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# INPUT AND STATES ESTIMATIONS OF BIOHYDROGEN PRODUCTION

**Cesar-Arturo Aceves-Lara, Eric Latrille,
Jean-Philippe Steyer**

*INRA, UR050, Laboratoire de Biotechnologie de l'Environnement,
Avenue des Etangs, Narbonne, F-11100, France.*

Abstract: This paper addresses the problem of estimating simultaneously the states and the input concentrations of an acidogenic process used for biohydrogen production. This process is described by a nonlinear model. The input and states estimations were solved using a state transformation and an asymptotic observer. Finally, the method was tested with experimental data and its interest was demonstrated. 

Keywords: Biohydrogen production, input estimation, dynamic modeling, asymptotic observer.

## 1 INTRODUCTION

One of the great challenges of the new century is to obtain a new source of renewable energy, capable of replacing fossil fuels. Hydrogen is a promising hope because of its great calorific value, *i.e.,* 122 $kJ.g^{-1}$. Unfortunately, even though biological processes have shown strong potentialities for sustainable $H_2$ production, hydrogen is presently mainly produced from the reforming of fossil materials (90% of world production, 45 billion tons) with a high level of pollution generated, *i.e.,* 10 tons $CO_2.ton^{-1}$ (Maddy *et al.* 2003).

Hydrogen can be produced by micro-organisms using two enzymes (*i.e.,* hydrogenase and nitrogenase) active in their metabolic pathways (Asada and Miyake 1999). However, up to now, the microbial production of hydrogen has still a low efficiency for substrate conversion, ranging between 16 and 24% (Woodward *et al.* 2000).

The involved processes can be classified into three main classes (Das and Veziroglu 2001): biophotolysis, photodecomposition and acidogenic fermentation of carbohydrates (acidogenesis). The two first processes are photobiological (*i.e.,* light is needed) while the acidogenesis step presents several advantages, such as a production yield higher than those obtained with photobiological processes and the capacity of working all day long (*i.e.,* even with no light) (Das and Veziroglu 2001).

In the acidogenic process, several perturbations can be present affecting the operating conditions and it is thus very important to have an efficient monitoring strategy. Unfortunately, only few measurements (*i.e.,* the biogas flowrate and biogas composition) are usually available online limiting the control possibilities (Hawkes *et al.* 2002). An interesting alternative is the use of observers for combined input (Theilliol *et al.* 2003) and states estimation (Bastin and Dochain 1990; Dochain 2003). However, compared with state estimation, less research has been carried out on simultaneous estimation of the state of a dynamic system and its input (Ha and Trinh 2004; Floquet and Barbot 2006; Nordberg and Gustafsson 2006; Pillonetto and Saccomani inpress).

The present study has, as main objective, the on-line estimation of the influent concentration and of the output states of a bioreactor used for the biohydrogen production from wastewater. Practical evaluation of the estimation scheme will be also performed from experimental data.

## 2. MATERIALS AND METHODS

### *2.1 Medium*

Molasses resulting from the industrial sugarbeet production were used as feeding substrate. They were diluted to concentrations ranging from 7 to 20 $g.L^{-1}$ by adding a nutritional medium rich in minerals and containing (in $mg.L^{-1}$): MgCl2.6H20, 150; NaCl, 1000; ZnCl2, 10; FeSO4.7H2O, 25; NH4Cl, 1000; CoCl2.5H2O, 5; CuCl2, 5; CaCl2.2H2O, 10; K2HPO4, 150; NiCl2.6H2O, 20; MnCl2.6H2O, 20.

### *2.2 Reactor Design*

Experiments were carried out in a continuous stirred tank reactor (Setric) of 2 L with a useful volume of

1200 ml. The reactor was equipped with a stirring system made of a Rushton turbine (on the top) and a marine propeller (on the bottom) in order to ensure a homogeneous mixture and an important agitation. A revolution counter was connected to access to the measurement of the stirring velocity. Stirring velocity was maintained at 300 rpm. Two additional sensors were connected to the reactor for measuring the redox potential (Pt 4805 - DXK-S8/225, Mettler Toledo) and pH (4010/120/Pt100, Mettler Toledo). The pH-meter (2300, Ingold) and the transmitter for redox potential (Mettler Toledo) were connected to a computer for on-line data acquisition. The pH was controlled at 5.5 by adding NaOH (2 M) with a peristaltic pump. Temperature in the reactor was also controlled using a platinum probe Pt100 and a heating electric resistance. The temperature of the culture media was maintained at 37°C. The quantity of NaOH added and the temperature were also recorded on-line.

*2.3 Off-line measurements*

Gas composition was analyzed using a gas chromatograph (GC-14A, Shimadzu, colon CTR I, Alltech). Operating conditions were: carrier gas, nitrogen; pressure of 335 KPa; temperature of the injector, 250°C; temperature of the detector, 275°C; temperature gradient for heating, 80 to 120 °C with levels of 10°C per minute.
Composition of volatile fatty acids (VFA) in the liquid phase, *i.e.,* acetic (Ace), propionic (Pro), butyric (Bu) and valeric acids, were determined by liquid injection into a gas chromatograph (GC 8000, Fisons Instruments). The residual absence or presence of sugar and other bioproducts such as organic acids (lactate), ethanol or acetone was confirmed by a HPLC (colon HPx 87H, BioRad). Operating conditions were: temperature of column, 35°C; temperature of refratometer, 40°C.
The biomass was determined through the volatile suspended solid (VSS) concentration measured according to the standard method (APHA, 1995).

*2.4 Inoculum preparation*

The inoculum was prepared with one liter of sludge taken from a pilot-scale fixed bed digester of $1m^3$ working volume and used for several years for the anaerobic treatment of wine distillery wastewater. The sludge was centrifuged for 15 minutes at 17700 g. The reactor was stripped with nitrogen for 15 minutes before continuous feeding during four days with a retention time of 6 h and a pH equal to 5.5. Finally, it was boiled at 98°C during 30 min.

*2.5 Experimental conditions*

The operating conditions applied during the experiments are showed in Table 1 where Sin and HRT represent respectively the feeding concentration in the influent and the hydraulic retention time (HRT=V/Qin, V being the working volume in the reactor).

Table1. Operating conditions

| Time of changes (h) | Sin ($g.L^{-1}$) | HRT (h) |
|---|---|---|
| 0 | 9.35 | 8.4 |
| 97.0008 | 9.35 | 6.48 |
| 244.9992 | 14.02 | 6.48 |
| 289.0008 | 14.49 | 4.8 |
| 343.6008 | 7.01 | 6.48 |
| 409.5 | 18.69 | 6.96 |

## 3. MATHEMATICAL MODEL

*3.1 Structure of Biochemical Reactions*

A general mass balance model of a continuous stirred tank reactor fed with glucose, (*i.e.* molasses contain only sugars: sucrose, fructose and glucose) and producing acetate, propionate, butyrate, biomass, carbon inorganic (*i.e., $CO_2$, $HCO^-_3$*, etc.) and hydrogen from sucrose uptake performed by a single micro-organism can be written according to equation (1):

$$\frac{d}{dt}\begin{bmatrix} Glu \\ Ace \\ Pro \\ Bu \\ X \\ CO_2 \\ H_2 \end{bmatrix} = K \cdot r - D\begin{bmatrix} Glu - Glu_{in} \\ Ace \\ Pro \\ Bu \\ X \\ CO_2 \\ H_2 \end{bmatrix} - \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ q_{CO2,gas} \\ q_{H2,gas} \end{bmatrix} \quad (1)$$

where *Glu*, *Ace*, *Pro*, *Bu*, *X*, $CO_2$ and $H_2$ represent, respectively, the concentrations in $g.L^{-1}$ of glucose, acetate, propionate, butyrate, biomass, carbon dioxide ($mol.L^{-1}$) and dissolved hydrogen in the liquid phase. The vector *r* describes the kinetics of the involved biological reactions (in $g.L^{-1}.h^{-1}$), *D* is the dilution rate ($h^{-1}$) and $q_{C02}$ and $q_{H2}$ the gas flow rates of carbon dioxide and hydrogen expressed in $g.d^{-1}.L^{-1}$. *K* represents the matrix of pseudo-stoichiometric coefficients (Bernard and Bastin 2004). The matrix of the pseudo-stoichiometric coefficients is of dimension 7x2 and its structure is determined as (Aceves-Lara *et al.* 2006):

$$\begin{bmatrix} K_{11} & K_{12} \\ 0 & K_{22} \\ K_{31} & K_{32} \\ K_{41} & K_{42} \\ K_{51} & K_{52} \\ K_{61} & K_{62} \\ K_{71} & 0 \end{bmatrix} = \begin{bmatrix} -1 & -1 \\ 0 & 0.2421 \\ 0 & 0.0047 \\ 0.3345 & 0.1984 \\ 0.2510 & 0 \\ 0.0108 & 0.0037 \\ 0.0285 & 0 \end{bmatrix}$$

Finally, the vector *r* is composed of the specific growth rate (assumed to be represented by Monod mathematical expressions) multipled by the biomass concentration in the reactor.

$$r = \begin{bmatrix} \frac{\rho_{\max,1} Glu}{K_{Glu1} + Glu} \\ \frac{\rho_{\max,2} Glu}{K_{Glu2} + Glu} \end{bmatrix} X$$

The kinetic coefficients are show in Table 2.

Table2. kinetic coefficients

| | $r_1$ | $R_2$ |
|---|---|---|
| $\rho_{\max}$ ($h^{-1}$) | 1.8296 | 1.5470 |
| $K_{Glu}$ ($g.L^{-1}$) | 0.18 | 0.22 |

*3.2 Physicochemical Processes*

The physicochemical processes (*i.e.,* the exchange of ions and the gas-liquid transfer) are important aspects that are considered in the present model. They are represented by a system of differential equations.

*3.2.1 Acid-base reactions*

The acid-base equilibrium reactions are modeled by the following differential equations:

$$\frac{dAce-}{dt} = -\rho_{A/B\,Ace-} \quad (2a)$$

$$\frac{dPro-}{dt} = -\rho_{A/B\,Pro-} \quad (2b)$$

$$\frac{dBu-}{dt} = -\rho_{A/B\,Bu-} \quad (2c)$$

$$\frac{dHCO_3^-}{dt} = -\rho_{A/B\,CO2} \quad (2d)$$

where

$$\rho_{A/B\,Ace-} = k_{A/B\,Ace}(Ace^- \cdot H^+ - K_{a,Ace} \cdot HAce)$$

$$\rho_{A/B\,Pro-} = k_{A/B\,Pro}(Pro^- \cdot H^+ - K_{a,Pro} \cdot HPro)$$

$$\rho_{A/B\,Bu-} = k_{A/B\,Bu}(Bu^- \cdot H^+ - K_{a,Bu} \cdot HBu)$$

$$\rho_{A/B\,CO2} = k_{A/B\,CO2}(HCO_3^- \cdot H^+ - K_{a,CO2} \cdot CO_2)$$

*3.2.3 Gas phase*

The differential equations for the gas phase with a constant gas volume are:

$$\frac{dCO_{2,gas}}{dt} = -\frac{CO_{2,gas} q_{gas}}{V_{gas}} + \rho_{T,CO2} \frac{V}{V_{gas}} \quad (3a)$$

$$\frac{dH_{2,gas}}{dt} = -\frac{H_{2,gas} q_{gas}}{V_{gas}} + \rho_{T,H2} \frac{V}{V_{gas}} \quad (3b)$$

where

$$\rho_{T,H2} = k_L a_{H2}(H_2 - 2K_{H,H2} p_{gas,H2})$$

$$\rho_{T,CO2} = k_L a_{CO2}(CO_2 - K_{H,CO2} p_{gas,CO2})$$

$$q_{gas,T} = \frac{RT}{P_{atm} - p_{vap,H2O}} V(\frac{\rho_{T,H2}}{2} + \rho_{T,CO2})$$

$$p_{gas,H_2} = \frac{H_{2,gas} \mathrm{R} * \mathrm{T}_{\mathrm{react}}}{2}$$

$$p_{gas,CO_2} = CO_{2,gas} \mathrm{R} * \mathrm{T}_{\mathrm{react}}$$

The term $V/V_{gas}$ is required since the gas transfer kinetic rate is liquid volume specific. The pressure of each gas component can be calculated using the ideal gas law for the two gases (in bar, factors in denominators are $g.L^{-1}$ equivalent for $H_2$ and $mol.L^{-1}$ for $CO_2$). *R* is the ideal gas constant ($bar.K^{-1}.M^{-1}$) and $T_{react}$ is the reactor temperature (K).

This model has some differences with respect to acidogenesis of carbohydrates in structured models such as ADM1 (Batstone *et al.* 2002). Only one biomass is indeed here assumed for the two biological reactions while in ADM1, a specific biomass is responsible of each reaction. Moreover, the specific growth rate is assumed not to be affected by pH nor by inorganic nitrogen limitation. Finally, valeric acid is not considered since it was measured as very close to 0 during the course of the performed experiments.

Finally the physicochemical constants are show in Table 3.

Table3. Physicochemical constants in the model

| Constant | Value |
|---|---|
| $K_{a,Ace}$ | $1.51\times10^{-8}$ M |
| $K_{a,Bu}$ | $1.74\times10^{-5}$ M |
| $K_{a,CO2}$ | $4.94\times10^{-7}$ M |
| $K_{a,Pro}$ | $1.32\times10^{-5}$ M |
| $K_{H,CO2}$ | 0.0271 $M.h^{-1}$ |
| $K_{H,H2}$ | $7.38\times10^{-4}$ $M.h^{-1}$ |
| $k_L a_{H2}$ | 127.7 $h^{-1}$ |
| $k_L a_{CO2}$ | 83.3 $h^{-1}$ |
| $P_{atm}$ | 1.013 bar |
| R | $8.314\times10^{-2}$ $bar.K^{-1}.M^{-1}$ |
| $p_{vap,H2O}$ | 0.0557 bar |
| $T_{react}$ | 310 K |
| $K_{A/B,i}$ | $1\times10^{8}$ $h^{-1}$ |

## 4. THE NON-LINEAR ASYMPTOTIC OBSERVER

In order to estimate online the influent concentration together with the states in the output of the bioreactor, a state transformation based on the methodology described by Bastin and Dochain (Bastin and Dochain 1990; Dochain 2003) will be used. To this end, the model needs to fulfill all the structural conditions for the observer design. This is obtained by reducing the model to its biochemical structure and by assuming that the gas phase is close to steady state. Then, the dissolved gas in the liquid phase can be calculated from the measurements in the gas phase:

$$p_{gas,H2} = \frac{\rho_{T,H2}}{\rho_{T,CO2} + \rho_{T,H2}}(P_{atm} - p_{vap,H2O}) \quad (4a)$$

$$p_{gas,CO2} = \frac{\rho_{T,CO2}}{\rho_{T,CO2} + \rho_{T,H2}}(P_{atm} - p_{vap,H2O}) \quad (4b)$$

$$H_2 = \rho_{T,H2} / k_L a_{H2} + 2K_{H,H2} p_{gas,H2} \quad (4c)$$

$$CO_2 = \rho_{T,CO2} / k_L a_{CO2} + 2K_{H,CO2} p_{gas,CO2} \quad (4d)$$

Assuming that the dilution rate, the gas flow rate and the gas composition are measured online, the following asymptotic observer can be proposed:

$$Z = A_o E_a + E_b \quad (5)$$

where $E_a = \begin{bmatrix} CO_2 \\ H_2 \end{bmatrix}$ $E_b = \begin{bmatrix} Glu \\ Ace \\ Pro \\ Bu \\ X \end{bmatrix}$

and $Ao*Ka+Kb=0$.

In such a case, we have:

$$K_a = \begin{bmatrix} 0.0108 & 0.0037 \\ 0.0285 & 0 \end{bmatrix}$$

and $K_b = \begin{bmatrix} -1 & -1 \\ 0 & 0.2421 \\ 0 & 0.0047 \\ 0.3345 & 0.1984 \\ 0.2510 & 0 \end{bmatrix}$

This leads to:

$$\frac{dGlu}{dt} = -A_o \frac{dE_a}{dt} - A_o(DE_a + Q_a) + DGlu_{in} - DGlu \quad (6)$$

where $Q_a = \begin{bmatrix} qCO_2 \\ qH_2 \end{bmatrix}$

In addition, the terms $Glu$ and $\frac{dGlu}{dt}$ can be neglected compared to the others terms (they are indeed close to zero in most of the experiments) and we obtain:

$$Glu_{in} = \left( A_o \frac{dE_a}{dt} + A_o(DE_a + Q_a) \right) / D$$

Finally, the asymptotic observer for the acetate, propionate, butyrate and biomass is:

$$\frac{dZ}{dt} = -DZ - A_o(Q_a) \quad (7)$$

with $E_b = Z - A_o E_a$

## 5. RESULTS

Figure 1 shows the hydraulic retention time applied to the process during the course of the experiments (fig. 1a) together with the measured gas flow of hydrogen and carbon dioxide (fig. 1b). These measurements were used by the asymptotic observer and the obtained results are shown in Figures 2 to 6.

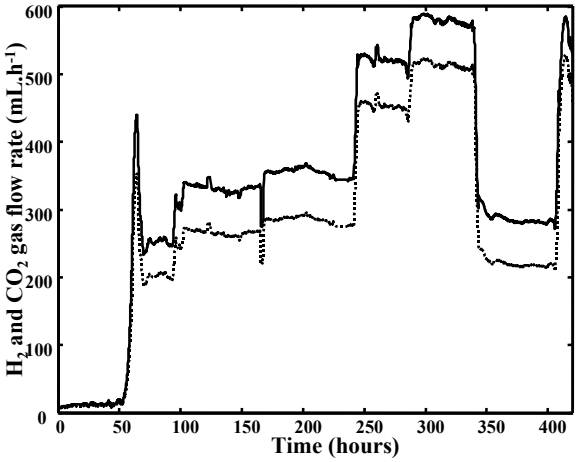


Fig. 1b. Measurements of the $H_2$.(– ) and $CO_2$ (- -) flow rates

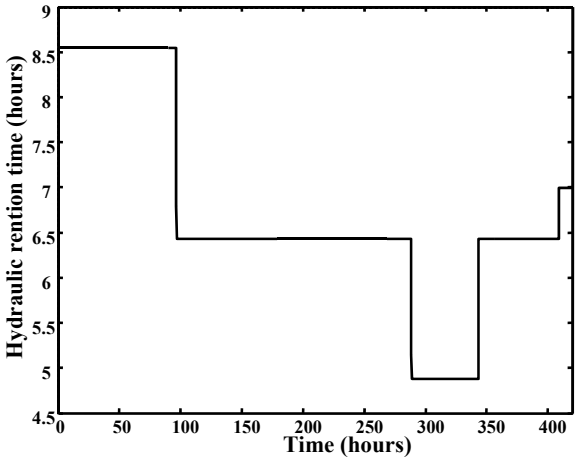


Fig. 1a. Hydraulic retention time applied during the course of the experiments

Figure 2 presents the applied input glucose concentration together with its estimation. Preparation of the input was manually performed and thus, the feeding concentration can be assumed to be very stable with sharp changes made on purpose to evaluate the observer performances. One can notice that the unknown input estimation is quite close to the expected values with only a small offset in steady

states. This offset is primarily due to the neglecting of the terms *Glu* and *dGlu/dt* in the observer design.
In Figures 3 to 6, the output liquid concentrations of the main process states are presented. Again, it can be seen that the proposed observer demonstrates very good performances with online estimations close to the offline manual measurements. It can be in particular noticed that the steady states values of these variables are very nicely determined but also that the observer allows us to precisely characterize the dynamic transitions between two steady states.

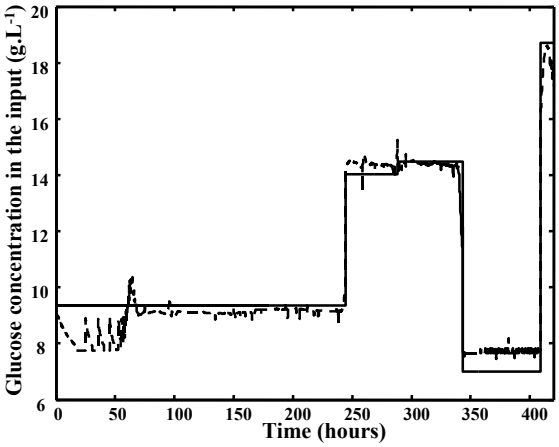


Fig. 2. Applied (– ) and estimated (- -) glucose concentration in the liquid inflow

This information is of the outmost importance to optimize the biohydrogen production with very few efforts spent on manual monitoring of the overall process.

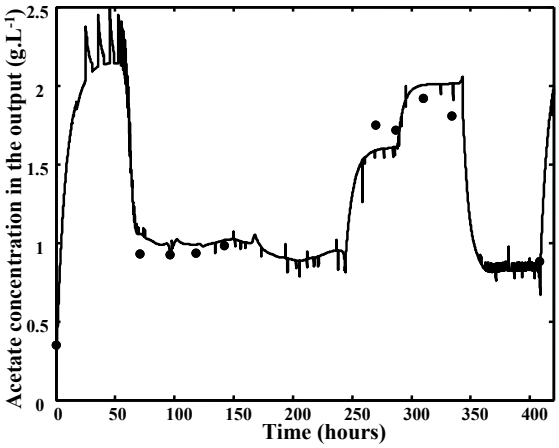


Fig. 3. Dynamic estimation (– ).and (•) offline measurements of the acetic acid in the output of the reactor.

There is another important aspect of the present study. Indeed, as previously said, the assumption of negligible glucose concentration and dynamics in the output of the reactor can be regarded as a strong assumption. However, as highlighted by our results, this assumption is valid when the process is running correctly and it leads to a small offset in steady state which does not affect the final process optimization.

But if a disturbance suddenly occurs on the process (*e.g.,* presence of a toxicant in the feeding line, problems of the temperature or pH regulation, etc.), this assumption will not be valid anymore and the present observer scheme will lead to poor results.

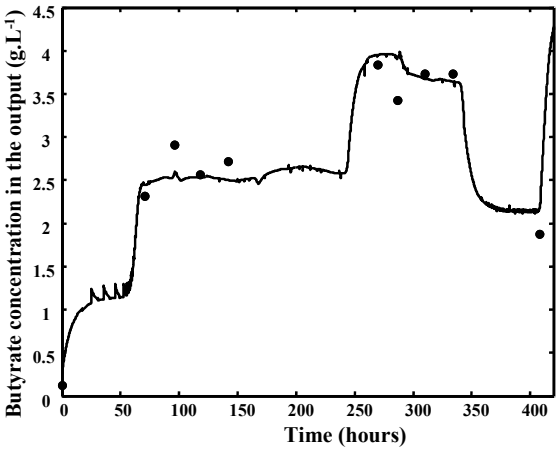


Fig. 4. Dynamic estimation (– ).and (•) offline measurements of the butyric acid in the output of the reactor.

However, it is important to emphasize that these disturbances – that can occur in many different locations in the process – will be easily detected by comparing the estimation of input glucose concentration with offline measurements in the feeding. The benefits of using the present observer is thus twofold: closed loop control of the bioreactor for optimization of hydrogen production and fault detection to guarantee long term operation of the process. These aspects are presently under consideration in our laboratory.

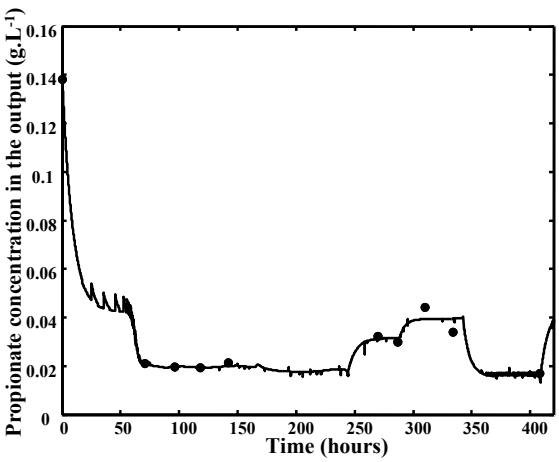


Fig. 5. Dynamic estimation (– ).and (•) offline measurements of the propionic acid in the output of the reactor.

## 6. CONCLUSION

This paper presented an approach of combined estimation of the output states and of the unknown

input concentration for an acidogenic process used for biohydrogen production.

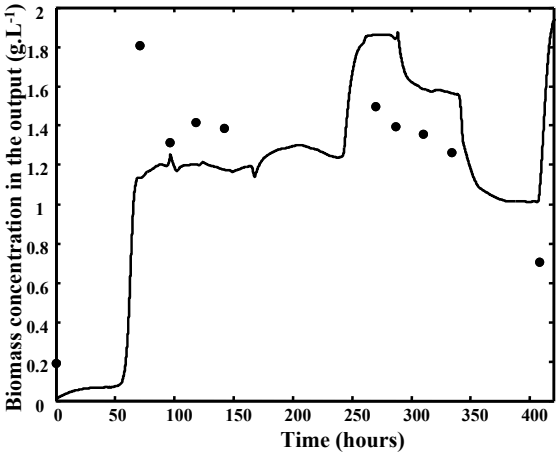


Fig. 6. Dynamic estimation (– ).and (•) offline measurements of the biomass concentration in the output of the reactor

The design and performance of the proposed method are illustrated using experimental data. This method was demonstrated to be able to reproduce accurately the dynamics and steady states of the influent and effluent concentrations. Many benefits can be withdrawn from this approach such as closed loop optimization and fault detection.

## 7. ACKNOWLEDGEMENTS

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