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Estimation of microalgal photobioreactor production based on total inorganic carbon in the medium

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Abstract: Microalgae biotechnology has been focusing on the use of algae in the production of high value compounds. During the last few decades, the intense research effort has been aiming at improving new controls and supervising tools as well as on a good process understanding. This requirement involves a large diversity and a better accessibility to process measurements. Probes or sensors are required to control the process. They are however relatively limited. Classical photobioreactors are usually equipped with temperature, dissolved oxygen and pH probes. These sensors actually provide very little online information on cell growth, viability, metabolic state and production. For the time being, sensors reliability cannot meet industrial bioprocessing requirements. In this context software sensors show numerous potentialities. The central axis of this work is the development of an extended Kalman filter (EKF) for the estimation of biomass concentration based on a dynamic process model in combination with total inorganic carbon measurement. A microalga *Porphyridium purpureum* was used as a model organism in this study. Numerical simulations and real-life experiments (batch and continuous mode) have been carried out and corresponding results are given in order to highlight the performance of the proposed estimator.

1. INTRODUCTION

Today, the microalgal biomass is recognized as an excellent source of proteins, carbohydrates, lipids and vitamins used as food and feed additives. Microalgae, as photosynthetic organisms, use light and carbon dioxide for their energy. Large-scale cultures also find applications in energy production (e.g. photobiological hydrogen, biofuel, methane) and environmental remediation (e.g. wastewater treatment, carbon dioxide fixation and greenhouse gas emissions reduction) (Benemann 1997, Hallenbeck *et al*., 2002). Microalgae also can absorb heavy metals and sequester or degrade many different classes of toxic compounds (Boussiba and Leu, 2007)

Current commercial technology for microalgae production involves both open ponds and closed photobioreactor productions system. Open ponds, although of lower cost than closed photobioreactors, have tendency to becoming rapidly contaminated with unwanted species. Commercial culture is usually carried out in closed photobioreactors which have several advantages such as high productivity and clean microalgal culture. A variety of closed devices have been proposed for generating microalgal biomass: tubular, bubble columns and airlift photobioreactors are widely used in the bioprocess industry (Camacho Rubio *et al*. 1999; Acién Fernandez *et al*.; 2001, Chisti, 2007). A good control over the growth environment results in a consistent product quality and the higher operating biomass densities. Dissolved oxygen, pH, temperature and light intensity are commonly monitored. The accessibility to process measurements and to culture physiological states is however limited.

The control system of bioreactor is a difficult task due to non-linear and time-varying nature of the system, slow response of the process and lack of reliable on-line sensors which can detect the important state variables (Shimizu, 1996).

Biomass is one of the most valuable variables to control. Such information may be collected by sampling and off-analysis such a cell counting, optical density or dry weight measurements. Other experimental methods are available to quantify and qualify the biomass: optical ones (visible near infrared absorption, scattering, etc) and those exploiting the electrochemical properties of the culture. Though, these methods are difficult to apply for online monitoring of biomass concentration in a photobioreactor. This is because these sensors are particularly sensitive to calibration problems and often need on-line cleaning. Aggregate formation and cell death can induce erroneous turbidity measurements.

In this context, considerable attention has been devoted to the development of on-line software sensors. The objective of a software sensor is indeed to provide an estimation of the system state variables and particularly those which are not obtained through in situ hardware sensor or which need laborious and expensive analysis.

Many studies have been made on the state estimation of unmeasured states variables. Several estimation techniques

have been proposed in the literature (Oliveira *et al*.,1996; Soroush, 1998; Bastin & Dochain, 1990; Bernard *et al*., 2001; Dochain, 2001).

In this paper, we report the development of an extended Kalman filter (EKF) for on-line estimation of biomass concentration, based on dynamic process model in combination with total inorganic carbon measurement. The process model is divided in two submodels: growth kinetics and gas-liquid mass transfer in the photobioreactor. A microalga *Porphyridium purpureum* was used as the model organism. This software sensor is capable of estimating biomass density in the photobioreactor bubbled with air containing a variable percentage of carbon dioxide and continuously illuminated with fluorescent lamps.

The paper is organized as follows. Section 2 presents the bioprocess and its components. The system modelling is then introduced in section 3 and the EKF in section 4. This estimator is applied on simulation and on a laboratory-scale reactor. Finally, some conclusions and perspectives are given in Section 5.

2. BIOPROCESS DESCRIPTION

2.1 Strain and growth conditions

The red microalga *Porphyridium purpureum* SAG 1830-1A was obtained from the Sammlung von Algenkulture Pflanzenphysiologister Institut der Universität Göttingen, Germany. The strain was grown and maintained on Hemerick medium (Hemerick, 1973). The pH was adjusted to 7.0 prior to autoclaving at 121 ºC for 20 min. Cultures were maintained at 25 ºC in 500 ml flask containing 400 ml culture under continuous light intensity of 70 μE m^{-2} s^{-1} and aerated with air containing 1% (v/v) CO_2 at 100 rpm on a orbital shaker. Every two weeks, 200 ml of a culture was transferred to a new flask containing fresh medium during the exponential phase of growth.

2.2 Photobioreactor culture conditions and measurements

The cultures of *P. purpureum* were performed in a bubble column photobioreactor (fig. 1) with a working height and diameter of 0.4 and 0.1 m, respectively. The total culture volume was 2.5 l. The cylindrical reactor was made of glass and had an illuminated area of 0.1096 m². Air containing 2 % (v/v) CO_2, was continuously supplied at a flow rate of 2.5 V.V.H. (gas volume per liquid culture volume per hour) at the bottom of the column for the agitation of the culture. The air flow rate entering the photobioreactor was filtered through 0.22 μm Millipore filters and was regulated using the suitable valves and flowmeters. An arrangement of four OSRAM white fluorescent tubes (L30W/72) and three OSRAM pink fluorescent tubes (L30W/77) around the bubble column was used as an external light source. The incident light intensity on the reactor surface was measured at 10 distinct locations using a flat-surface quantum sensor LI-COR LI-190SA. The average light intensity was calculated by taking the weighted average of all measurements. The optimal value of irradiance on surface for our reactor is 120 μE m^{-2} s^{-1}.The reactor was equipped with a transparent jacket connected to a thermostat unit, which allowed controlling the temperature at 25 ºC. The photobioreactor was also equipped with a pH sensor (Radiometer Analytical) and a dissolved oxygen sensor (Ingold type 170).

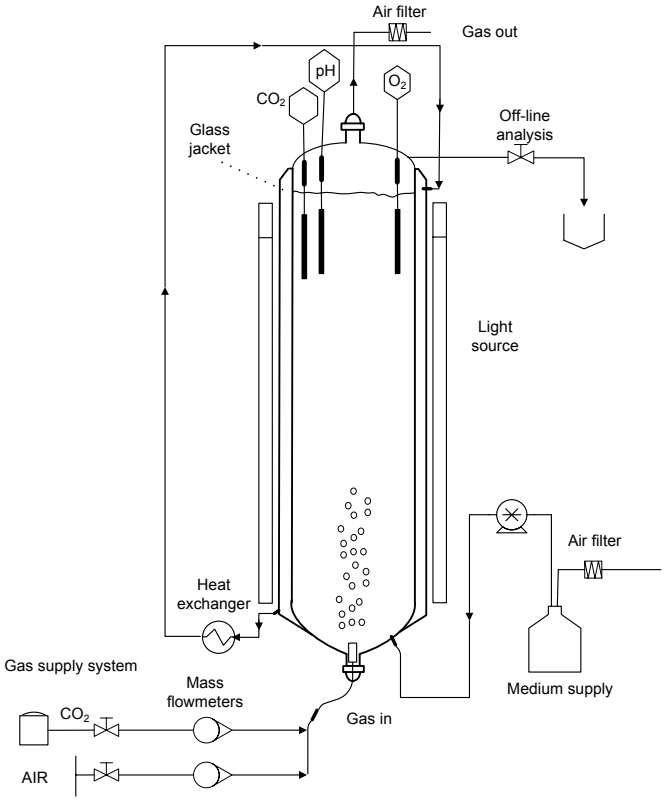

Fig. 1. Schematic representation of the photobioreactor system

The photobioreactor has been equipped with one sampling port in the top of the column. Samples for off-line analysis were collected at regular intervals (every 6, 8, and 12 hours). Cells number was counted under an optical microscope ZEISS Axioplan-2 on Malassez cells. Four determinations were made for each sample and the mean and the standard deviation calculated. Total inorganic carbon (T.I.C.) in the culture medium was determined by gas phase chromatography. The method proposed by Marty *et al.* (1995) is used to measure low inorganic carbon concentrations (down 10^{-6} mol l^{-1}) and gives results with accuracy within 10 % and thus appears reliable and sensitive.

3. BIOPROCESS MODELLING

Mathematical modelling of a photobioreactor requires knowing the coupling between the metabolism of microorganisms, the light transfer inside the culture and the fluid dynamics of the reactor. In this paper, the process model proposed by (Baquerisse, 1999) is considered. It is made of two submodels: growth kinetics and gas-liquid mass transfer in the photobioreactor. Thus, the dynamic process model considers two equations, namely biomass and total inorganic carbon balances. The inorganic carbon concentration is associated with increasing cell density.

Evolution of cell number can be expressed as:

$$\frac{dX}{dt} = \frac{F_{IN}}{V}.X_{IN} + \mu.X - \frac{F_{OUT}}{V}.X_{OUT} \quad (1)$$

where μ, X, F, and V are the specific growth rate, biomass concentration per unit culture volume, medium flow rate, and culture volume, respectively.

The balance of the concentration of total inorganic carbon (*TIC*) in the aqueous solution may be calculated as follow:

$$\frac{d[TIC]}{dt} = \frac{F_{IN}}{V}.[TIC]_{IN} - \frac{F_{OUT}}{V}.[TIC]_{OUT} - \mu.\frac{X}{Y_{X/S}} - m.X + k_L a.\left([CO_2^*] - [CO_2]\right) \quad (2)$$

where $Y_{X/S}$ is the mass conversion yield, m is the maintenance coefficient, and k_La is the gas-liquid transfer coefficient.

By definition, the carbon dioxide concentration in the medium fresh $[CO_2^*]$ is expressed as:

$$[CO_2^*] = PCO_2 / H \quad (3)$$

where PCO_2 is the partial pressure of carbon dioxide and H denotes the Henry's constant for Hemerick medium. Carbon concentration in the medium culture is given by:

$$[CO_2] = [TIC] \Big/ \left[1 + \frac{K_1}{[H^+]} + \frac{K_1.K_2}{[H^+]^2}\right] \quad (4)$$

where K_1 and K_2 are the kinetic constants and $[H^+]$ is the concentration of hydrogen ions in the culture media which is defined as:

$$[H^+] = 10^{-pH} \quad (5)$$

We have also integrated a light transfer model (Krystallidis, 1994) that describes the evolution of incident and outgoing light intensity. The amount of light intensity accessible per cell (E) and the outgoing light intensity (I_{OUT}) were calculated using (6) and (7).

$$E = \frac{(I_{IN} - I_{OUT}).Ar}{V.X} \quad (6)$$

$$I_{OUT} = C_1.I_{IN}.X^{C_2} \quad (7)$$

where Ar is the reactor area, C_1 and C_2 are the constants which depend on the reactor geometry.

The specific growth rate μ is predominantly influenced by the light intensity and the total inorganic carbon concentration. Substrates limitation effect has been incorporated in the rate equation. Therefore, the rate expression for biomass growth is given by:

$$\mu = \mu_{\max}.\left(\frac{E}{E_{opt}}\right).\exp\left(1 - \frac{E}{E_{opt}}\right).\left(\frac{TIC}{TIC_{opt}}\right).\exp\left(1 - \frac{TIC}{TIC_{opt}}\right) \quad (8)$$

where μ_{max}, E_{opt}, and TIC_{opt} are the parameters of the model and were identified from the data batch experiments.

The aim of this study is estimate the biomass and *TIC* concentrations, based on the measurement of *TIC*. This will be achieved thanks to an extended Kalman filtering.

4. EXTENDED KALMAN ESTIMATOR

The Kalman filter and is the most widely adopted state estimation technology for non-linear systems. This estimator is a recursive filter which has two steps: prediction step between observations in which the uncertainty in the estimates increases with time, and correction step which takes place when observations occur.

In the case of nonlinear system, Extended Kalman filter is based on process dynamic (9) and measurement equation (10). Both these models include noise terms that serve to account for unmodelled dynamics in the process equations and measurement errors in the observation equations, mostly assumed as Gaussian white noises.

$$\dot{x}(t) = \varphi[x(t)] + \omega(t), \quad x(t)\big|_{t=0} = x_0 \quad (9)$$

$$y(t) = h[x(t)] + \upsilon(t) \quad (10)$$

where $\varphi[*]$is the dynamic matrix and is function of the state to be estimated, $x(t)$ is the state vector with an initial value of x_0, $y(t)$ the measurement, $\omega(t)$ is the dynamic noise with the covariance matrix Q, $h[*]$ is the measurement matrix, and $\upsilon(t)$ is the measurement noise which has the covariance matrix R.

In the case of bioprocess systems, observations occur at discrete times (in general with large intervals). Thus, A continuous-discrete version of the EKF has been proposed.

For the discrete-time case, the one-step ahead prediction of the measurement $\hat{y}_{k|k-1}$ is computed as:

$$\hat{y}_{k|k-1} = y_{k-1}(t_k) = h\left[t_k, \hat{x}_{k|k-1}\right] \quad (11)$$

The Kalman filter gain is given by:

$$K_\varphi = P_{k|k-1}.C_k^T.R_{k|k-1}^{-1} \quad (12)$$

With $C_k = \left.\frac{\partial h[x(t)]}{\partial x(t)}\right|_{\hat{x}_{k|k-1}}$.

The filtered state $\hat{x}_{k|k}$ is calculated on the basis of available measurements and its covariance matrix $P_{k|k}$ is computed by:

$$\hat{x}_{k|k} = \hat{x}_{k|k-1} + K_\varphi.\left(y_k - \hat{y}_{k|k-1}\right) \quad (13)$$

$$P_{k|k} = P_{k|k-1} - K.R_k.K_\varphi^T \quad (14)$$

In the continuous-discrete time case, the one-step ahead propagation of the state estimate $\hat{x}_{k+1|k}$, and its covariance $P_{k+1|k}$ are computed as the solution to the system of differential equations:

$$\frac{d\hat{x}_k(t)}{dt} = \varphi(t, \hat{x}_k(t)) \quad (15)$$

$$\frac{dP_k(t)}{dt} = A(t).P_k(t) + P_k(t).A(t)^T \quad (16)$$

where $A(t) = \left.\frac{\partial\varphi[x(t)]}{\partial x(t)}\right|_{\hat{x}_k(t)}$, and with the initial conditions

$$\hat{x}_k(t_k) = \hat{x}_{k|k} \quad \text{and} \quad P_k(t_k) = P_{k|k} \quad (17)$$

Equations (15) and (16) are integrated thanks to an Euler integration scheme. Observability of the model is checked after system linearization. Covariance matrices Q and R are taken constant, and are tuning parameters depending on the wanted estimator performance.

5. RESULTS AND DISCUSSION

In order to illustrate the performance of the proposed software sensor, the estimator is applied in the cases of a batch and continuous modes. Its efficiency is shown thanks to simulation and experimental results.

5.1 Model identification

First, the model parameters are identified thanks to experimental data. These data were obtained from an experimental run on the real laboratory-scale photobioreactor in a bath mode. The effects of the incident light intensity and percentage of carbon dioxide in the air flow were studied separately. Figure 2 shows the typical growth of *P. purpureum* in Hemerick medium.

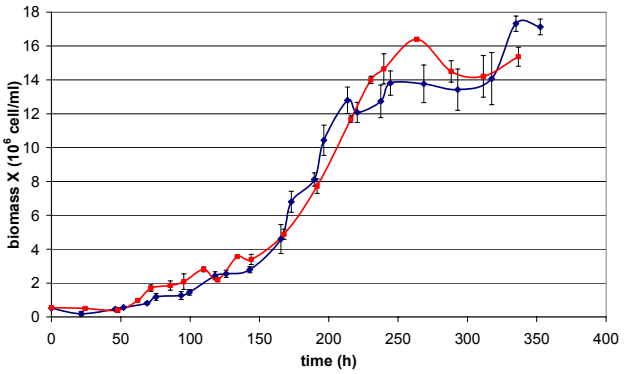

Fig. 2. Typical growth of *P.purpureum* in a photobioreactor with Hemerick medium in batch mode.

Parameter values for the microalgae growth are listed in Table 1. These values were obtained by fitting the experimental data acquired along the exponential phase of batch cultivation (Fig. 2) of *P. purpureum* under the different conditions in the same photobioreactor from a set of four experiments. The correlation coefficient was calculated to be 0.985.

Table 1. Model parameter values calculated for *Porphyridium purpureum* at 25 ºC

Parameters	Units	Value
μ_{max}	h^{-1}	0.03
E_{opt}	$\mu E\ s^{-1}10^9 cell^{-1}$	1.20
TIC_{opt}	mmole l^{-1}	12.93
k_La	h^{-1}	41.40
C_1		0.65
C_2		-1.02

The model parameters for the TIC dynamic have been obtained by (Baquerisse, 1999) and are given in Table 2.

Table 2. Model parameters of TIC dynamic

Parameters	Units	Value
K_1		$1.02.\ 10^{-6}$
K_2		$8.32.\ 10^{-10}$
m	h^{-1}	0.004
$Y_{X/S}$	10^9 cell per mole *TIC*	198.1
H	atm.l.mole^{-1}	34.03

The value of k_La has been obtained by the dynamical method (Leveau and Bouix, 1988) with a dissolved oxygen sensor (Ingold type 170). Because in a microalgal photobioreactor, the absorption rate was shown to be independent of the chemical reaction taking place in the liquid phase, the $k_La(CO_2)$ could be directly related to the $k_La(O_2)$ by a single factor obtained from (18) which take into account the difference in aqueous diffusivities of the two gases (Camacho Rubio *et al*. 1999).

$$k_La(CO_2) = \sqrt{\frac{D_{CO_2}}{D_{O_2}}}k_La(O_2) \quad (18)$$

where D_{CO_2} and D_{O_2} are the diffusion coefficients or diffusivities in Hemerick medium of carbon dioxide and oxygen respectively.

5.2 Batch mode

First, the model simulation was performed in batch mode under the following conditions:

$X_{(0)} = 2.44\ 10^9$ cells l^{-1}, $[TIC]_{(0)} = 2.55.\ 10^{-3}$ mole l^{-1},

$F_{IN} = F_{OUT} = 0$, $[TIC]_{IN} = 0$, and $X_{IN} = 0$.

The measurement of total inorganic carbon concentration is corrupted with a noisy signal with a standard deviation equal to 3 and 5% of the corrupted quantity. The simulation results given in Fig. 3 show that the estimated biomass concentration coincides with the true values issued from the process simulation.

The measurement of total inorganic carbon concentration is supposed to be available at sampling times equally spaced (Ts = 0.5 h). These measurements are obtained by simulating the discrete model of the process. The experiences show the importance of the sampling time Ts and the noise level utilized. Many numerical simulations have been carried out, showing a good performance of the estimator and a very satisfactory noise rejection.

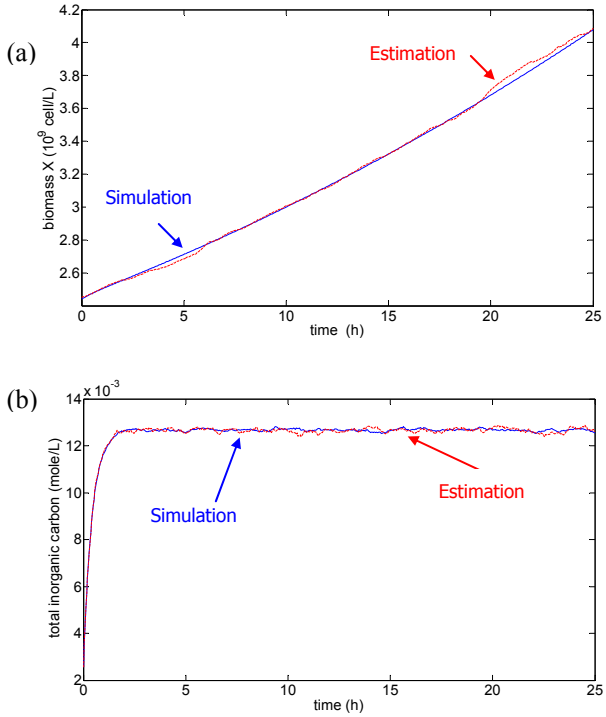

Fig. 3. Simulation studies: estimation of cell concentration based on noisy data of total inorganic carbon.

In order to investigate the practical interest of the approach, a number of experiments were carried out to implement and verify the performance of the software sensor algorithm. The objective of this experimental work was to study the performance of extended Kalman filter for the cultures with changes in operating conditions of the photobioreactor.

The choice of covariance matrix is important for the estimation. Here, the system noise covariance matrix Q and the covariance matrix of the initial estimation error P_0 were chosen in a diagonal form according to the usual assumption that the individual components in the system noise vector are uncorrelated. P_0, Q, and R were determined empirically. The covariance matrix R was assumed to be characterized by a Gaussian noise with a standard deviation of 5%.

The performance of extended Kalman filter was examined under different growth conditions: low and high light intensity, excess of CO_2 in the air injection. Some experimental results are presented in Fig. 4.

It can be seen that the EKF has good performance in the case of a batch culture.

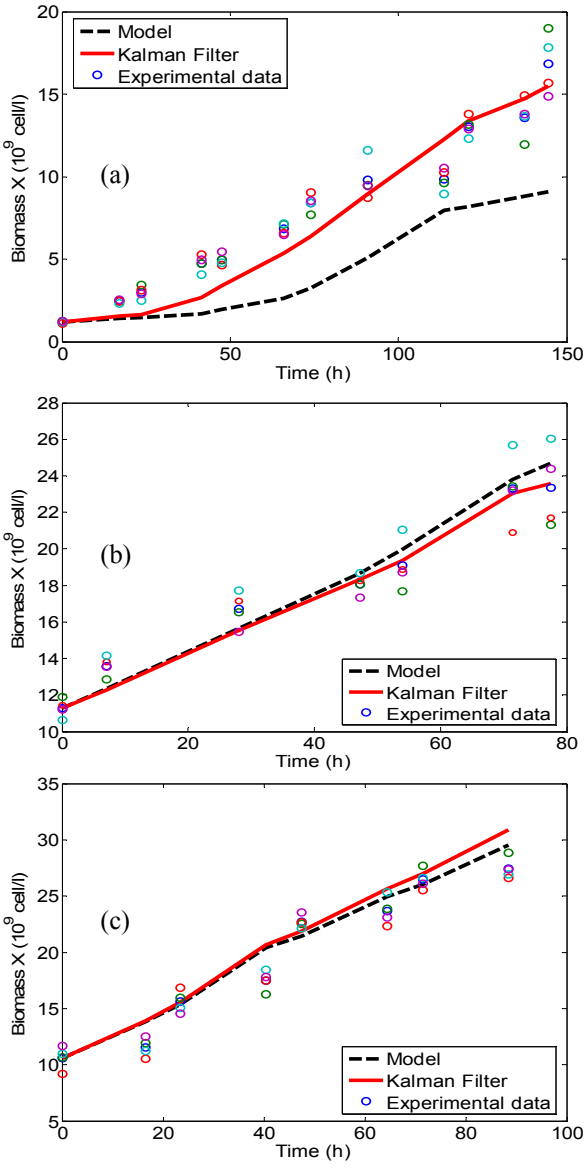

Fig. 4. Experimental studies: cell concentration estimation in batch culture with (a) 5% CO_2, (b) low light intensity, (c) high light intensity.

5.3 Continuous cultures

In the sequel, the performance of this estimator was examined in continuous mode ($F_{IN} = F_{OUT} \neq 0$). The feed-rate profile is given in Fig. 5(a). The EKF was tested in simulation and on experimental conditions.

Figure 5 shows the performances of extended Kalman filter for the continuous culture which was performed under the following conditions: $X_{(0)}$ = 2 10^9 cells l^{-1} , $[TIC]_{(0)}$ = 4.51. 10^{-3} mole l^{-1}. A slight error in the guess of the initial conditions of the filter was considered: $X_{(0)}$ = 2.25 10^9 cells.l^{-1}. As illustrated by Fig. 5, the proposed estimator has good performances in the case of continuous cultures of microalgae.

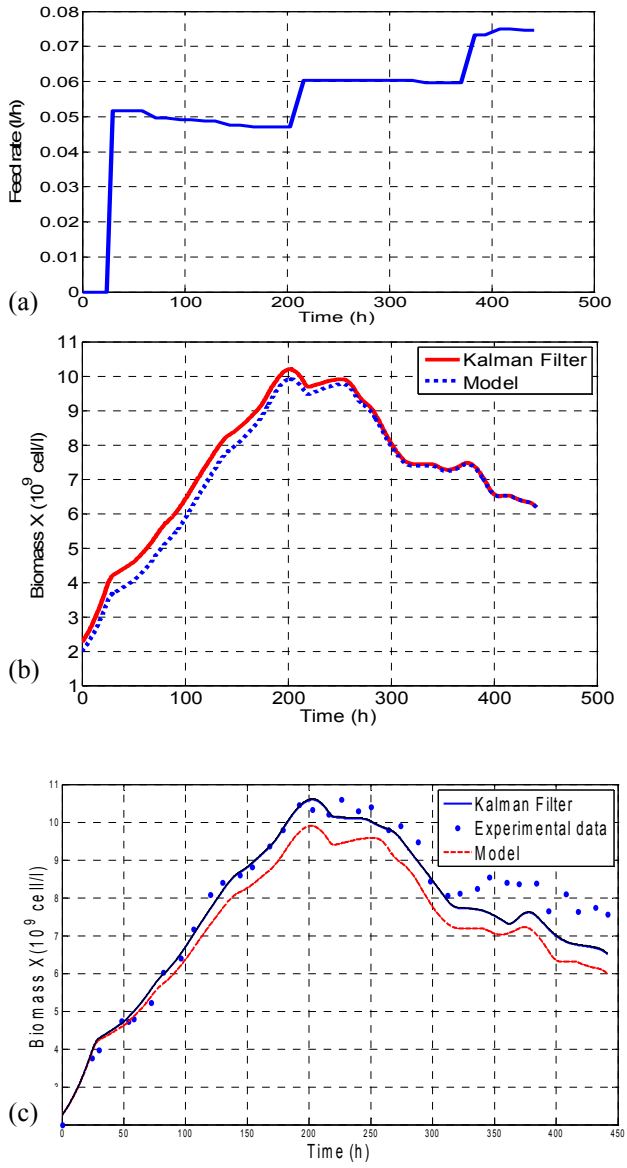

Fig. 5. Estimator performance for continuous culture: (a) culture medium feeding profile, (b) results in simulation, and (c) experimental results.

6. CONCLUSIONS

The proposed software sensor is very successful in estimating the biomass concentration. It was designed in continuous as well as in discrete time and the convergence of each of these versions is clearly established. Numerical simulations and experimental data show a satisfactory performance of the proposed methodology. The developed state estimator offers a cost-effective alternative and an easier estimation of biomass concentration in microalgal photobioreactors. Further studies will consider the validation on a full-scale one. The development of a combined parameter and state estimator will be also investigated.

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