

USE OF NON LINEAR SOFTWARE SENSOR TO MONITOR THE  
INTERNAL STATE OF A CULTURE OF MICROALGAE

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**Abstract.** In this paper we construct nonlinear software sensors in order to monitor the internal state of a culture of microalgae. We use the Droop model to represent the growth of phytoplanktonic cells limited by a nutrient in bioreactor. We show that this model is an element of a general class of systems for which the conditions for the exponential convergence of high gain observers are proved. The software sensors is then designed and applied in a nitrate-limited microalgae chemostat experiment performed in a computer controlled fluctuating environment. The efficiency of the software sensor is discussed.

**Keywords :** ecology, phytoplankton, Droop model, nonlinear systems, observers.

## 1. INTRODUCTION

The key role played by the phytoplankton in the oceanic carbon cycle (in particular in relation with the greenhouse effect) has enhanced the studies aiming at improving the understanding and the modeling of the phytoplanktonic growth. In the oceanographic laboratory of Villefranche-sur-mer (France), the algae are studied in bioreactors where some *in vivo* marine conditions are reproduced. The observation of these algal cultures is used to better understand the physiological processes associated with algal growth and photosynthesis (*i.e.* absorption of CO<sub>2</sub> and release of O<sub>2</sub>). It is therefore important to have sensors that can monitor the internal state of the phytoplanktonic cultures during the experiments. However, when dealing with biosystems the monitoring and control task is difficult to achieve. It appears that only few concentrations of the components involved and critical for a physiological study are available from on-line measurements. Besides, there is still a lack of appropriate sensors which are at the same time reliable and cheap. Therefore, there is a clear incentive to develop and apply new dedicated sensors in order to get closer to the internal working of the system, and finally to improve the monitoring and control. A way to overcome this lack of sensor consist in combining

the limited set of measurements together with a mathematical model of the process. This leads to the development of software sensors (or observers) *i.e.* algorithms that are used to reduce the effects of noise on measurements as well as to estimate non measured variables.

In this paper we propose to design a non linear software sensor for a phytoplanktonic culture of the green algae *Dunaliella tertiolecta* in bioreactor. In Section 2 we present the Droop model, which is the classical model describing the growth of phytoplanktonic cells under nutrient limitations in continuous reactor (chemostat). Contrary to simple microorganisms (bacteria, yeast, ...) whose growth can be represented by the well known 2-dimensional Monod model [1], micro algae are autotrophic organisms far more complex, whose behavior has to be described with this more complicated 3-dimensional model. This classical model has a great ecological importance because it appears in complex ecological models of trophic nets or carbon fluxes. In Section 3.1 we recall some classical results on observability of nonlinear systems. Then we define a class of systems that find numerous applications in biology and biotechnology. These systems fulfill the appropriate conditions that guaranty the exponential convergence of the nonlinear observers presented in Section 3.2.

tems. In Section 3.3 we derive thus a high gain observer based on this model for the monitoring of the phytoplankton cells. After a review of the material and methods used (Section 4), we apply in Section 5 the software sensor to real experiments, and we draw the conclusions on the validity and on the efficiency of the software sensor.

## 2. THE DROOP MODEL

The modeling of phytoplankton growth under substrate limitation has been under development during the past 30 years. In contrast to bacteria whose growth can be represented by the simple Monod model [1], phytoplankton shows a strong uncoupling between nutrient uptake and growth [2, 3]. A modeling of this phenomenon has been proposed by Droop [4] and Caperon and Meyer [5] who represented growth as dependent not on external substrate (of concentration  $S$ ), but on an internal quota ( $Q$ ) defined as the quantity of nutrient per biomass unit. This empirical law, combined with Dugdale's [6] term for nutrient uptake, allowed Burmaster [7] to compose an ordinary differential equation based model for phytoplanktonic cells (biomass  $X$ ) growing in a bioreactor under a single nutrient limitation:

$$(\Sigma_D) \begin{cases} \dot{S}(t) = D[S_{in} - S(t)] - \rho_m \frac{S(t)N(t)}{K_\rho + S(t)} \\ \dot{N}(t) = \bar{\mu} \left(1 - \frac{K_Q}{Q(t)}\right) N(t) - DN(t) \\ \dot{Q}(t) = \rho_m \frac{S(t)}{K_\rho + S(t)} - \bar{\mu}(Q(t) - K_Q) \end{cases} \quad (1)$$

The parameters  $\rho_m$  and  $k_s$  represent respectively the maximum uptake rate and the half-saturation constant for the substrate.  $k_Q$  is the minimum internal quota allowing growth and  $\bar{\mu}$  is the hypothetical growth rate obtained for an infinite quota. The dilution rate  $D$  is the quotient of the medium inflow rate over the bioreactor volume. The substrate concentration in the renewal medium is denoted  $S_{in}$ . The model parameters have been calibrated using chemostat steady state conditions and batch experiments (Table 1).

The on-line measurement ( $y$ ) is the biomass, estimated by the measurements of total biovolume (see Section 4). We will consider experiments where the concentration of nitrate in the renewal medium  $S_{in}$  varies under the following way:  $S_{in} = s_i(1 + u)$ ,  $u$  being the input applied to the system ( $u > -1$ ), and  $s_i$  the nitrate concentration in the influent without input ( $u = 0$ ).

Let us do the following change of variable:

- $x_1 = \frac{\rho_m N}{s_i}$ ;  $x_2 = \frac{Q}{K_Q}$ ;  $x_3 = \frac{S}{s_i}$
- $a_1 = \frac{k_s}{s_i}$ ;  $a_2 = \bar{\mu}$ ;  $a_3 = \frac{\rho_m}{K_Q}$

$\rho_m$	$\mu\text{mol}.\mu\text{m}^{-3}.\text{d}^{-1}$	$8.3810^{-9}$	$1.2010^{-9}$
$k_s$	$\mu\text{mol}.\text{l}^{-1}$	0.12	0.10
$\bar{\mu}$	$\text{d}^{-1}$	1.85	0.30
$k_Q$	$\mu\text{mol}.\mu\text{m}^{-3}.\text{d}^{-1}$	$1.510^{-9}$	$0.210^{-9}$
$D$	$\text{d}^{-1}$	0.96	0.02
$T$	$d$	1.0	0.0
$s_i$	$\mu\text{mol}.\text{l}^{-1}$	$f(t)$	1.0

Table 1: Parameters of the Droop model for *Dunaliella tertiolecta* grown at 20°C. With  $f(t) = 35 [1 + 0.3 \sin(\frac{2\pi}{T}t)]$ . (\* Standard deviation.)

The model reduces now to:

$$(\Sigma_D) \begin{cases} \dot{x} = f(x) + ug(x) \\ y = h(x_1) \end{cases} \quad (2)$$

with:

$$f(x) = \begin{pmatrix} a_2(1 - \frac{1}{x_2})x_1 - Dx_1 \\ a_3 \frac{x_3}{a_1 + x_3} - a_2(x_2 - 1) \\ D(1 - x_3) - \frac{x_1 x_3}{a_1 + x_3} \end{pmatrix}, \quad (3)$$

$$g(x) = \begin{pmatrix} 0 \\ 0 \\ D \end{pmatrix}, \quad h(x_1) = x_1$$

## 3. OBSERVABILITY AND HIGH-GAIN OBSERVER FOR THE DROOP MODEL

### 3.1. Recall on the observability of nonlinear systems

Let  $\mathcal{U}$  be the set of admissible controls. We consider the differential system  $(\Sigma)$  defined on a domain  $\Omega \subset \mathbf{R}^n$ :

$$(\Sigma) \begin{cases} \dot{x}(t) = F(x(t), u(t)) \\ y(t) = h(x(t)) \\ x(0) = x_0, u \in \mathcal{U} \end{cases} \quad (4)$$

Where  $F$  is a smooth function  $\mathbf{R}^n \times \mathbf{R} \rightarrow \mathbf{R}^n$ ,  $h$  the observation function is also supposed smooth  $\mathbf{R}^n \rightarrow \mathbf{R}$ . Note that we consider here only the SISO case.

**Definition 1 (Observability)** *A system  $(\Sigma)$  is said to be observable if for any pair of different initial states  $(x_0, x_1)$   $x_0 \neq x_1$  there exists an admissible control  $u(\cdot) \in \mathcal{U}$ , a time  $t \geq 0$  such that:*

$$y(x_0, u(\cdot), t) \neq y(x_1, u(\cdot), t)$$

**Definition 2 (Observability for any input)** *A system  $(\Sigma)$  is said to be uniformly input observable if for any input  $u(\cdot) \in \mathcal{U}$  and for any  $(x_0, x_1)$ ,  $x_0 \neq x_1$ , there exists a time  $t \geq 0$  such that:*

$$y(x_0, u(\cdot), t) \neq y(x_1, u(\cdot), t)$$

biology have a specific structure which guarantees the observability for any input. This class of system has been called the Strictly Linked Lower Hessenberg (SL<sub>2</sub>H) systems. It is defined as follows:

**Definition 3 (SL<sub>2</sub>H systems)** *A system  $(\Sigma)$  is said to be SL<sub>2</sub>H if it verifies the following conditions for any  $(x, u) \in \Omega \times U$ :*

1. for any indexes  $(i, j)$  such that  $j > (i + 1)$ :  $\frac{\partial F_i}{\partial x_j}(x, u) = 0$
2. for any index  $i$ :  $\frac{\partial F_i}{\partial x_{i+1}}(x, u) \neq 0$
3.  $h(x) = h(x_1)$ , with  $\frac{dh}{dx_1}(x_1) \neq 0$ .

Now we give an important result that holds for the SL<sub>2</sub>H systems.

**Theorem 1** *The SL<sub>2</sub>H systems are uniformly input observable.*

**Proof:** This is a consequence of the theorem 3.2 of [9].

In the next section, we see that the SL<sub>2</sub>H systems verify (under additional reasonable hypotheses) conditions ensuring the exponential convergence of the high gain observers [10, 11].

### 3.2. Observers for the SL<sub>2</sub>H systems

Let us restrict the considered class of system to control affine systems, *i.e.*  $F(x, u) = f(x) + u.g(x)$ , where  $f$  and  $g$  are smooth vector fields on  $\mathbf{R}^n$ .

We assume also that the trajectories of  $(\Sigma)$  verify the following property:

**(H1):** there exists two compact sets  $K_0, K$  with  $K_0 \subset K \subset \Omega$  such that  $(\Sigma)$  is SL<sub>2</sub>H on  $K$ , and for any  $(x_0, u(\cdot), t) \in K_0 \times \mathcal{U} \times \mathbf{R}^+$  we have  $x(t) \in K$ .

Now we propose the following observer [10] for the system  $(\Sigma)$ :

**Property 1 (High Gain observer [10])** *If the system  $(\Sigma)$  satisfy the property (H1), then, for  $\theta$  large enough, the following differential system  $(\Sigma_{HG})$  is an exponential observer for  $(\Sigma)$ :*

$$\Sigma_{HG} : \dot{\hat{x}} = f(\hat{x}) + u g(\hat{x}) - \left[ \frac{\partial \Phi}{\partial x} \right]_{x=\hat{x}}^{-1} S_\theta^{-1} {}^t C (h(\hat{x}) - y) \quad (5)$$

with  $C = [1, 0, \dots, 0]$ . The elements of the matrix  $S_\theta$  can be analytically computed as follows:

$$S_\theta(i, j) = \frac{(-1)^{i+j}}{\theta^{i+j-1}} \frac{(i+j-2)!}{(i-1)!(j-1)!}$$

$\Phi$  is the following diffeomorphism, globally defined on  $\Omega$  ( $L_f$  denotes the Lie derivative of  $h$  along the field  $f$ ):

$$\Phi : x \longrightarrow {}^t \left( h(x), L_f h(x), \dots, L_f^{(n-1)} h(x) \right)$$

conditions presented in [10] for the convergence of the observer can be found in [8].

Note that an extended Kalman observer [11] can also be designed for the SL<sub>2</sub>H systems [8]. It is not presented here for sake of simplicity, but from extensive simulation tests there is no superiority of any observer for the Droop model. In theory the Kalman observer should be more robust to noise, but the high gain observer presents the advantage of less calculus, which makes it easier to bring into operation.

### 3.3. Observability and observer of the Droop model

From Section 3, provided that the property (H1) is satisfied, a high gain observer can be derived for the Droop model which is SL<sub>2</sub>H. The delicate point consist now in finding the two compact sets  $K_0$  and  $K$  such that the property (H1) holds. These compact sets have been determined in the case where the input of the system is the dilution rate [8]. We propose here to show how to construct these compact sets when  $S_{in}$  is the input.

**Proposition 1** *Let us denote  $u_{min}$  and  $u_{max}$  the lower and the upper bound of  $u$  (with  $u_{min} > -1$ ). We denote  $v = x_3 + \frac{x_1 x_2}{a_3}$  which represent the total quantity of nutrient in the bioreactor. We have for all time  $t$ :*

$$\min(1 + u_{min}, v(0)) \leq v(t) \leq \max(1 + u_{max}, v(0))$$

**Proof:**  $v$  satisfies a linear first order equation:

$$\dot{v} = D(1 - v) + Du$$

We have then:

$$D(1 - v) + Du_{min} \leq \dot{v} \leq D(1 - v) + Du_{max}$$

which gives the result.

Now we choose the compact  $K_0$  of initial conditions which have a reasonable biological meaning:

$$K_0 = \left\{ x \in \mathbf{R}_+^3 : x_{1min} \leq x_1 \leq a_2 v_{max}, \right. \\ \left. 1 \leq x_2 \leq 1 + \frac{a_3}{a_2}, x_3 \geq x_3^*, x_3 + \frac{x_1 x_2}{a_3} \leq v_{max} \right\}$$

where  $v_{max} \geq 1 + u_{max}$ , and  $x_3^* > 0$  verifies:

$$D(1 + u_{min} - x_3^*) - a_2 v_{max} \frac{x_3^*}{a_1 + x_3^*} > 0 \quad (6)$$

(it is straightforward to check that such an  $x_3^*$  exists, and also that it can be chosen as small as needed).

**Proposition 2** *There exists a compact set, with  $K_0 \subset K \subset \mathbf{R}_+^3$  such that for all the trajectories initiated in the compact set  $K_0$ , for any input  $u$ , for any time  $t$ ,  $x(t)$  remains in  $K$ .*

ing properties:

- for all time  $t$ :  $1 \leq x_2(t) \leq 1 + \frac{a_3}{a_2}$  and  $x_3(t) > 0$ . The proof of these elementary facts can be found in [12].
- For all time  $t$ :  $x_1(t) \leq a_2 v_{max}$ . Indeed,  $v \leq v_{max}$  which implies that:  $x_1 x_2 \leq a_3 v_{max}$ , while  $x_2(t) \geq 1$ , we have the result.
- For all time  $t$ ,  $x_3(t) \geq x_3^*$ . If we compute  $\dot{x}_3$  for  $x_3 = x_3^*$ , we have by definition of  $x_3^*$  (cf Eq. (6)):  $\dot{x}_3 > 0$ .
- Finally it remains to prove that there exists an  $x_{1min} > 0$  such that if  $x_1(0) \geq x_1^*$ , then for any time:  $x_1(t) \geq x_{1min}$ . This is a bit more delicate to prove, and it can be done with the same methodology than in [8] although the input applied to the system in this paper was the dilution rate  $D$ .

It is now easy to verify that the Droop model is  $SL_2H$  on the compact set  $K$ .

From Property 1, the high gain observer for the Droop model can be designed. The differential system for this software sensor is the following:

$$(\hat{\Sigma}) \{ \dot{\hat{x}} = f(\hat{x}) + ug(\hat{x}) + C(\hat{x})(y - \hat{x}_1) \quad (7)$$

with

$$C(\hat{x}) = \begin{pmatrix} \left[ 3\theta \frac{\hat{x}_2}{\hat{x}_1} \left[ 1 - \left( 1 - \frac{D}{a_2} \right) \hat{x}_2 \right] + 3\theta^2 \frac{\hat{x}_2^2}{a_2 \hat{x}_1} \right] \\ \left[ 3\theta \hat{B}_{31} + 3\theta^2 \hat{B}_{32} + \theta^3 \frac{\hat{x}_2^2 (a_1 + \hat{x}_3)^2}{a_1 a_2 a_3 \hat{x}_1} \right] \end{pmatrix}$$

where:

$$\hat{B}_{31} = \frac{1}{a_1 a_3 \hat{x}_1} \left[ \frac{a_3 \hat{x}_3}{a_1 + \hat{x}_3} + 2a_2 + \hat{x}_2^2 \left( 2a_2 - 3D - \frac{D^2}{a_2} \right) - \hat{x}_2 \left( 2 \frac{a_3 \hat{x}_3}{a_1 + \hat{x}_3} \left( 1 - \frac{D}{a_2} \right) + 4a_2 - 4D \right) \right]$$

$$\hat{B}_{32} = \frac{\hat{x}_2 (a_1 + \hat{x}_3)^2}{a_1 a_2 a_3 \hat{x}_1} \left[ \hat{x}_2 (2D - 3a_2) + 4a_2 + 2 \frac{a_3 \hat{x}_3}{a_1 + \hat{x}_3} \right]$$

#### 4. MATERIAL AND METHODS

The basic culture system has been described in [13]. The bioreactors consisted of 1.8 liter double-jacketed glass vessels.

To ensure the automation of a Technicon Auto-analyser, a set of pumps and electric valves are controlled by a PC computer. For each bioreactor

and output signal acquisition from both colorimeters (nitrite ( $NO_2$ ) and  $NO_3$ ) are programmed in time with specified intervals. The system standardizes itself by calculating the respective gains of the colorimeters and the Cadmium-Copper column efficiency [13].

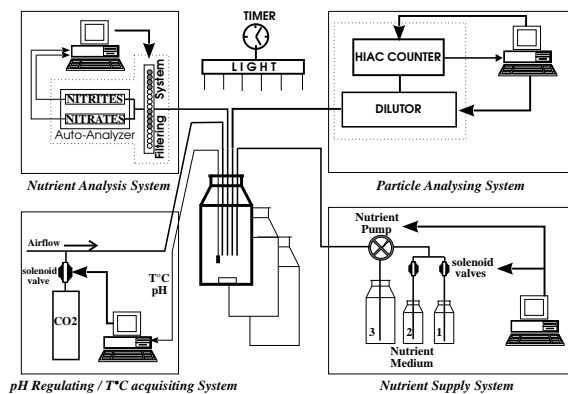


Figure 1: Synoptic schema of the culturing system. The three tanks for the Nutrient Supply System are: 1: sterile f/2 medium without nitrate, 2: sterile seawater with high nitrate concentration, 3: sterile seawater without nitrate.

Size spectra and cell concentrations are obtained by the particle counter HIAC/ROYCO PACIFIC. The system, constituted by an optical sensor (Laser sensor HRLD-400), a high-speed digital counter (Model 9064) and an automatic sampler (Model 3000), is monitored by a computer using particle distribution analysis software (PDAS). Before counting, dilution of concentrated phytoplankton cultures is necessary. This is routinely performed by an automatic system constituted by peristaltic pumps (GILSON), solenoid valves, and a syringe commanded by another computer.

To estimate the internal quota, measures of particulate nitrogen were performed, with a CHN analyzer (LECO 900) after filtration through Whatman GFF filters. These off-line measures are used to estimate the total quantity of particulate nitrogen  $N_T$  in the bioreactor. The internal quota  $Q$  is then estimated as follows:  $Q = \frac{N_T}{X}$

Peristaltic pumps (GILSON) provide axenic enrichment medium into the bioreactors after mixing the f/2 medium without  $NO_3$  and a concentrated solution of  $NaNO_3$ . For this, a dedicated computer controlled system drives a double solenoid valve allowing the concentrated solution of  $NaNO_3$  to be replaced with the sea water without  $NO_3$ . This way of monitoring the pump is used to give to the renewal medium concentration any dynamic pattern by computing every 5 minutes the proportion of time sea water replaces the concentrated  $NaNO_3$  solution [13].

The high gain software sensor (Equation (7)) was tested on experiments with the autotrophic chlorophyceae *Dunaliella tertiolecta* grown under nitrate limitation in a fluctuating chemostat environment controlled by computers. From the on-line biomass measurements, the software sensor computes the nitrate concentration in the medium and the internal quota of the phytoplankton. A periodic pattern of the influent nitrate concentration has been applied in order to reproduce certain marine hydrodynamical conditions. These experiments was used to test the software sensor when a dynamical forcing of the system is maintained.

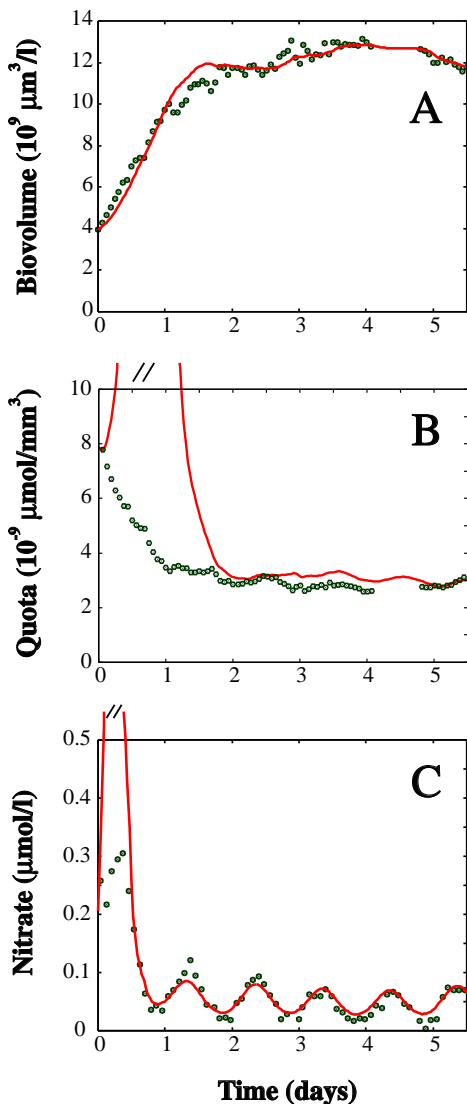


Figure 2: Comparison between measurements (●) and software sensors predictions (—) for the Droop model: (A) Biomass described by total biovolume of algae. (B) Internal Quota. (C) Nitrate concentration. The gain of the software sensor is  $\theta = 1$ .

In spite of its intensive use, the validation works of the Droop model have been done only at equi-

libria obtained in different stable nutrient environments. The main question of this work is to know whether the software sensor based on the Droop model can accurately estimate the variables for a nutrient fluctuating environment.

The software sensor firstly acts as a filter for the biomass  $X$  (Fig. 2). Indeed, it smoothes the noisy signal of the biomass measurements (estimated with total biovolume). Its main function is nevertheless to compute unmeasured variables. It can be seen that the software sensor substrate estimations are very close to the direct measurements which currently required sophisticated and delicate devices under the supervision of a micro-computer. In this case it can replace or back up fastidious measurements. In regard to the internal quota, it can provide an estimation, at least for established conditions (after day 2).

At the beginning of experiment, where algae were submitted to a deep starvation, the soft sensor points out two modeling defects. First, the internal quota predictions are overestimated during the 2 first days of experiment. This lack of observer convergence indicates a bias in growth rate modeling: the rapid evolution of biovolume at the beginning of experiment is above the maximum expected growth rate  $\mu_{max}$ . This theoretical threshold can be calculated when considering algae growing in an unlimited environment. The hypothesis  $S \ll k_S$  can then be stated, so that it is straightforward from Eq. (1) that  $Q$  satisfies a first order linear differential equation with the limit:

$$Q_{max} = \frac{\rho_m}{\bar{\mu}} + k_Q$$

This gives, therefore, the value of the theoretical maximal growth rate:

$$\mu_{max} = \frac{\rho_m \bar{\mu}}{\rho_m + k_Q \bar{\mu}}$$

This maximal growth rate can be computed using parameters value in Table 1,  $\mu_{max} = 1.4 \text{ day}^{-1}$ , it corresponds actually to the growth rate  $\mu$  achieved in batch experiments without nutrient limitation. This is much lower than the value measured here where  $\mu$  reaches  $2.2 \text{ day}^{-1}$ . The software sensor compensates this high value of  $\mu$  by overestimating  $Q$  by values larger than the maximum authorized by the model.

The second defect of the model concerns the substrate  $S$ . The software sensor leads us to suspect another phenomenon which is not taken into account by the model for these algae which were strongly limited before the experiment: an emergency uptake [16, 17] at the very beginning of experiments may explain the largely overestimated nitrate concentration. This was confirmed by es-

higher than the expected one.

## 6. CONCLUSION

Finally if we remember that the Droop model has been elaborated at equilibrium, the transient behavior of the software sensor build with this model is remarkable if the input does not vary too fast. In particular, observations of substrate show that the prediction of the nitrate concentration evolution is reliable, even at the beginning of the experiment after conditions of strong starvation of the algae, where the observer does not predict properly the internal quota  $Q$ .

As lots of biological models have the structure treated in this paper, this methodology could thus be extended to various fields of ecology and biology, where sensors for living material are very expensive, difficult to set up and suffer from a lack of reliability.

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