(\tau(t), t)$ when the current time is t .

5.2. A second observer for m and x

We come back in time t and consider the measured variable $z = y_1 + y_2$. When the parameters α and β are known, the dynamics of the vector $\zeta = [z \ \dot{z} \ \ddot{z}]^T$ can be written as follows:

$$\dot{\zeta} = A\zeta + \begin{pmatrix} 0 \\ 0 \\ \psi(y_1, \zeta, \alpha, \beta) \end{pmatrix} \text{ with } z = C\zeta$$

$$\text{and } \psi(y_1, \zeta, \alpha, \beta) = \frac{\zeta_3^2}{\zeta_2} + \zeta_2^2 \mu'(y_1) (\beta \mu(y_1) - \alpha)$$

Parameter m and variable $x(\cdot)$ can then be made explicit as functions of y_1 and ζ :

$$m = l_m(y_1, \zeta) = \mu(y_1) - \frac{\zeta_3}{\zeta_2}, \quad x = -\beta \zeta_2$$

Functions $\psi(y_1, \cdot, \alpha, \beta)$ and $l_m(y_1, \cdot)$ are not well defined in \mathbb{R}^3 but along the trajectories of the dynamics (3), one has $\zeta_3/\zeta_2 = \mu(y_1) - m$ and $\zeta_2 = -x/\beta$ that are bounded. These functions can be extended as (globally) Lipschitz functions w.r.t. ζ :

$$\begin{aligned} \tilde{\psi}(y_1, \zeta, \alpha, \beta) &= h_3(y_1, \zeta) \zeta_3 + \min(\zeta_2^2, z(0)^2 / \beta^2) \mu'(y_1) (\beta \mu(y_1) - \alpha) \\ \tilde{l}_m(y_1, \zeta) &= \mu(y_1) - h_3(y_1, \zeta) \end{aligned} \tag{10}$$

with

$$h_3(y_1, \zeta) = \text{sat} \left(\mu(y_1) - m^+, \mu(y_1) - m^-, \frac{\zeta_3}{\zeta_2} \right).$$

Proposition 4. *When α and β are known, there exists numbers $b_2 > 0$ and $c_2 > 0$ such that the observer*

$$\frac{d}{dt} \hat{\zeta} = A \hat{\zeta} + \begin{pmatrix} 0 \\ 0 \\ \tilde{\psi}(y_1, \hat{\zeta}, \alpha, \beta) \end{pmatrix} - \begin{pmatrix} 3\theta_2 \\ 3\theta_2^2 \\ \theta_2^3 \end{pmatrix} (\hat{\zeta}_1 - y_1 - y_2) \quad (11)$$

$$(\hat{m}, \hat{x}) = (\tilde{l}_m(y_1, \hat{\zeta}), -\beta \hat{\zeta}_2)$$

guarantees the exponential convergence

$$\max (|\hat{m}(t) - m|, |\hat{x}(t) - x(t)|) \leq b_2 e^{-c_2 \theta_2 t} \|\hat{\zeta}_2(0) - \zeta_2(0)\|$$

for any θ_2 large enough and $t \geq 0$.

Proof. As for the proof of Proposition 3, it is a straightforward application of the result given in [10]. ■

5.3. Coupling the two observers

We consider now the coupling of observer (11) with the estimation $(\hat{\alpha}, \hat{\beta})$ provided by observer (8). This amounts to study the robustness of the second observer with respect to uncertainties of parameters α and β .

Proposition 5. *Consider the observer (11) with (α, β) replaced by $(\tilde{\alpha}(\cdot), \tilde{\beta}(\cdot))$ such that*

$$(\tilde{\alpha}(t), \tilde{\beta}(t)) \in [\alpha^-, \alpha^+] \times [\beta^-, \beta^+], \quad \forall t \geq 0,$$

then there exists positive numbers $\bar{b}_2, \bar{c}_2, \bar{d}_2$ such that for any $\epsilon > 0$ there exists θ_2 large enough to guarantee the inequalities

$$|\hat{m}(t) - m| \leq \epsilon + \bar{b}_2 e^{-\bar{c}_2 t} \|\hat{\zeta}(0) - \zeta(0)\| \quad (12)$$

$$|\hat{x}(t) - x(t)| \leq \epsilon + \bar{d}_2 |\tilde{\beta}(t) - \beta| + \bar{b}_2 e^{-\bar{c}_2 t} \|\hat{\zeta}(0) - \zeta(0)\| \quad (13)$$

for any $t \geq 0$.

Proof. As for the proof of Proposition 3, we fix an initial condition of system (3) and consider the bounded set $O_1 = \{y_1(t)\}_{t \geq 0}$. The dynamics of $e = \hat{\zeta} - \zeta$ is

$$\dot{e} = (A + K_{\theta_2} C)e + (\tilde{\psi}(y_1, \hat{\zeta}, \tilde{\alpha}, \tilde{\beta}) - \tilde{\psi}(y_1, \zeta, \alpha, \beta))v$$

where (A, C) in the Brunovsky's form (7), $v = (0 \ 0 \ 1)^T$ and $K_{\theta_2} = -P_{\theta_2}^{-1}C^T$ with

$$P_{\theta_2} = \begin{pmatrix} \theta_2^{-1} & -\theta_2^{-2} & \theta_2^{-3} \\ -\theta_2^{-2} & 2\theta_2^{-3} & -3\theta_2^{-4} \\ \theta_2^{-3} & -3\theta_2^{-4} & 6\theta_2^{-5} \end{pmatrix} \quad (14)$$

solution of the algebraic equation

$$\theta_2 P_{\theta_2} + A^T P_{\theta_2} + P_{\theta_2} A = C^T C. \quad (15)$$

Consider then $V(t) = \|e(t)\|_{P_{\theta_2}}^2 = e^T(t)P_{\theta_2}e(t)$. Using (15), one has

$$\begin{aligned} \dot{V} &= -\theta_2 e^T P_{\theta_2} e - e^T C^T C e + 2\delta e^T P_{\theta_2} v \\ &\leq -\theta_2 \|e\|_{P_{\theta_2}}^2 + 2\delta \|e\|_{P_{\theta_2}} \|v\|_{P_{\theta_2}} \end{aligned} \quad (16)$$

where $\delta = |\tilde{\psi}(y_1, \hat{\zeta}, \tilde{\alpha}, \tilde{\beta}) - \tilde{\psi}(y_1, \zeta, \alpha, \beta)|$.

One can easily compute from (14) $\|v\|_{P_{\theta_2}} = \sqrt{6}\theta^{-5/2}$.

From the expression (10) and the (globally) Lipschitz property of the map $\zeta \mapsto \tilde{\psi}(y_1, \zeta, \alpha, \beta)$ uniformly in $y_1 \in O_1$, we deduce the existence of two positive numbers c and L such that

$$\begin{aligned} \delta &\leq |\tilde{\psi}(y_1, \hat{\zeta}, \tilde{\alpha}, \tilde{\beta}) - \tilde{\psi}(y_1, \hat{\zeta}, \alpha, \beta)| + |\tilde{\psi}(y_1, \hat{\zeta}, \alpha, \beta) - \tilde{\psi}(y_1, \zeta, \alpha, \beta)| \\ &\leq |\tilde{\psi}(y_1, \hat{\zeta}, \alpha^-, \beta^+) - \tilde{\psi}(y_1, \hat{\zeta}, \alpha^+, \beta^-)| + L\|e\| \\ &\leq c + L\|e\| \end{aligned} \quad (17)$$

Notice that one has $\|e\|_{P_{\theta_2}} = \theta_2 \|\tilde{e}\|_{P_1}$ with $\tilde{e}_i = \theta_2^{-i} e_i$ and $\|\tilde{e}\|^2 \geq \theta_2^{-6} \|e\|^2$ for any $\theta_2 \geq 1$. The norms $\|\cdot\|_{P_1}$ and $\|\cdot\|$ being equivalent, there exists a number $\eta > 0$ such that $\|\tilde{e}\|_{P_1} \geq \eta \|\tilde{e}\|$, and we deduce the inequality

$$\|e\|_{P_{\theta_2}} \geq \eta \theta_2^{-5/2} \|e\|. \quad (18)$$

Finally, gathering (16), (17) and (18), one can write

$$\frac{d}{dt} \|e\|_{P_{\theta_2}} \leq \left(-\frac{\theta_2}{2} + \frac{\sqrt{6}L}{\eta} \right) \|e\|_{P_{\theta_2}} + \sqrt{6}\theta_2^{-5/2} c$$

For θ_2 large enough, one has $-\theta_2/2 + \sqrt{6}L/\eta < 0$ and then, using again (18), obtains

$$\frac{d}{dt} \|e\| \leq \left(-\frac{\theta_2}{2} + \frac{\sqrt{6}L}{\eta} \right) \|e\| + \frac{\sqrt{6}}{\eta} c$$

from which we deduce the exponential convergence of the error vector e toward any arbitrary small neighbourhood of 0 provided that θ_2 is large enough.

The Lipschitz continuity of the map $l_m(\cdot)$ w.r.t. ζ uniformly in $y_1 \in O_1$ provides the inequality (12).

For the estimation of $x(\cdot)$, one has the inequality

$$|\hat{x} - x| = |\hat{\beta}\hat{\zeta}_2 - \beta\zeta_2| \leq |\hat{\beta} - \beta||\zeta_2| + \beta^+|\hat{\zeta}_2 - \zeta_2|$$

provided the estimation (13), the variable ζ_2 being bounded. ■

Corollary 2. *At any time $t > 0$, the coupled observer*

$$\begin{aligned} \frac{d\hat{\zeta}_1}{ds_1} &= A\hat{\zeta}_1 + \begin{pmatrix} 0 \\ 0 \\ \tilde{\varphi}(y_1, \hat{\zeta}_1) \end{pmatrix} - \begin{pmatrix} 3\theta_1 \\ 3\theta_1^2 \\ \theta_1^3 \end{pmatrix} (\hat{\zeta}_1 - y_1) \\ \frac{d\hat{\zeta}_2}{ds_2} &= A\hat{\zeta}_2 + \begin{pmatrix} 0 \\ 0 \\ \tilde{\psi}(y_1, \hat{\zeta}_1, \hat{\alpha}(s_2), \hat{\beta}(s_2)) \end{pmatrix} - \begin{pmatrix} 3\theta_2 \\ 3\theta_2^2 \\ \theta_2^3 \end{pmatrix} (\hat{\zeta}_2 - y_1 - y_2) \end{aligned}$$

integrated for $s_1 \in [0, \min(t, \tau(t))]$ and $s_2 \in [0, t]$, with

$$\begin{aligned} \tau(t) &= y_1(0) - y_1(t) + y_2(0) - y_2(t), \\ \hat{\alpha}(s_2) &= \text{sat}(\alpha^-, \alpha^+, \tilde{l}_\alpha(y_1(\min(s_2, \tau(t))), \hat{\zeta}_1(\min(s_2, \tau(t))))), \\ \hat{\beta}(s_2) &= \text{sat}(\beta^-, \beta^+, \tilde{l}_\beta(y_1(\min(s_2, \tau(t))), \hat{\zeta}_1(\min(s_2, \tau(t))))), \end{aligned}$$

provides the estimations

$$\begin{aligned} \hat{m}(t) &= \tilde{l}_m(y_1(t), \hat{\zeta}_1(t)), \\ (\hat{x}(t), \hat{x}_d(t)) &= (-\hat{\beta}(t)\hat{\zeta}_2(t), y_2(t) + \hat{\beta}(t)\hat{\zeta}_2(t)). \end{aligned}$$

The convergence of the estimator is exponentially practical, provided θ_1 and θ_2 to be sufficiently large.

6. Numerical simulations

We have considered a Monod's growth function (2) with the parameters $\mu_{max} = 1$ and $K_s = 100$ and the initial conditions $s(0) = 50$, $x(0) = 1$, $x_d(0) = 0$. The parameters to be reconstructed have been chosen, along with a priori bounds, as follows:

parameter	δ	k	m
value	0.2	0.2	0.1
bounds	[0.1, 0.3]	[0.1, 0.3]	[0.05, 0.2]

Those values provide an effective growth that is reasonably fast ($s(0)$ is about $K_s/2$), and a value $\bar{\tau}$ (see (6)) we find by numerical simulations is not too small. For the time interval $0 \leq t \leq t_{max} = 80$, we found numerically the interval $0 \leq \tau \leq \tau_{max} = \tau(t_{max}) \simeq 37.22$ (see Figure 1). For the first observer, we have chosen a gain parameter $\theta_1 = 3$ that provides

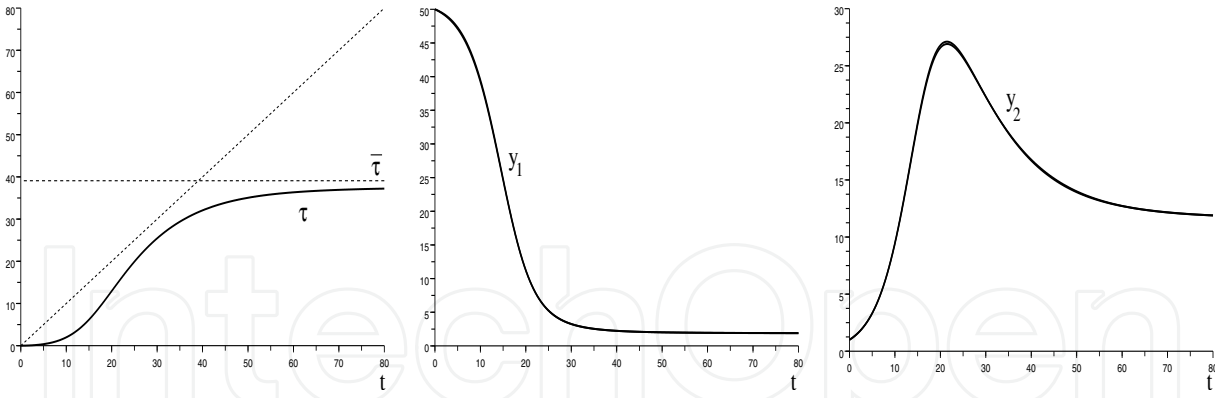


Figure 1. Graphs of function τ and observations y_1, y_2 .

a small error on the estimation of the parameters α and β at time τ_{max} (see Figures 2 and 3). These estimations have been used on-line by the second observer, with $\theta_2 = 2$ as a choice

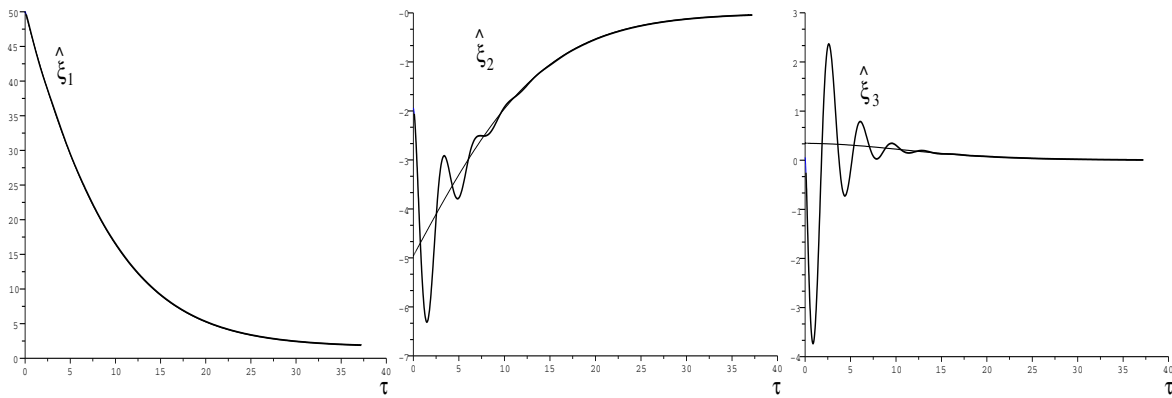


Figure 2. Internal variables $\hat{\xi}$ of the first observer in time τ (variables ξ of the true system in thin lines).

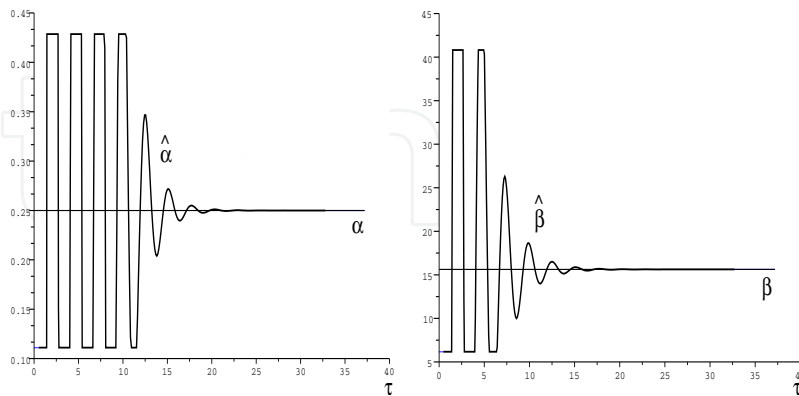


Figure 3. On-line estimation of parameters α and β .

for the gain parameter. On Figures 4 and 5, one can see that the estimation error get small when the estimations provided by the first observer are already small. Simulations have been also conducted with additive noise on measurements y_1 and y_2 with a signal-to-noise

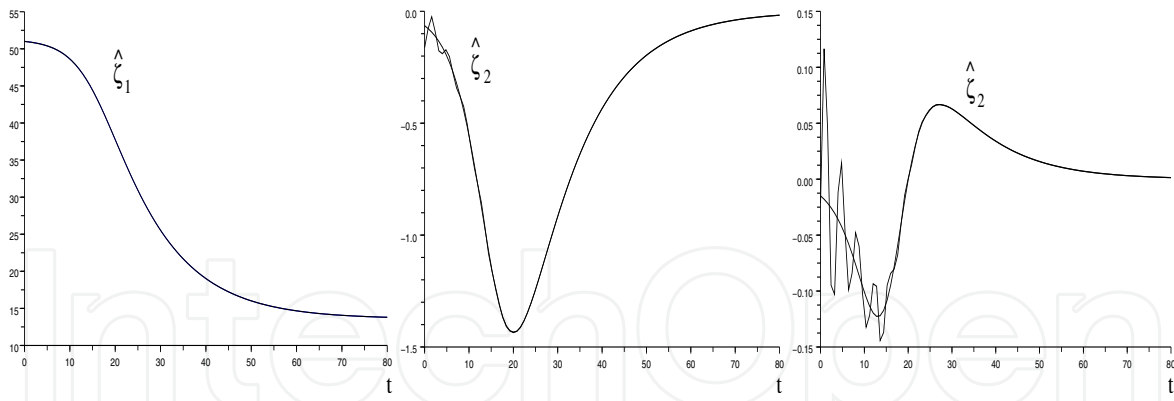


Figure 4. Internal variables $\hat{\zeta}$ of the second observer in time t (variables ζ of the true system in thin lines).

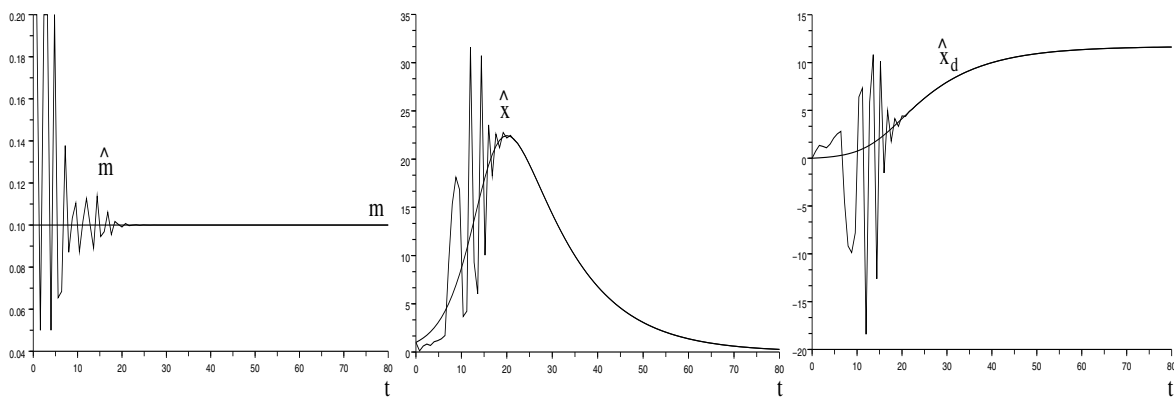


Figure 5. On-line estimation of parameter m and state variables x and x_d .

ratio of 10 and a frequency of 0.1Hz (see Figures 6 and 7). In presence of a low frequency

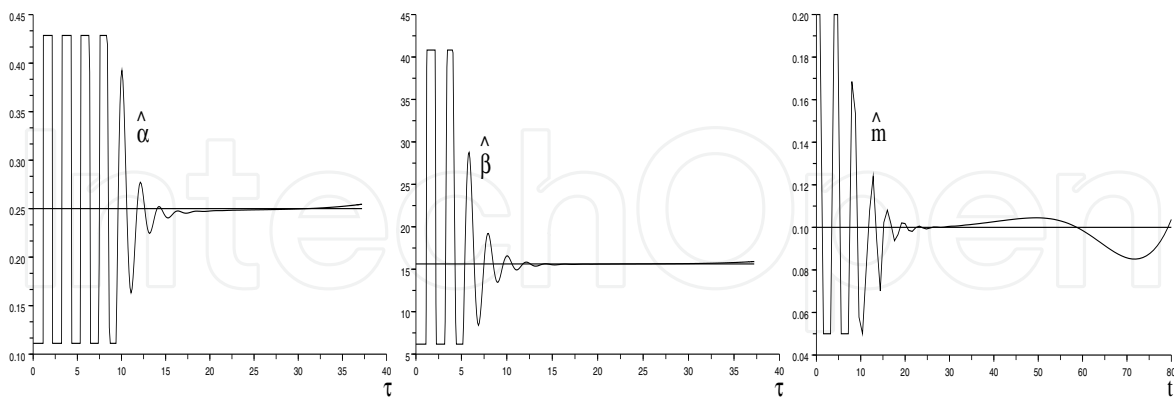


Figure 6. Estimation of the parameters α , β and m in presence of measurement noise.

noise (as it can be usually assumed in biological applications), one finds a good robustness of the estimations of parameters α , β and variables x and x_d . Estimation of parameter m is more affected by noise. This can be explained by the structure of the equations (5): the estimation of m is related to the second derivative of both observations y_1 and y_2 , and consequently is more sensitive to noise on the observations.

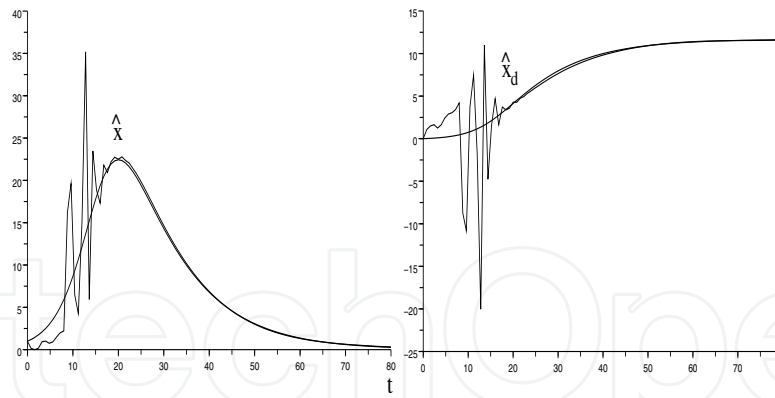


Figure 7. Estimation of the state variables x and x_d in presence of measurement noise.

7. Conclusion

The extension of the Monod's model with an additional compartment of dead cells and substrate recycling terms is no longer identifiable, considering the observations of the substrate concentration and the total biomass. Nevertheless, we have shown that the model can be written in a particular cascade form, considering two time scales. This decomposition allows to design separately two observers, and then to interconnect them in cascade. The first one works on a bounded time scale, explaining why the system is not identifiable at steady state, while the second one works on unbounded time scale. Finally, this construction provides a practical convergence of the coupled observers. Each observer has been built considering the variable high-gain technique proposed in [10] with an explicit construction of Lipschitz extensions of the dynamics, similarly to the work presented in [19]. Other choices of observers techniques could have been made and applied to this particular structure. We believe that such a decomposition might be applied to other systems of interest, that are not identifiable or observable at steady state.

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