ADAPTIVE SMOOTH OBSERVER DESIGN FOR STATE ESTIMATION IN *Desulfovibrio alaskensis* 6SR CULTURES

DISEÑO DE UN OBSERVADOR SUAVE ADAPTIVO PARA ESTIMACION DE ESTADOS EN CULTIVOS DE *Desulfovibrio alaskensis* 6SR

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Abstract

In this work the dynamics of a sulfate reducing bacteria is predicted by estimating the biomass concentration and sulfide production using only sulfate (substrate) concentration measurements. The estimation process is developed on batch, fed-batch and continuous cultures of *Desulfovibrio alaskensis* 6SR, where a mathematical dynamic model of the bioreactor is presented and tuned with experimental data. The design of the adaptive smooth state observer takes into account the model's system structure and an adaptive gain of the output feedback which contain a hyperbolic tangent of the estimation error, an theoretical frame is provide in order to show the convergence characteristics of the proposed method. The results of the proposed estimation methodology are generated via numerical simulation in order to show its performance.

Keywords: bounded error observer, hyperbolic tangent feedback, adaptive observer gain, bioreactor model, *Desulfovibrio alaskensis* 6SR.

Resumen

En este trabajo la dinámica de una bacteria sulfato reductora es predicha vía estimación de la concentración de la biomasa y la producción de sulfuro usando únicamente mediciones de la concentración de sulfato (sustrato). El proceso de estimación es aplicado en cultivos por lotes, lote alimentado y continuo de *Desulfovibrio alaskensis* 6SR, donde el modelo dinámico del biorreactor es presentado y sintonizado con datos experimentales. El diseño del observador de estados adaptable toma en consideración la estructura del modelo del sistema y una retroalimentación con una ganancia adaptable que contiene una tangente hiperbólica del error de estimación, un marco teórico es propuesto con el fin de mostrar algunas de las característica de la convergencia del observador propuesto. Los resultados de la estrategia de estimación propuesta son generados por medio de simulaciones numéricas con el fin de mostrar su desempeño.

Palabras clave: observador con error acotado, retroalimentación con tangente hiperbólica, ganancia del observador adaptable, modelo del biorreactor, *Desulfovibrio alaskensis* 6SR.

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1   Introduction

In standard bioprocess operation, on-line measurements are often limited to basic variables such as temperature, pH, and dissolved oxygen O_2. Measurements of component concentrations, i.e. essential substrates, biomass and products of interest are obtained for off-line laboratory analysis at different discrete times. In recent years, on-line probes for measuring component concentrations, e.g. biomass probes based on capacitance measurements, have been developed, but their use is still very limited due to high costs. In particular, in sulfate-reducing bioreactors the biomass growth and products are difficult of measure on-line, given the anaerobic process conditions. Different methods for detection and enumeration of sulfate reducing bacteria (SRB) in natural and industrial environments have been developed, they have been grouped in: (i) direct detection methods and (ii) culture methods (APHA, 1989). The detection direct involved the using antibodies raised against SRB (Daly *et al.*, 2000), and the uses of molecular biology tools as 16S rRNA, both techniques may be using in situ but required of a bigger knowledge and in some case is not possible by the nature of the considered sample (Vester and Ingvorsen, 1998). Culture methods for enumeration of SRB requires of strict anaerobic conditions and special culture medium, experience of handling of these bacterial, the incubation times are sometimes very large, etc (Neria-González *et al.*, 2006).

In this context, the design of "software sensors" based on state estimation techniques takes on particular importance (Bastin and Dochain, 1990). Software sensors allow the online reconstruction of non-measured variables (i.e. component concentrations) based on a process model and some available measurements (from "hardware sensors" or from on-line sampling).

From kinetic studies point of view, is important to determine the kinetic parameters and especially of kinetic rates inside bioreactor (Soroush, 1997). The estimates of these rates are used for advanced control strategies (Aguilar-López, 2003). The procedure to estimate the kinetic rates based on the *adaptive systems theory*, consist of the estimation of unmeasured state with asymptotic observers (software sensor); later, the measurements (hardware sensor) and the estimates of the state variables (software

estimator) are used for on-line estimation (Kazantzis and Kravaris, 1998; Aguilar-López *et al.*, 2010; Espinoza-Salgado *et al.*, 2008). This method is useful, but in some cases, when many reactions are involved, the implementation requires the calibration of too many parameters. A relative modern approach (Marin, 2002; Ohsumi *et al.*, 2002) is the distribution based on *identification method*. In this approach the set of nonlinear differential equations that describe the state evolution is mapped into a set of linear algebraic equations respect to the model parameters.

The design of a stable and convergent state estimator appropriates for a particular bioprocesses is a complex task, and an adequate response is only obtained studying each bioprocess (Aguilar *et al.*, 2004). For example, sliding mode control can be used to design an observer that brings one estimated state's error to zero in finite time even in the presence of measurement error (Selişteanu, *et al.*, 2007; Hong, *et al.*, 2002); the other states have error that behaves similarly to the error in a Luenberger observer after peaking has subsided. Sliding mode observers also have attractive noise resilience properties that are similar to a Kalman filter. As discussed for the linear case above, the peaking phenomenon present in Luenberger observers justifies the use of a sliding mode observer. The sliding mode observer uses non-linear high-gain feedback to drive estimated states to a hypersurface where there is no difference between the estimated output and the measured output (Levant, 2001). The non-linear gain used in the observer is typically implemented with a scaled switching function, like the signum (i.e., sign) of the estimated-measured output error. Hence, due to this high-gain feedback, the vector field of the observer has a crease in it so that observer trajectories *slide along* a curve where the estimated output matches the measured output exactly. So, if the system is observable from its output, the observer states will all be driven to the actual system states. Additionally, by using the sign of the error to drive the sliding mode observer, the observer trajectories become insensitive to many forms of noise (Boiko and Fridman, 2005). Hence, some sliding mode observers have attractive properties similar to the Kalman filter but with simpler implementation. However, as it is well known, this switching happens at any

instant the state trajectories cross the switching hyper-plane, this leads to the named *chattering phenomenon* caused by the discontinuity of the input injection, undesirable in most applications (Davila, *et al.*, 2006). In order to avoid the named *chattering phenomenon*, several methodologies have been proposed, as the high order sliding-mode observers, which provides some smoothness to the estimation methodology diminishing the corresponding chattering, however to avoid the presence of the sign function (i.e., discontinuous function) a continuous one have been proposed (Bertoni and Punta, 2000).

From the above in this work it is proposed a class of adaptive smooth bounded observer, where the feedback term is related with the hyperbolic tangent of the estimation error, coupled with an adaptive gain which is close to high order sliding-mode observers but avoiding the discontinuous structure of the sliding-modes, which allows a smooth convergence of the proposed observer, avoiding the chattering phenomena. The considered observer allows infers biomass and product concentrations under the assumption that the substrate measurements are available. The proposed observer is applied to kinetic model for cell growth for *Desulfovibrio alaskensis* 6SR. *Desulfovibrio alaskensis* was described in the first time by Feio (Feio *et al.*, 2004) and subsequently studies of bacteria associated at biocorrosion, have reported that D. alaskensis is present in oil fields from Gulf of Mexico (Neria-González *et al.*, 2006, Hernández-Gayoso *et al.*, 2004; Padilla-Viveros *et al.*, 2006). The strain 6SR was isolated of biofilm in oil pipeline, which have high resistance to heavy metals (Cd^{+2} and Cr^{+6}) in relation at other species, is tolerant at oxygen, grows at pH 5.5-9 (7), 15-55 (45) °C and in 30% (w/v) NaCl, and produces extracellular polymeric substances. These bacterial characteristics are important in environmental process and other as corrosion.

2 Experimental

2.1 Organism, culture maintenance and purity test

Desulfovibrio alaskensis 6SR was isolated of a developed biofilm inside face of oil pipeline (Neria-González *et al.*, 2006). The strain was maintained routinely in Hungate tubes with 5 mL of Postgate's medium B (Hungate, 1969). To evaluate the organism purity decimal dilution in plates of anaerobic agar supplemented with 6 mL of lactate (60% w/w), 4.5 g of $NaSO_4$, 0.004 mg of $FeSO_4$, and 27.5 g of NaCl were carried-out. The plates were placed in anaerobic jar (BBL^{TM} $GasPak^{TM}$ Anaerobic Systems) and incubated until to colonies appear. The presence of black colonies indicated the growth of sulfate reducing bacteria. One black colony well definite and isolated was picked and quickly transferred at 45 mL sterile Postgate's C medium in anaerobic conditions (Postgate, 1979), and subcultures ware made subsequently.

The media was inoculated with 5 mL of culture and incubated at 37 °C. Each medium was prepared and dispended in anaerobic conditions under a N2 (99.998% purity) atmosphere, 120 and 160 mL serum bottles were filled with 45 and 95 mL of medium, respectively, and autoclaved at 121°C.

2.2 Growth kinetics

The inoculum for kinetic study was cultured in 45 mL of Postgate's C medium for 25 h at 37°C (logarithmic phase). A 5 mL aliquot was taken from Postgate's C medium to inoculate 95 mL of fresh medium at 37 °C. The experiment was done using two series of triplicate independent cultures; each set of triplicate cultures were inoculated with12 hours separated each other, the experimental run time was 72 hours.

2.3 Analytic methods

The bacterial growing, consuming of sulfate and the sulfide production were monitored each 3 or 4 hours, the samples were taken carefully, avoiding contact with oxygen. The bacterial growing was followed through Optical Density (OD) methodology, the OD reading for cell growing was transformed into dry mass (mg/L) through a standard growth curve. The consuming sulfate in the medium was measured by the turbid metric method based on the precipitation of barium (Kolmert *et al.*, 2000). Also, the production of sulfide was measured by a colorimetric method (Cord-Ruwisch, 1985).

2.4 Data analysis and mathematical model

The experimental data of biomass, sulfate, and sulfide from two series of sulfate reducing culture were analyzed using the average values of each measurement point. As is well known, in sulfate reducing culture the accumulation of sulfide (product) in the medium has an inhibitory effect on bacterial growth. Therefore, to approximate the average experimental data of bacterial growth in batch bioreactor, the Levenspiel's inhibition product model (Levenspiel, 1999) be used to represent the specific growth rate (Eq. 1). Basically, this is an unstructured model that describes the bacterial growth rate as a function of substrate, product and biomass concentrations as the unique biological state variable.

$$\frac{dx}{dt} = \mu\left(S, P\right)\left(x\right) = \mu_{\max}\left(1 - \frac{P}{P^*}\right)^n \left(\frac{S}{K_S + S}\right)(x) \tag{1}$$

Where: μ_{max} represents the maximum growing rate; K_S represents the affinity substrate; P^* corresponds to inhibition concentration product, and n is the reaction order; meanwhile S, P, and x are substrate, product, and biomass concentrations, respectively.

2.5 Estimation of the kinetic parameters

Levenspiel growth kinetic parameters ware estimated by the rate of change of biomass production, using central finite differences according to the following equation:

$$\left(\frac{dt}{dt}\right)_{ti} \cong \left(\frac{\Delta X}{\Delta t}\right) = \left(\frac{X_{i+1} - X_i}{t_{i+1} - t_i}\right) \tag{2}$$

and a nonlinear multivariable regression for the rates of change of biomass production and experimental data (X, S, and P) was done. POLYMATH 6.0 Professional software was used, the program allow applying effective numerical analysis techniques, and Levenberg-Marquardt algorithm was using for this case (see Table 1).

The mathematical model was simulated using the same software. Besides, a linear regression between the experimental and the predicted data were obtained, and overall correlation coefficient was calculated, see figs. 1-2 (Tejeda, 2007).

Table 1. Growth kinetic parameters and yields for *Desulfovibrio alaskensis.*

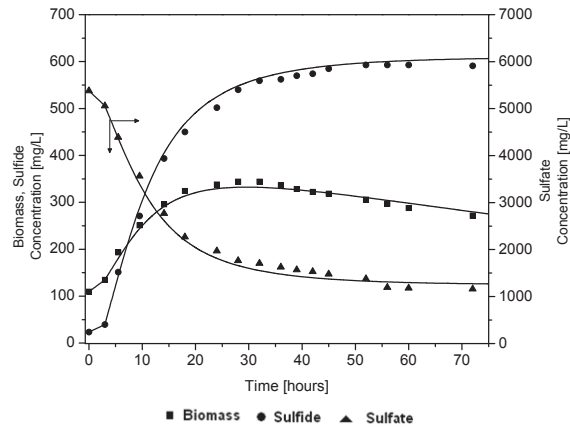| Parameter	Value
μ_{max}, h^{-1}	0.188
K_S, mg L^{-1}	5699.86
μ_d, h^{-1}	0.0038
P^*, mg L^{-1}	735.0
n	0.89
$Y_{(S/X)}$, mg S mg^{-1} X	14.13
$Y_{(P/X)}$, mg P mg^{-1} X	2.14

Fig. 1: Comparison of the experimental and predicted kinetics of growth of *Desulfovibrio alaskensis* on Postgate's C medium. The solid line represents predicted data for each response variables.

3 Bioreactor mathematical model

The unstructured models are using nowadays as the main tool for the bioprocess modeling, but also for being applied in overall computer control. Considering the above kinetic model, it is proposed by following the mathematical model for a class of continuous stirred bioreactor, which is based on classical mass balances for biomass, sulfate (substrate) and sulfide (product) concentrations:

Sulfate (S).-

$$\frac{dS}{dt} = D\left(S_{in} - S\right) - \mu(S)\frac{X}{Y_{S/X}} \tag{3}$$

Biomass (X).-

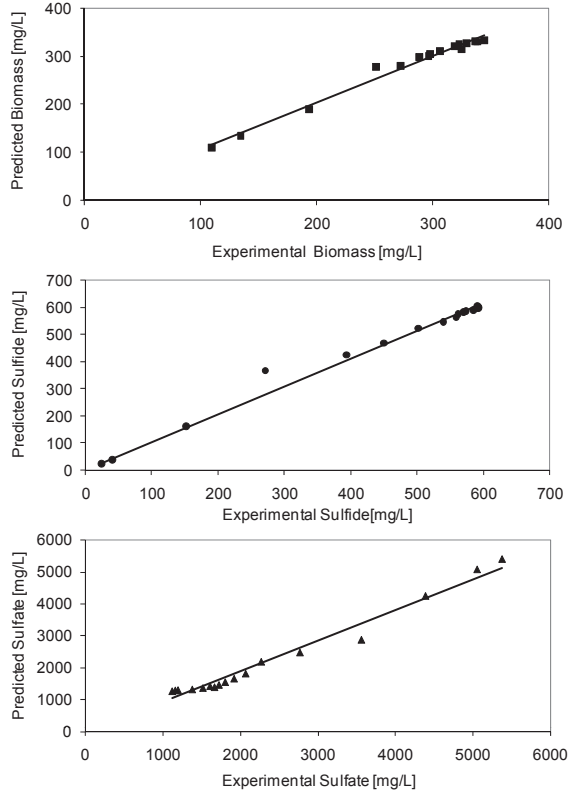$$\frac{dX}{dt} = -DX + \mu(S)X \tag{4}$$

Fig. 2: Linear regressions for experimental and predicted data in a batch anaerobic growth with initial substrate concentrations of 5379 mg/L. a) experimental Biomass (■), predicted biomass, $R^2 = 0.982$; b) experimental Sulfide (●), predicted Sulfide, $R^2 = 0.985$; c) experimental Sulfate (▲), predicted Sulfate, $R^2 = 0.976$. Overall coefficient was 0.979.

Sulfide (P).-

$$\frac{dP}{dt} = -DP + \mu(S)\frac{X}{Y_{P/X}} \qquad (5)$$

Here D is the dilution rate, μ is the specific growth rate, $Y_{S/X}$ is the sulfate coefficient yield and $Y_{P/X}$ is the sulfide coefficient yield. In accordance with the specific experimental setup the following initial conditions are considered for the batch culture and model validation purposes $X_o = 100$ mg/L, $S_o = 5200$ mg/L, $P_o = 10$ mg/L. Figure 1 shows the performance of the kinetic model considering a comparison with the experimental data which looks satisfactory. The above model predicts batch operation when $D = 0$, the fed-batch operation is considered when $D = f(t)$ and the output flow terms are null and finally the

continuous operation which considers inputs and outputs flow terms.

4 Methodology for the observer design

Consider a canonical control representation form of the bioreactor model:

$$\begin{aligned} \overset{\bullet}{x} &= f(x) \\ y &= Cx \end{aligned} \qquad (6)$$

Now, the following state observer is proposed:

$$\begin{aligned} \overset{\bullet}{\hat{x}} &= f(\hat{x}) + l\tanh(y - \hat{y}) \\ \overset{\bullet}{l} &= -\alpha \; abs(y - \hat{y})^{1/m} \end{aligned} \qquad (7)$$

where the observer's gain l is given by an update adaptation algorithm and α is a parameter design. To prove the convergence of the proposed observer, let us to consider the dynamic equation of the estimation error ($\varepsilon = x - \hat{x}$), as follows:

$$\begin{aligned} \overset{\bullet}{\varepsilon} &= \overset{\bullet}{x} - \overset{\bullet}{\hat{x}} = f(x) - (f(\hat{x}) + l\tanh(\varepsilon)) \\ \overset{\bullet}{l} &= -\alpha \; abs(\varepsilon)^{1/m} \end{aligned} \qquad (8)$$

under the following assumptions:
A1. $f(x) - f(\hat{x}) - \leqslant L(x - \hat{x})$ Taking norms to both sides of Eq. (8) and applying A1 it is obtained:

$$\left|\overset{\bullet}{\varepsilon}\right| \leqslant L\left|\varepsilon\right| - l\left|\tanh(\varepsilon)\right| \qquad (9)$$

Now, suppose that the function $abs(\varepsilon)^{1/m}$ is a positive continuous function on the integration interval $[a, b]$; then H is the maximum of the function on the domain $[\delta, \gamma]$, then $abs(\varepsilon)$ is bounded, i.e. $abs(\varepsilon) \leqslant H \; \forall \; t \in [\delta, \gamma]$, such that: $abs(\varepsilon)^{1/n} \leqslant H^{1/n} \; n > 0 \Rightarrow \int_{\delta}^{\gamma} abs(\varepsilon)^{1/n} \leqslant H^{1/n}(\gamma - \delta)$, considering n an odd number i.e. $n = 2p + 1$, $p \in Z^+$, therefore; $\lim \; \sup \int_{\delta}^{\gamma} abs(\varepsilon)^{1/(2p+1)} \leqslant \lim \sup H^{1/(2p+1)}(\gamma - \delta) \leqslant (\gamma - \delta)$ for p large enough.

From the above, Eq. (9) can be rewritten as:

$$\left|\overset{\bullet}{\varepsilon}\right| \leqslant L\left|\varepsilon\right| - \alpha(\gamma - \delta) \qquad (10)$$

Solving Eq. (9) and considering $t \rightarrow \infty$, can be concluded that the estimation error belongs to ball:

$$|\varepsilon| \leqslant \frac{\alpha\,(\gamma - \delta)}{L} \qquad (11)$$

5 Numerical experiments and discussion

Besides to show the performance of the proposed estimation methodology, an important additional issue concerns with the evaluation of the capacities *Desulfovibrio alaskensis* 6SR for biotechnological applications. Both two of these possible applications are related with wastewater treatment for degradation of sulfate compounds and heavy metal mobility. From the kinetic model presented in Section 3, the proposed observer is applied to batch, continuous and fed-batch operating modes; each one of the above mentioned operating modes is related with a specific process task, on the batch operation an optimal trajectory must to be reached in order to provide an adequate performance, besides a finite time operation politics must be considered; related with the continuous operation process the main tasks is to lead the bioreactor trajectories to an optimal and stable steady state, where the convergence of observers and controllers can be designed with asymptotic convergence and finally the fed-batch operation can be considered as a batch operation with input disturbances, therefore a robust monitoring and controlling methodologies are needed. The initial concentration conditions for the batch culture was above mentioned and the corresponding for the continuous and fed-batch operating modes are $X_o = 980$ mg/L, $S_o = 6850$ mg/L and $P_o = 15$ mg/L. The initial conditions imposed to the proposed observer are 5000 mg/L, 96 mg/L and 8 mg/L, respectively, for the batch operation and 950 mg/L, 6250 mg/L and 20 mg/L for biomass, sulfate and sulfide concentrations, respectively, for continuous and fed-batch operation modes. The estimation procedure considers the sulfate concentration (substrate) as measured output in order to infer the biomass and sulfide concentrations.

Batch operation is considered from figs. 3-5, where the performance of the proposed observer is satisfactory, a fast convergence to the named real concentrations is achieved, without large
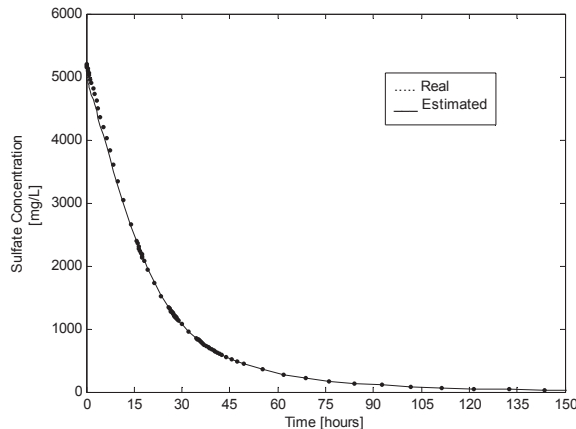

Fig. 3: Sulfate Concentration filtering with the proposed observer (batch operation).
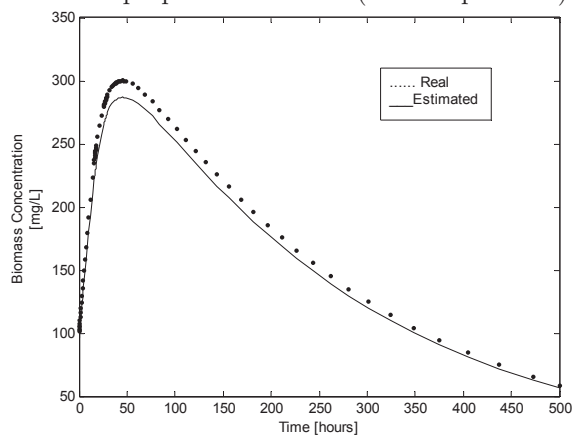

Fig. 4: Biomass concentration estimation with the proposed observer (batch operation).
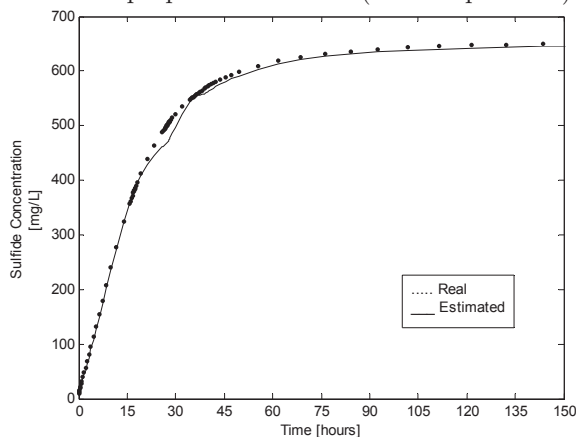

Fig. 5: Sulfide concentration estimation with the proposed observer (batch operation).

overshoots and settling times.

Figures 6-8 concern to continuous operating mode can be observed an adequate performance of the proposed estimation methodology from the
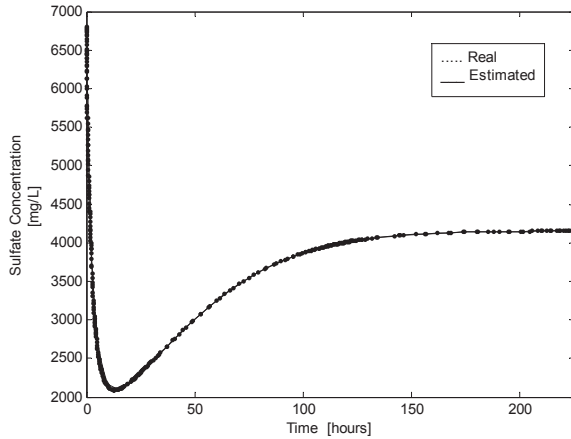
Fig. 6: Sulfate concentration filtering with the proposed observer (continuous operation).
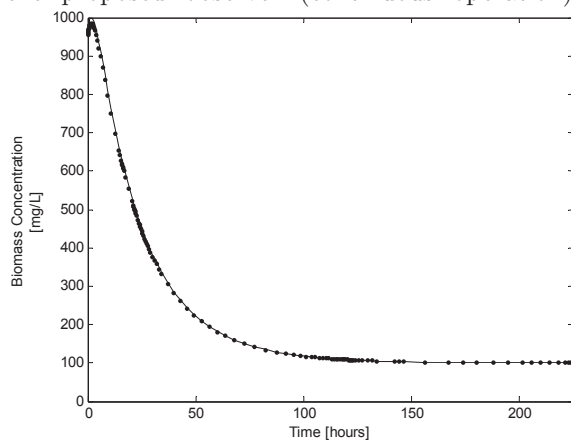

Fig. 7: Biomass concentration estimation with the proposed observer (continuous operation).
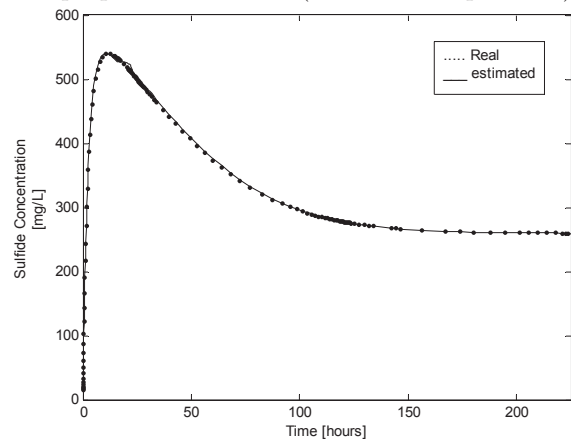

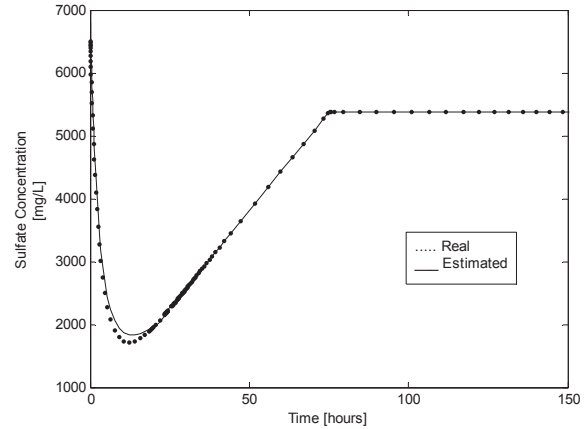Fig. 8: Sulfide concentration estimation with the proposed observer (continuous operation).

Fig. 9: Sulfide concentration estimation with the proposed observer (fed-batch operation).
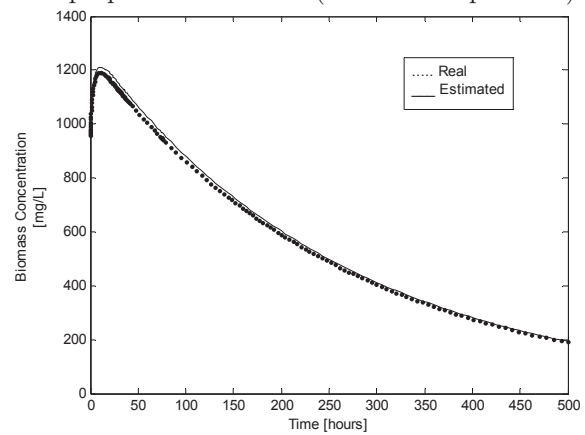

Fig. 10: Biomass concentration estimation with the proposed observer (fed-batch operation).

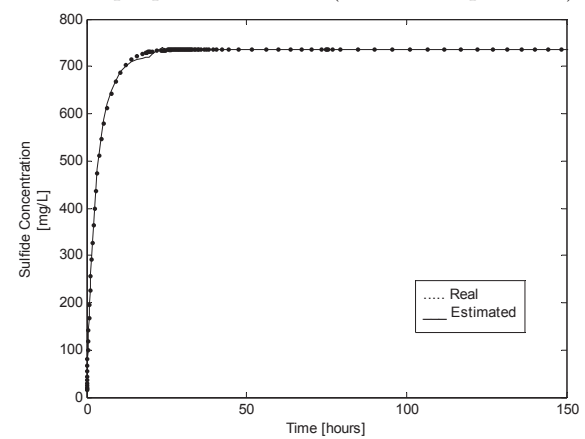Fig. 11: Sulfide concentration estimation with the proposed observer (fed-batch operation).

time series.

Finally, figs. 9-11 are related with the observer's performance when a fed-batch operation is considered; for this case a dilution rate of $D = 0.01$ 1/h is considered from the start-

up of the bioreactor operation to 75 hours, later the input flow is null ($D = 0$). Despite of this sudden disturbance the proposed observer keeps a satisfactory performance following the named real concentrations.

Conclusions

In this work is modeled the biomass growth and sulfide production of a sulfate reducing bacteria *Desulfovibrio alaskensis*, which has been recently described, and its biotechnological properties are not studied yet enough. For the above mentioned, a nonlinear state observer to infer biomass and sulfide concentration from sulfate concentration measurements is implemented for a batch, fed-batch and continuous operating bioreactor modes, where can be observed a satisfactory performance. A mathematical analysis to show the convergence characteristics of the proposed methodology is done. Future work will be oriented to analyze the effect of model uncertainties and noisy measurements in order to try to design robust estimation methodologies.

Acknowledgment

Nomenclature

D	dilution rate (1/h)
f	nonlinear smooth function
L	Lipchitz constant (1/h)
K_S	substrate affinity constant, inhibition constant, term inhibition (mg/L)
n	exponential term for Levenspiel model
P	product concentration (mg/L)
P^*	inhibitory product concentration (mg/L)
r_d	death rate (mg-death biomass/L per h)
r_X	growth rate (mg-biomass/L per h)
S	substrate concentration (mg /L)
S_{in}	inlet substrate concentration (mg/L)
x	state variables vector, (mg/ L)
X	biomass concentration (mg/L)
X_d	measured output (mg/L)
X_o, S_o and P_o	initial concentration of biomass, substrate and product (mg/L)
y	measured output (mg/L)
$Y_{S/X}$	substrate-biomass yield coefficient (mg-sulfate/mg-biomass)
$Y_{P/X}$	product-biomass yield (mg-sulfide/mg-biomass)

Greek symbols

α	observer gain (1/h)
ε	estimation error (mg/L)
ι	adaptive observer gain (1/h)
μ, μ_d, μ_{max}	specific growth rate, specific death rate, maximum rate growth (1/h)

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