

Observer-based virtual sensors for microalgae cultures monitoring

Vélez-Landa, L.¹, Astorga-Zaragoza, C. M.^{2,*}, Hernández de-León, H. R.¹, Osorio-Gordillo, G. L.² & Bermúdez-Hernández, J. R.¹

¹ Tecnológico Nacional de México/Instituto Tecnológico de Tuxtla Gutiérrez; Tuxtla Gutiérrez, Chiapas, México.

² Tecnológico Nacional de México/Centro Nacional de Investigación y Desarrollo Tecnológico; Cuernavaca, Morelos, México.

* Corresponding author: astorga@cenidet.edu.mx; Tel.: (777) 362-770 ext. 1406

Received: 21 September 2019 Accepted: 13 November 2019

Abstract: In this paper, a nonlinear observer design is presented for simultaneous parameter estimation and state variables estimation. The case of study is microalgae cultures for biodiesel generation, where reaction rates, biomass concentration, intracellular quota and nitrogen concentration are critical variables that provide information about the state of the process. However, these variables might be difficult to measure due to the lack of specific instruments, high sensor costs or infeasibility of installation in the process. Therefore, two observer-based virtual sensors are presented in this paper as an analytical alternative to perform estimation of the main important variables or parameters of the process: a nonlinear adaptive observer and a nonlinear high-gain observer. The observers are based on the Droop's mathematical model that describes the ability of microalgae to store nutrients and the decoupling between substrate uptake and biomass growth. Numerical simulations are made in order to evaluate the performance of the proposed observers.

Keywords: Microalgae; biodiesel; virtual sensors; mathematical model

Nomenclature

X	Biomass concentration, mg L ⁻¹
S	Nitrogen concentration, mgN L ⁻¹
Q	Intracellular quota, gN gD.W ⁻¹
μ_m	Maximum growth rate, d ⁻¹
ρ_m	Maximum inorganic nitrogen uptake rate of limiting substrate, gN gD.W ⁻¹ d ⁻¹
k_s	Half saturation constant of substrate, mg L ⁻¹
Q_0	Minimum cell quota, mgN mgD.W ⁻¹
S_{in}	Inlet substrate, mgN L ⁻¹
D	Dilution rate, d ⁻¹

Introduction

The hard reliance on fossil fuels to cover energy demand has become increasingly important. However, fossil fuels are not renewable, their combustion causes emission of large amounts of carbon dioxide (CO₂), which is one of the greenhouse gases responsible for climate change and, in addition, they could be depleted within a few decades (Leonard et al., 2020; Barreto, 2018; Amalina et al., 2018). In fact, fuel oil reserves are expected to be scarce by 2050, so that as production decreases costs will increase (Shih et al., 2013).

Therefore, there is an urgent need to find sustainable, renewable, readily available and affordable alternative energy sources (Deshmukh et al., 2019). Biodiesel is a promising option to fuel derived from fossils, because it has a high flash point, it is biodegradable, ecological and it emits low concentrations of polluting gases during its combustion (Ching et al., 2015; Rincón et al., 2014).

Microalgae is one of the raw materials that has received attention by the scientific community for the biodiesel production and that could potentially resolve the current energetic. Microalgae are photosynthetic organisms that use a source of light, carbon dioxide, water and some nutrients to generate biomass (Uganeeswary et al., 2019; Chun et al., 2011). The oil content in microalgae biomass is around 20-50% by weight of dry mass (Spolaore et al., 2006). They also have the quality of overcoming various limitations, since they are cultivated in infertile lands or lands using water from low quality. Algae production does not cause food problems because the cultivation process does not necessarily require fresh water for cultivation. Algae-based biofuels could satisfy a great amount of the global demand for transportation fuels (Mohammadmatin et al., 2018; Demirbas, 2010; Chisti, 2007).

Unlike terrestrial crops, microalgae grow 10-15 times faster (Chun et al., 2011) and biodiesel production is expected to be 15-300 times higher (Amalina et al., 2018). They commonly double their mass in 24 hours (Chisti, 2007) and their

harvest cycle is very short (around 12 days, depending on the species). This allows to obtain biomass during the year. It is possible to obtain oil production from 19000 to 57000 liters per hectare in a year, that is a higher volume of lipids than any other raw material for biodiesel (Demirbas et al., 2011).

In this context, continuous performance monitoring of the process in order to prevent interruptions and stoppages of the plant, is of utmost importance. Unfortunately, most of supervision attempts are hampered by the lack of appropriate devices, high instrumentation costs and unfeasibility of installing sensors inside the system (Benavides et al., 2015; Mohd et al., 2015; de Battista et al., 2011; Jenzsch et al., 2006). An alternative to mitigate this difficulty is to design and implement robust and reliable analytical methods (by combining the information available through process models and available measurements) to perform on-line estimation of process variables and parameters (Benavides et al., 2015 and Dochain, 2003).

The estimation of variables and parameters in bioprocess is a challenging task given its complex and nonlinear dynamic behavior, the lack of reliable sensors, unmodeled dynamics, unpredictable parameters variations among other factors (Dochain, 2003; de Battista et al., 2011). In this regard, kinetic parameters are directly correlated with process performance but they are not measurable with commercially available sensors.

For this reason, it is of great importance to implement robust methods to determine the specific characteristics in a short period of time and to select industrially relevant microorganisms (Dietzsch et al., 2011; Jenzsch et al., 2006).

The main contribution of this work is the implementation of state observers as virtual sensors in biotechnological applications such as microalgae cultures for online monitoring of reaction rates and nutrient concentration based on the knowledge of available measurements. This development is an extension of the work published in Vélez et al., (2019). The main difference consists in the use of a variable dilution rate for the simultaneous estimation of two parameters that change during the process: the maximum growth rate and the maximum absorption rate of nitrogen. In addition, the nutrient concentrations (which is difficult to measure online) and the intracellular quota (which cannot be controlled online) (Coutinho et al., 2019) are estimated for monitoring purposes.

Materials and Methods

Mathematical model

Droop model (Droop, 1983) is one of the most accepted and used models for design, analysis, operation and control of complex processes such as microalgae cultures. This model expresses the mass balance in a continuous photobioreactor. The model describes the dynamics of three state variables: biomass concentration (X), substrate concentration (S) and intracellular quota (Q). The extracellular nitrogen is considered as a limiting substrate. It is of particular interest when microalgae biomass is required for the biodiesel production. The accumulation of lipids within microalgae is triggered by subjecting them to stress due to nitrogen depletion in the culture media (Solimeno et al., 2017; Yuan et al., 2014; Yoo et al., 2014; Bougaran et al., 2010).

The Droop model for microalgae cultures is given in the following equations:

$$\dot{X} = \mu_m \left(1 - \frac{Q_0}{Q}\right) X - DX \quad (1)$$

$$\dot{S} = -\rho_m \left(\frac{S}{S + k_s}\right) X - DS + DS_{in} \quad (2)$$

$$\dot{Q} = \rho_m \left(\frac{S}{S + k_s}\right) - \mu_m \left(1 - \frac{Q_0}{Q}\right) Q \quad (3)$$

The dilution rate D is the quotient between the inflow and volume of the culture, k_s is a half saturation constant of substrate, ρ_m is the maximum uptake rate, Q_0 is the minimum intracellular quota at which microalgae no longer grow and μ_m is the maximum growth rate.

Assumption 1. The volume and illumination are constant, temperature is uniformly distributed within the photobioreactor, pH is maintained at 7.3, culture media is perfectly mixed and nitrogen is limited.

The system given in Eqs. (1)-(3) can be rewritten as:

$$\dot{x}_1 = \theta_1 \left(1 - \frac{Q_0}{Q}\right) x_1 - ux_1 \quad (4)$$

$$\dot{x}_2 = -\theta_2 \left(\frac{x_2}{x_2 + k_s}\right) x_1 - ux_2 + uS_{in} \quad (5)$$

$$\dot{x}_3 = \theta_2 \left(\frac{x_2}{x_2 + k_s}\right) - \theta_1 \left(1 - \frac{Q_0}{x_3}\right) x_3 \quad (6)$$

where $x_1 = X$, $x_2 = S$, $x_3 = Q$, $u \in R$ is the control input of the system, $\theta_1 = \mu_m$ and $\theta_2 = \rho_m$ are unknown parameters (the maximum growth rate and the maximum nitrogen rate, respectively).

Table 1 shows the parameters description, as well as their values, presented in Benavides et al., (2015) for microalga *Dunaliella tertiolecta*.

Table 1. Parameters description and their values

Parameter	Value	Definition
θ_1	1.8102 d ⁻¹	Maximum growth rate
θ_2	0.0916 gN gD. W. ⁻¹ d ⁻¹	Maximum absorption rate of nitrogen
k_s	1.7499 mgN L ⁻¹	Half saturation constant of substrate
Q_0	0.0369 mgN mgD.W. ⁻¹	Minimum intracellular quota
S_{in}	15 mgN L ⁻¹	Inlet substrate

Adaptive observer

In general, a state observer is a dynamic system that provides an asymptotic estimate of the real state of a system, based on knowledge of the measured inputs and outputs of a process. An adaptive observer is a recursive algorithm for simultaneous estimation of the state of the system and unknown parameters. The adaptive observer used in this work was firstly developed by (Besançon, 2000) and it has been successfully used in various practical applications (Astorga et al., 2007; Ortiz et al., 2013).

Consider the following nonlinear system, written in the adaptive form presented in Besançon, (2000):

$$\dot{y} = \alpha(y, \zeta, u, t) + \beta(y, \zeta, u, t)\theta \quad (7)$$

$$\dot{\zeta} = Z(y, \zeta, u, t) \quad (8)$$

where $y \in R^p$ is the output vector of the system (measured variables), $\zeta \in R^r$ is the vector of unmeasured variables, $u \in R^m$ is the input vector and $\theta \in R^q$ is the vector of unknown parameters. Functions α and β are Lipschitz on the domain of the state variables and the input.

An adaptive observer for the system given in Eqs. (7)-(8):

$$\hat{y} = \alpha(y, \hat{\zeta}, u, t) + \beta(y, \hat{\zeta}, u, t)\hat{\theta} - k_y(\hat{y} - y) \quad (9)$$

$$\dot{\hat{\zeta}} = Z(y, \hat{\zeta}, u, t) \quad (10)$$

$$\dot{\hat{\theta}} = -k_{\theta}\beta^T(y, \hat{\zeta}, u, t)(\hat{y} - y) \quad (11)$$

The symbol “^” represents the estimated values. The superscript “T” denotes the transpose of a matrix. $k_y > 0$, and $k_{\theta} > 0$ are the observer gains.

In order to demonstrate that the estimation errors $\|\hat{y}(t) - y(t)\|$ and $\|\hat{\zeta}(t) - \zeta(t)\|$ asymptotically tend to zero when t tends to infinity, while $\|\hat{\theta}(t) - \theta\|$ remains bounded, the following dynamics of the estimation errors are considered:

$$\dot{e}_y = \hat{y} - \dot{y} \quad (12)$$

$$\dot{e}_{\zeta} = \hat{\zeta} - \dot{\zeta} \quad (13)$$

$$\dot{e}_{\theta} = \hat{\theta} - \dot{\theta} \quad (14)$$

By considering the following Lyapunov's candidate function

$$V_e(t, e_y, e_{\zeta}, e_{\theta}) = \left(\frac{\varepsilon}{2}\right) e_y^T e_y + V(t, e_{\zeta}) + (\varepsilon/2k_{\theta}) e_{\theta}^T e_{\theta} > 0 \quad (15)$$

it can be easily demonstrated that its derivative is given by

$$\dot{V}_e \leq -\varepsilon k_y \|e_y\|^2 + \varepsilon (\gamma_{\alpha} + \gamma_{\beta}) \|e_y\| \sqrt{\kappa(e_{\zeta})} - \kappa(e_{\zeta}) > 0 \quad (16)$$

If $k_y > 0$ and $k_{\theta} > 0$ then, $\dot{V}_e$ is negative definite and thereby it ensures the convergence of the observer.

Evaluation of the adaptive observer

- *Simultaneous estimation of the maximum growth rate θ_1 and the maximum absorption rate θ_2 from measurements of biomass concentration, nitrogen concentration and intracellular quota.*

In order to design the nonlinear adaptive observer, the system given in Eqs. (4)-(6) can be rewritten in the form of Eqs. (7)-(8) as follows:

$$\underbrace{\begin{bmatrix} \dot{y}_1 \\ \dot{y}_2 \\ \dot{y}_3 \end{bmatrix}}_{\dot{\hat{y}}} = \underbrace{\begin{bmatrix} -uy_1 \\ uy_2 + uS_{in} \\ 0 \end{bmatrix}}_{\alpha(y, \hat{\zeta}, u, t)} + \underbrace{\begin{bmatrix} y_1 \left(1 - \frac{Q_0}{y_3}\right) \\ 0 \\ -y_3 \left(1 - \frac{Q_0}{y_3}\right) \end{bmatrix}}_{\beta_1(y, \hat{\zeta}, u, t)} \theta_1 + \underbrace{\begin{bmatrix} 0 \\ -y_1 \left(\frac{y_2}{y_2 + k_s}\right) \\ \left(\frac{y_2}{y_2 + k_s}\right) \end{bmatrix}}_{\beta_2(y, \hat{\zeta}, u, t)} \theta_2 \quad (17)$$

Based on Eqs. (9)-(11), the adaptive observer design can be expressed as is given in Eqs. (18)-(20). It can be seen that simultaneous estimation of the maximum growth rate (θ_1) and the maximum absorption rate (θ_2) can be performed. These parameters are time-varying throughout the process, given that biological systems are naturally uncertain and they change over time depending on external conditions (Countinho et al., 2019). In this case, it is considered that all states are measurable, therefore no unmeasurable states ζ exist. We are only interested to obtain the online estimations of parameters θ_1 and θ_2 .

$$\dot{\hat{y}} = \alpha(y, u, t) + \beta_1(y, u, t)\hat{\theta}_1 + \beta_2(y, u, t)\hat{\theta}_2 - k_y(\hat{y} - y) \quad (18)$$

$$\dot{\hat{\theta}}_1 = -k_{\theta 1}\beta_1^T(y, \hat{\zeta}, u, t)(\hat{y} - y) \quad (19)$$

$$\dot{\hat{\theta}}_2 = -k_{\theta 2}\beta_2^T(y, \hat{\zeta}, u, t)(\hat{y} - y) \quad (20)$$

- Intracellular quota estimation from biomass and nitrogen concentrations measurements

In this case, we are interested to estimate the unknown state variables due to its complex measurement in microalgae cultures. To design the observer, the system given in Eqs. (4)-(6), is rewritten in the form of Eqs. (7) and (8), as is shown in Eqs. (21) and (22). All parameters (θ) are considered to be known. The biomass and nitrogen concentrations are measured with physical sensors.

$$\begin{bmatrix} \dot{y}_1 \\ \dot{y}_2 \end{bmatrix} = \begin{bmatrix} -uy_1 \\ uy_2 + S_{in} \end{bmatrix} + \begin{bmatrix} y_1 \left(1 - \frac{Q_0}{\zeta}\right) \\ 0 \end{bmatrix} \theta_1 + \begin{bmatrix} 0 \\ -y_1 \left(\frac{y_2}{y_2 + k_s}\right) \end{bmatrix} \theta_2 \quad (21)$$

$$\dot{\zeta} = \underbrace{-\zeta \left(1 - \frac{Q_0}{\zeta}\right) \theta_1 + \left(\frac{y_2}{y_2 + k_s}\right) \theta_2}_{Z(y, \zeta, u, t)} \quad (22)$$

Based on Equations (9)-(11), the adaptive observer design is as given in Eqs. (23)-(25):

$$\dot{\hat{y}}_1 = \alpha(y, \hat{\zeta}, u, t) + \beta_1(y, \hat{\zeta}, u, t) \theta_1 - k_y(\hat{y}_1 - y_1) \quad (23)$$

$$\dot{\hat{y}}_2 = \alpha(y, \hat{\zeta}, u, t) + \beta_2(y, \hat{\zeta}, u, t) \theta_2 - k_y(\hat{y}_2 - y_2) \quad (24)$$

$$\dot{\hat{\zeta}} = Z(y, \hat{\zeta}, u, t) \quad (25)$$

High-gain observer

A high-gain observer for nonlinear systems was proposed by Gauthier et al., (1992) and, since then, it has been successfully applied for estimation of various nonmeasurable or difficult to measure variables, for instance, in ethanol-water distillation columns (Téllez et al., 2009), heat exchangers (Astorga et al., 2008), steam generators of thermal power plants (Astorga et al., 2018), among others.

Consider the following nonlinear control affine system

$$\dot{x} = f(x(t)) + g(x(t))u(t) \quad (26)$$

$$y(t) = h(x(t)) \quad (27)$$

where $x(t) \in R^n$ is the state vector, $u(t) \in R$ is input of the system, $h(x(t)) \in R$ is output function, $f(x(t)) \in R^n$ and $g(x(t)) \in R^n$ are smooth nonlinear functions.

Assuming that the system given by Eqs. (26) and (27) is observable and by considering a transformation of coordinates $z(t) = \Phi(x(t)) = [h(x) \ L_f h(x) \ \dots \ L_f^{n-1} h(x)]^T$, where $L_f^i h(x)$ is the i -th successive Lie derivate of $h(x)$ along $f(x)$, then the system becomes

$$\dot{z}(t) = \tilde{A}z(t) + \Psi(z(t)) + \phi(z(t))u(t) \quad (28)$$

$$y(t) = \tilde{C}z(t) \quad (29)$$

$$\text{where } z(t) \in R^n, \tilde{A} = \begin{bmatrix} 0 & 1 & 0 & 0 \\ \vdots & \ddots & 0 & 0 \\ 0 & \ddots & 0 & 1 \\ 0 & \dots & 0 & 0 \end{bmatrix}, \Psi(z(t)) = \begin{bmatrix} 0 \\ \vdots \\ 0 \\ \Psi_n(z(t)) \end{bmatrix}, \phi(z(t)) = \begin{bmatrix} \phi^1(z(t)) \\ \phi^2(z_1(t), z_2(t)) \\ \vdots \\ \phi^n(z_1(t), \dots, z_n(t)) \end{bmatrix} \text{ and } \tilde{C} = [1 \ 0 \ \dots \ 0]$$

An observer for the system given by Eqs. (28) and (29) is given by Gauthier et al., (1992)

$$\dot{\hat{z}}(t) = \tilde{A}\hat{z}(t) + \Psi(\hat{z}(t)) + \phi(\hat{z}(t))u(t) + S_\theta^{-1}\tilde{C}^T(\tilde{C}z(t) - \tilde{C}\hat{z}(t)) \quad (30)$$

where S_θ is a positive definite symmetric matrix calculated from the Lyapunov equation.

$$\theta S_\theta + A^T S_\theta + S_\theta A = \tilde{C}^T \tilde{C} \quad (31)$$

where $\theta > 0$ is the observer calibration parameter.

By considering a third order system (which is the case of the system representing the microalgae cultures, the Droop model), the matrix S_θ is expressed as follows

$$S_\theta = \begin{bmatrix} \frac{1}{\theta} & -\frac{1}{\theta^2} & \frac{1}{\theta^3} \\ -\frac{1}{\theta^2} & \frac{2}{\theta^3} & -\frac{3}{\theta^4} \\ \frac{1}{\theta^3} & -\frac{3}{\theta^4} & \frac{6}{\theta^5} \end{bmatrix} \quad (32)$$

In the original coordinates $x(t) = \phi^{-1}(z(t))$, the observer given in Eq. (30) becomes

$$\dot{\hat{x}}(t) = f(\hat{x}(t)) + g(\hat{x}(t))u(t) + \left[\frac{\partial \phi(\hat{x}(t))}{\partial \hat{x}} \right]^{-1} S_\theta^{-1} \tilde{C}^T (y(t) - \hat{y}(t)) \quad (33)$$

$$\hat{y} = h(\hat{x}(t)) \quad (34)$$

where $J(\hat{x}(t)) = \frac{\partial \phi(\hat{x}(t))}{\partial \hat{x}}$ is the $n \times n$ Jacobian matrix of $\phi(\hat{x}(t)) = \phi(x(t))|_{x(t)=\hat{x}(t)}$.

A condition for the existence of an observer for the system given by Eqs. (26) and (27) is that $\text{rank}(J(x(t))) = n$.

Performance evaluation of the high-gain observer

In order to design the high-gain observer, the system given by Eqs. (4)-(6) is rewritten as is shown in Eqs. (26)-(27). It can be seen that the biomass concentration (x_1) is the available measurement.

$$\dot{x} = \underbrace{\begin{bmatrix} \mu_m x_1 - \frac{\mu_m Q_0 x_1}{x_3} \\ -\frac{\rho_m x_1 x_2}{x_2 + k_s} \\ \frac{\rho_m x_2}{x_2 + k_s} - \mu_m x_3 + \mu_m Q_0 \end{bmatrix}}_{f(x(t))} + \underbrace{\begin{bmatrix} -x_1 \\ -x_2 + S_{in} \\ 0 \end{bmatrix}}_{g(x(t))} u(t) \quad (35)$$

$$y(t) = x_1 \quad (36)$$

It can be verified that for the system given by Eqs. (35) and (36), $\text{rank}(J(x(t))) = 3$, therefore, the system is observable. Then the proposed high-gain observer is:

$$\dot{\hat{x}}(t) = f(\hat{x}(t)) + g(\hat{x}(t))u(t) + \left[\frac{\partial \phi(\hat{x}(t))}{\partial \hat{x}} \right]^{-1} S_\theta^{-1} \tilde{C}^T (x_1(t) - \hat{x}_1(t)) \quad (37)$$

$$\hat{y} = \hat{x}_1 \quad (38)$$

$$\text{where } S_\theta^{-1} = \begin{bmatrix} 3\theta & 3\theta^2 & \theta^3 \\ 3\theta^2 & 5\theta^3 & 2\theta^4 \\ \theta^3 & 2\theta^4 & \theta^5 \end{bmatrix}$$

Results and Discussion

Two scenarios are presented for the supervision of microalgae cultures through the online monitoring of parameters and characteristic variables of the process as explained below.

Scenario 1. Adaptive observer

- *Simultaneous estimation of the maximum growth rate and the maximum absorption rate are performed based on the measurements of the biomass and nitrogen concentration and the intracellular quota.*

The simulations were carried out in Matlab R2018 using the Euler integration method to solve the differential equations, with an integration time of 0.001s. The initial conditions of the adaptive observer were same as those of the system, that is $\hat{y}_1(0) = 140 \text{ mgD.W. L}^{-1}$, $\hat{y}_2(0) = 16.5 \text{ mgN L}^{-1}$ and $\hat{y}_3(0) = 0.045 \text{ gN gD.W}^{-1}$. This is an acceptable consideration due to the assumption that all states are measurable. The initial condition of the parameters to be estimated were zero, that is $\hat{\theta}_1(0) = 0 \text{ d}^{-1}$ and $\hat{\theta}_2(0) = 0 \text{ gN gD.W}^{-1} \text{ d}^{-1}$ by taking into account that they are completely unknown.

Likewise, the tuning gains for the observer $k_y = 120$, $k_{\theta_1} = 2$ and $k_{\theta_2} = 0.1$ were chosen such that error converges asymptotically to zero. On the other hand, it was considered that the maximum growth rate varies throughout the process from 1.8102 to 1.6, and from 1.3 to 2.2 d^{-1} and in the same way, the maximum absorption rate varies from 0.0916 to 0.01, and from 0.3 to 0.05 $\text{gN gD.W}^{-1} \text{ d}^{-1}$, as is shown in Fig. 2.

In Fig. 1 the input signal of the system is shown. Three impulses lasting one day were applied, one on day 2 with $u=0.9 \text{ d}^{-1}$, another on day 4 with $u=0.5 \text{ d}^{-1}$ and the last one on day 6 with $u=0.7 \text{ d}^{-1}$, in order to validate the performance of the observers in continuous cultures.

Figs. 2a and 2b present the estimates of the two kinetic parameters. This is achieved by using biomass concentration, nitrogen concentration and intracellular quota as available measurements. Given that two parameters are included in three equations of the Droop model, it is not possible to estimate any of these state variables, because the parameters must not be present in the vector of unmeasured variables according to Eq. (10) proposed in Besançon (2000). In Fig. 2a it is clearly observed that the observer estimates adequately the maximum growth rate. However, as soon as a change in the value of the parameter is detected, the estimated value (dotted line) tries to follow the true value (solid line) without achieving asymptotic convergence during the variation, once the value remains constant for a period of considerable time estimation error tends to zero.

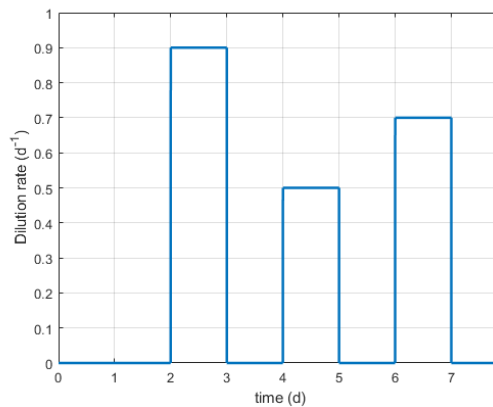


Fig. 1. System input.

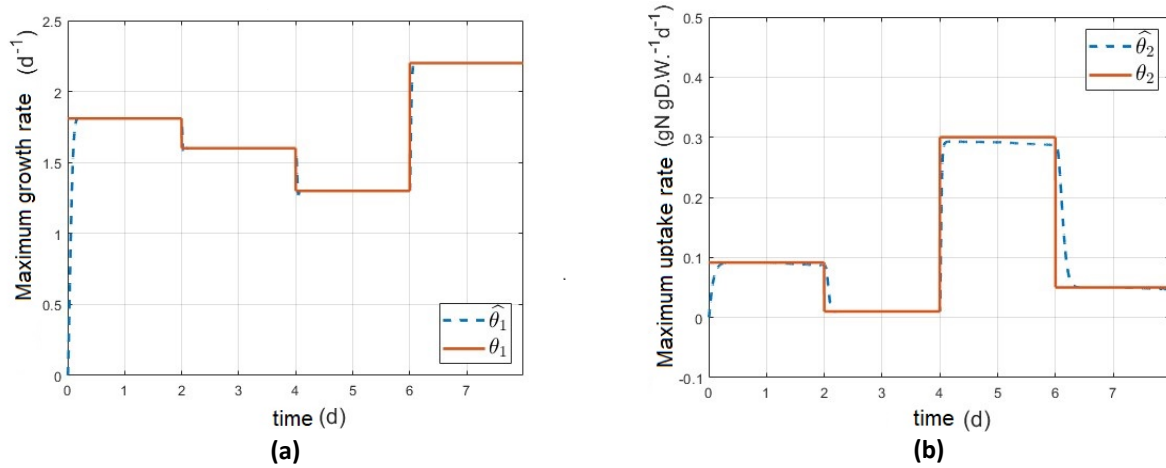


Fig. 2. True and estimated values of the parameters: (a) maximum growth rate, and (b) maximum uptake rate.

In Fig. 2b it can be seen that the estimated value (dotted line) of the maximum uptake rate before detecting the first change begins to diverge from the true values (solid line), until it remains constant for two days, it achieves convergence, but detecting a larger change presents more noticeable discrepancy without recovering even when occurs a decrease in value.

- *Estimation of intracellular quota from biomass and nitrogen concentrations measurements*

Simulations were carried out in Matlab R2018a by using the Euler integration method to solve the differential equations, with an integration time of 0.001s. The constant parameters mentioned in Table 1 were used. The profile of the input signal is given in Fig. 1. Initial conditions for the observer were $\hat{y}_1(0) = 140\ mgD.W.L^{-1}$, $\hat{y}_2(0) = 16.5\ mgN.L^{-1}$ due to the assumption that they are measurable with physical sensors. It was considered that $\hat{\zeta}(0) = 0.025\ gN\ gD.W^{-1}$ since it is an unknown variable. Likewise, gain $k_y = 100$ was chosen, according to the stability conditions imposed by the Lyapunov stability method. Therefore, the estimation error converges to zero. Figs. 3 and 4 show the biomass and nitrogen concentrations respectively. It can be seen that the estimated values (dotted line) quickly converge from the beginning of the process to the true values (solid line) inasmuch as we consider that measurements are obtained with physical sensors. In Fig. 5 it can be seen that the intracellular quota estimation is adequately achieved with the adaptive observer and it reaches its true value before the middle of the process.

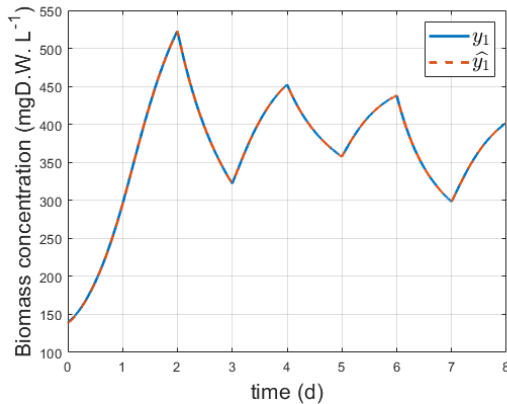


Fig. 3. True and estimated values of biomass concentration.

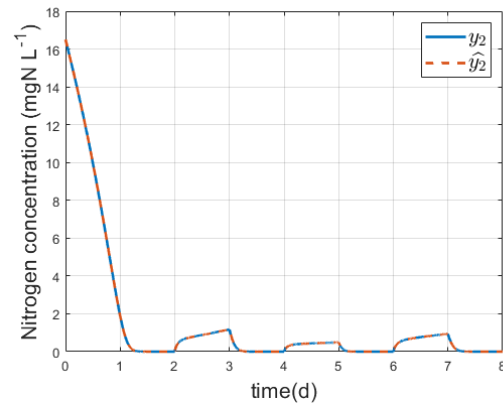


Fig. 4. True and estimated values of nitrogen concentration.

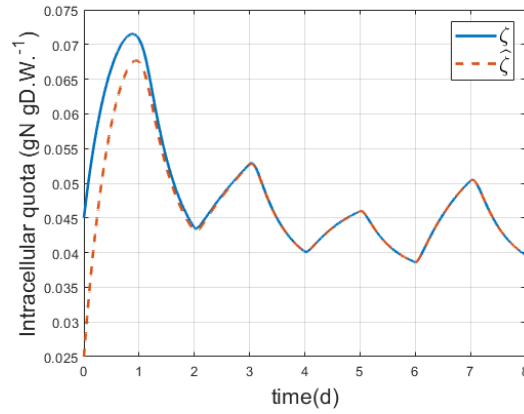


Fig. 5. True and estimated values of intracellular quota.

Scenario 2. Estimation of nitrogen concentration and intracellular quota from the available measurement of biomass concentration by means of a high-gain observer

Simulations were carried out in Matlab R2018 by using the Euler integration method to solve the differential equations, with an integration time of 0.001 s. Initial conditions for the system were $x_1(0) = 140 \text{ mgD.W. L}^{-1}$, $x_2(0) = 16.5 \text{ mgN L}^{-1}$ and $x_3(0) = 0.045 \text{ gN gD.W}^{-1}$ and the initial conditions of the observer were $\hat{x}_1(0) = 145 \text{ mgD.W. L}^{-1}$, $\hat{x}_2(0) = 20 \text{ mgN L}^{-1}$ and $\hat{x}_3(0) = 0.025 \text{ gN gD.W}^{-1}$. The same input illustrated in Fig. 1 was used. The calibration parameter was $\theta = 2$.

Fig. 6 shows the estimated value (dotted line) of the biomass concentration compared to its true value (solid line). It can be that the estimation converges asymptotically as expected, in as much as they are the measured variables of the process.

In Fig. 7 it is observed that the estimation of the nitrogen concentration quickly converges to the true value. In Fig. 8 it can be appreciated the estimated value of the intracellular quota which requires less time to reach the true value compared with the estimated value obtained with the adaptive observer. This demonstrates the effectiveness of the high-gain approach to predict this variable, which in practice, it is difficult to measure with physical sensors.

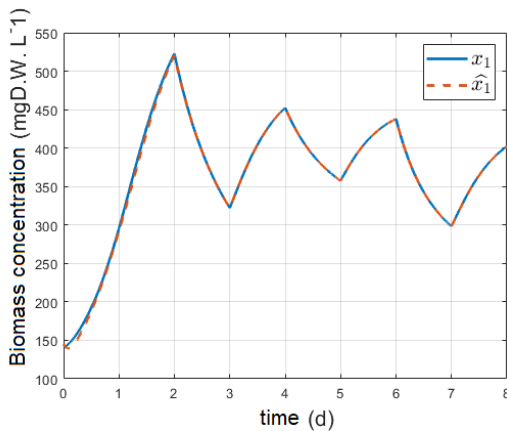


Fig. 6. True and estimated values of biomass concentration.

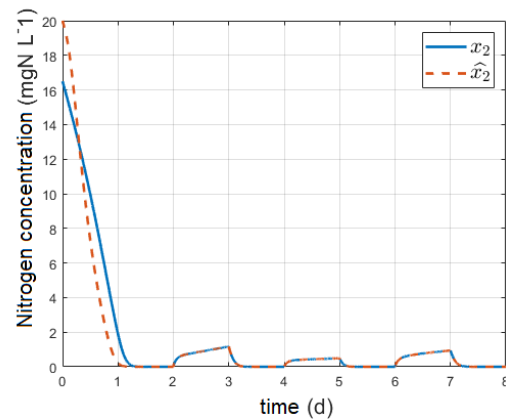


Fig. 7. True and estimated values of nitrogen concentration.

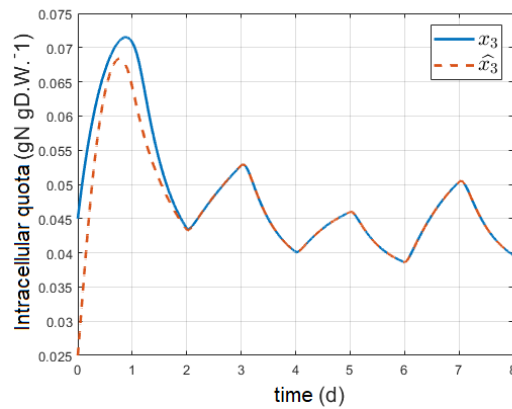


Fig. 8. True and estimated values of intracellular quota.

Conclusions

The nonlinear adaptive observer proposed for estimation of the intracellular quota and simultaneous estimation of the maximum growth rate and the maximum uptake rate turned out to be efficient and easy to implement. The observer gains guarantee the convergence of the estimation values towards the true values. These gains are explicitly proposed as positive values without the need to solve any other dynamic system. The online estimation of the state variables or the process parameters offers the opportunity to optimize the control and productivity of the bioprocess. Also, it allows to provide timely diagnosis in case of problems during the cultivation stage. The estimation of the maximum growth rate is achieved despite the fact that it undergoes significant changes. In contrast, estimation of the maximum absorption rate is not possible when there are very large changes in its values, however for small changes or constant values, the estimated value converges asymptotically to the true value.

On the other hand, by using high-gain observers it is possible to know the values of the nitrogen concentration and the intracellular quota based on available measurements of biomass concentration. This observer converges faster than the adaptive observer when intracellular quota is estimated. In conclusion, the use of virtual sensors such as those presented in this work are very useful to monitor continuous microalgae cultures.

Acknowledgments

The authors gratefully acknowledge to Consejo Nacional de Ciencia y Tecnología (CONACYT) for supporting Laura Vélez-Landa with a national mobility grant to carry out a staycation at Tecnológico Nacional de México (TecNM) / Centro Nacional de Investigación y Desarrollo Tecnológico (CENIDET).

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