MAXIMUM LIKELIHOOD ADAPTIVE OBSERVER FOR BIOPROCESSES

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Abstract: A particularity of cell culture processes is the relatively restricted number of valuable and accurate measurements for process control. Software sensors are an interesting solution in response to this problem since it provides non measured state estimation combining the available measurements to a mathematical model. But, due to the complexity of cell culture processes, the mathematical model itself may present some uncertainties particularly in the kinetic description. Such a difficulty has lead to the development of adaptive observers which are designed to jointly estimate state variables and model parameters. However those observers may become particularly difficult to design and to tune as the process complexity increases. In this contribution, an adaptive observer based on the theory of the full horizon and the asymptotic observers is proposed.

Keywords: State observers, biotechnology, fermentation processes, nonlinear systems, maximum likelihood estimators

INTRODUCTION

Products from the biotechnology industry becomes particularly complex in terms of reactor design, biocatalysts, product quality, etc. As this complexity increases, the needs of information about the dynamics of the main constituents becomes crucial for control, optimization and supervision. However, such a requirement is most often limited by the availability, and the efficiency of hardware sensors. For instance, knowing and supervising the biomass growth during the process may be very important. However biomass measurement involves most often, off line analyses (optical density measurements, cell counting, etc.). On line optical density sensors are available but are particularly expensive and the measures may be rapidly biased due to the high cell density. A solution to these latter problems can be found through the design of software sensors, which combine some available hardware sensor signals and a mathematical model, in order to provide estimates of non-measured variables.

Several estimation techniques considering the non-linear models involved in bioprocesses have been proposed in the literature. Many state observers belong to the so called class of exponential observer. These observers are characterized by an adjustable rate of convergence towards the true state, which is defined by one or several tuning parameters. The extended Kalman filter, the extended Luenberger observer, the high gain observer and the full horizon observer belong to this class since the convergence rate may be freely tuned. However the main drawback of these exponential observers is that their efficiency strongly rely on the model quality. Dealing with model uncertainty was, and still is, an area of intensive research particularly for kinetic uncertainties

(structure and/or parameters) which may be particularly important in the field of bioprocesses. A first solution to cope with model uncertainty is to design an asymptotic observer as proposed in (Bastin and Dochain 1990). Such an observer uses a state transformation in order to provide a model which is independent of the kinetics. However, the price to pay is that the rate of convergence is completely determined by the experimental conditions (namely the dilution rate). This may lead to very slow convergence in the case of low dilution rate or no converge at all in the case of batch cultures. Hence, when experimental conditions and model structure are appropriate, the asymptotic observer is a good solution for state estimation with uncertain kinetic model. However, this observer does not provide any information on the kinetic model which was supposed to be uncertain. Using observers as estimators of the kinetics or model parameters was the objective of the subsequently developed observer-based estimators.(OBE). OBE were initially developed to estimate on-line the specific growth rates in bioprocesses (Bastin and Dochain 1986) then extended to the on-line estimation of kinetic parameters (Bastin and Dochain 1990). The OBE stability properties are well exposed and improvements of tuning procedure has been proposed in (Oliveira *et al.* 1996), (Perrier *et al.* 2000), (Oliveira *et al.* 2002). Another approach, improving the tuning procedure of an OBE was also proposed in (Farza *et al.* 1997). However these algorithms were focused on the model parameter estimation. A natural extension was therefore to jointly estimate the state and the uncertain model parameters. These algorithms are known as adaptive observers since the state observer is evolving by correcting the uncertain parameters. Several approaches in this field are presented in (Bastin and Dochain 1990). The most common designs consist in considering the parameters as extra states with no dynamics and to introduce it in an extended Kalman or Luenberger observer. These designs present the advantage that they follow a standard and well-known solution. However they present the inherent drawbacks of both observers (stability properties, tuning procedure, linearization, etc.). Thus other adaptive observer designs have been proposed as in (Dochain 2003). In this contribution an adaptive observer is proposed by combining the asymptotic observer and the theory of the full horizon observer (Bogaerts and Hanus 2001). Such an algorithm will be used to estimate and to correct the uncertain parameters in order to provide a state estimation with the corrected model. Using the full horizon theory allows also to achieve this objective by using an algorithm which is particularly suitable for bioprocesses, does not require any tuning parameter and involves an estimation technique previously developed for the identification of bioprocess model parameters (Bogaerts (1999)), (Bogaerts and Hanus 2000).

In the following sections the mathematical framework of bioprocess modelling is presented. The theory of the asymptotic and the full horizon observers are then briefly recalled. In a fourth section the adaptive observer is described in detailed. Illustrations on simulated cell cultures are then presented. Finally a last section is devoted to some conclusions.

1. MACROSCOPIC REACTION SCHEMES AND MASS BALANCES FOR BIOPROCESS MODELLING

A bioprocess can be described by a reaction scheme defined by a set of M reactions (Bastin and Dochain 1990). Such a reaction scheme can be expressed by :

$$\sum_{i\in R_k}(-\nu_{i,k})\xi_i \overset{\varphi_k}{\rightarrow} \sum_{j\in P_k}\nu_{j,k}\xi_j \qquad k\in[1,M] \quad (1)$$

where

- $\nu_{i,k}$ and $\nu_{j,k}$ are the pseudo-stoichiometric coefficients or yield coefficients;
- φ_k is the reaction rate;
- ξ_i is the i^{th} component;
- $R_k(P_k)$ is the set of ξ_i which are reactants (products) in the reaction k;
- M is the number of reactions.

Assuming that the bioprocess takes place in a perfectly stirred bioreactor, the system dynamics can be described by a model resulting from mass balances for the macroscopic species involved in the reaction scheme:

$$\frac{d\boldsymbol{\xi}(t)}{dt} = \boldsymbol{K}\boldsymbol{\varphi}(\boldsymbol{\xi}(t)) - D(t)\boldsymbol{\xi}(t) + \boldsymbol{F}(t) - \boldsymbol{Q}_g(t) \quad (2)$$

where

- $\boldsymbol{\xi}\in\Re^N$ is the vector of component concentrations;
- $\boldsymbol{K}\in\Re^{N\times M}$ is the pseudo-stoichiometric coefficient matrix ($M\leq N$);
- $\boldsymbol{\varphi}\in\Re^M$ is the vector of reaction rates;
- $D\in\Re$ is the dilution rate;
- $\boldsymbol{F}\in\Re^N$ is the vector of external feed rates;
- $\boldsymbol{Q}_g\in\Re^N$ is the vector of gaseous outflow rates.

In the sequel, the external feed rates and gaseous outflow rates are put together in a vector

$$\boldsymbol{u}(t) = \boldsymbol{F}(t) - \boldsymbol{Q}_g(t) \quad (3)$$

In the context of state observation the state vector can be subdivided into two vectors :

$$\boldsymbol{\xi}(t)^T = [\boldsymbol{\xi}_1^T \quad \boldsymbol{\xi}_2^T] \quad (4)$$

where $\boldsymbol{\xi}_1\in\Re^L(L\leqslant N)$ contains the elements of $\boldsymbol{\xi}$ which are measured :

$$\boldsymbol{\xi}_1 = \boldsymbol{C}\boldsymbol{\xi} = [\boldsymbol{I}_L \quad \boldsymbol{0}_{L,N-L}]\boldsymbol{\xi} \quad (5)$$

These measures are in the form of discrete samples:

$$\boldsymbol{y}(t_k) = \boldsymbol{C}\boldsymbol{\xi}(t_k) + \boldsymbol{\varepsilon}(t_k) \quad (6)$$

$\boldsymbol{\varepsilon} \in \Re^L$ being a white noise sequence normally distributed with zero mean:

$$E[\boldsymbol{\varepsilon}(t_i)] = 0 \quad (7)$$

$$E[\boldsymbol{\varepsilon}(t_i)\boldsymbol{\varepsilon}^T(t_j)] = \delta_{i,j}\boldsymbol{Q}(t_j) \quad (8)$$

The other elements $\boldsymbol{\xi}_2 \in \Re^{(N-L)}$ of $\boldsymbol{\xi}$ are the state variables which are not measured.

2. THE ASYMPTOTIC OBSERVER

The principle of the asymptotic observer (AO) (Bastin and Dochain 1990) is to use a state transformation in order to provide at each sampling time an estimation of the non-measured state from the measured one without any knowledge of the kinetic model. The derivation of the AO is based on the following conditions : $\boldsymbol{\varphi}(\boldsymbol{\xi})$ is unknown, the yield coefficients matrix $\boldsymbol{K}$ is known, $L = dim(\boldsymbol{\xi}_1) \geqslant p = rank(\boldsymbol{K})$. Hence, there always exists a partition

$$\boldsymbol{\xi}^T = [\boldsymbol{\xi}_a^T \ \boldsymbol{\xi}_b^T] \quad (9)$$

so that the corresponding partition

$$\boldsymbol{K}^T = [\boldsymbol{K}_a^T \ \boldsymbol{K}_b^T] \quad (10)$$

involves a matrix $\boldsymbol{K}_a \in \Re^{p\times M}$ of full row rank. Given such a partition of $\boldsymbol{K}$, the following matrix equation

$$\boldsymbol{A}_0\boldsymbol{K}_a + \boldsymbol{K}_b = \boldsymbol{0}_{N-p,M} \quad (11)$$

has always a unique solution $\boldsymbol{A}_0 \in \Re^{(N-p)\times p}$. It is therefore possible to define an auxiliary vector $\boldsymbol{Z} \in \Re^{(N-p)}$.

$$\boldsymbol{Z} = \boldsymbol{A}_0\boldsymbol{\xi}_a + \boldsymbol{\xi}_b \quad (12)$$

whose dynamics is independent of the kinetics $\boldsymbol{\varphi}(\boldsymbol{\xi})$:

$$\frac{d\boldsymbol{Z}(t)}{dt} = -D(t)\boldsymbol{Z}(t) + \boldsymbol{A}_0\boldsymbol{u}_a(t) + \boldsymbol{u}_b(t) \quad (13)$$

where $\boldsymbol{u}^T = [\boldsymbol{u}_a^T \ \ \boldsymbol{u}_b^T]$ is the partition of $\mathbf{u}$ corresponding to the partition of $\boldsymbol{\xi}$.

It is also possible to write the vector $\mathbf{Z}$ as a linear combination of the vectors $\boldsymbol{\xi}_1$ and $\boldsymbol{\xi}_2$ of measured and non-measured states :

$$\boldsymbol{Z}(t) = \boldsymbol{A}_1\boldsymbol{\xi}_1(t) + \boldsymbol{A}_2\boldsymbol{\xi}_2(t) \quad (14)$$

where
$\mathbf{A}_1 \in \Re^{(N-p)\times L}$ and $\mathbf{A}_2 \in \Re^{(N-p)\times(N-L)}$.

The AO is finally defined by:

$$\frac{d\hat{\boldsymbol{Z}}(t)}{dt} = -D(t)\hat{\boldsymbol{Z}}(t) + \boldsymbol{A}_1\boldsymbol{u}_1(t) + \boldsymbol{A}_2\boldsymbol{u}_2(t) \quad (15)$$

$$\hat{\boldsymbol{\xi}}_2(t) = \boldsymbol{A}_2^+(\hat{\boldsymbol{Z}}(t) - \boldsymbol{A}_1\boldsymbol{\xi}_1(t)) \quad (16)$$

where $\boldsymbol{A}_2^+$ is a left pseudo inverse of the matrix $\boldsymbol{A}_2$.

The dynamics of the state estimation error $\tilde{\boldsymbol{\xi}}_2$ is given by:

$$\frac{d\tilde{\boldsymbol{\xi}}_2(t)}{dt} = -D(t)\tilde{\boldsymbol{\xi}}_2(t) \quad (17)$$

It is therefore obvious that the convergence of the AO is function of the experimental conditions (D). This observer may therefore not converge (batch process) or converge very slowly (low dilution rate).

The above description of the AO shows that it provides a discrete time observation (in the case of discrete time measurements), it is a deterministic approach, and its convergence is defined by the dilution rate However, its main advantage is that it does not require the kinetic model.

3. THE MOST LIKELY INITIAL CONDITIONS OBSERVER

The most likely initial conditions observer (MLICO) (the full horizon observer in (Bogaerts and Hanus 2001)) is a stochastic observer that consists in integrating the simulation model between two measures (prediction) starting with the most likely initial conditions identified on the basis of all the available measures (correction).

The prediction equation of the full horizon observer is defined as

$$\frac{d\hat{\boldsymbol{\xi}}(t)}{dt} = \boldsymbol{K}\boldsymbol{\varphi}(\hat{\boldsymbol{\xi}}, t) - D(t)\hat{\boldsymbol{\xi}}(t) + \boldsymbol{u}(t) \quad (18)$$

$$\hat{\boldsymbol{\xi}}(t) = \boldsymbol{g}(t, \boldsymbol{u}(t), \hat{\boldsymbol{\xi}}_{0/k}) \quad \forall t \in [t_k, t_{k+1}] \quad (19)$$

where $\boldsymbol{g}(t, \boldsymbol{u}(t), \hat{\boldsymbol{\xi}}_{0/k})$ is the prediction of $\boldsymbol{\xi}(t)$ on the time interval $[t_k, t_{k+1}]$ deduced from the integration of (18) from the most likely initial conditions $\hat{\boldsymbol{\xi}}_{0/k}$. These latter are identified by solving a nonlinear optimization problem on the basis of all the measures $\boldsymbol{y}(t_j), j \in [1, k]$ available up to time k :

$$\hat{\boldsymbol{\xi}}_{0/k} = \frac{1}{2}\underset{\xi_0}{Argmin} \sum_{j=1}^{k} \boldsymbol{\delta}^T(t_j)\boldsymbol{Q}^{-1}(t_j)\boldsymbol{\delta}(t_j) \quad (20)$$

where
$\boldsymbol{\delta}(t_j) = \boldsymbol{y}(t_j) - \boldsymbol{C}\boldsymbol{g}(t_j, \boldsymbol{u}(t), \hat{\boldsymbol{\xi}}_{0/k})$ and
$\boldsymbol{Q}$ is the measurement error covariance matrix.

Note that a necessary condition of existence of the solution (20) is that the number of available measures is greater or equal to the number of initial states to identify.

As pointed out in (Bogaerts and Hanus 2001), the full horizon observer provides a continuous-time estimation even on the basis of on rare and asynchronous measurements, consists of a stochastic approach, is a true nonlinear approach (no approximation such as linearization), does not

require any tuning, may be theoretically analyzed (e.g., state estimation correction) and provides confidence intervals for the state estimates. However, as classical exponential observers, it requires the full knowledge of the model structure (including the kinetics).

4. THE ADAPTIVE MOST LIKELY INITIAL CONDITIONS OBSERVER

Suppose that some of the kinetic parameters $\boldsymbol{\vartheta}_{cin}$ are unknown or uncertain. The vector of kinetic parameters may therefore be subdivided into a known part ($\boldsymbol{p}_{cin}$) and an unknown one ($\boldsymbol{p}'_{cin}$):

$$\vartheta_{cin}^T = \left[\boldsymbol{p}_{cin}^T \;\; \boldsymbol{p}_{cin}'^T \right] \tag{21}$$

The principle of the adaptive MLICO is to extend the vector of initial conditions to be estimate with the unknown or uncertain kinetic parameters. These parameters will therefore be jointly estimated with the initial conditions. The parameter vector to be identified with the most likely estimator at each measurement time becomes therefore:

$$\hat{\boldsymbol{\vartheta}}_k^T = \left[\hat{\boldsymbol{\xi}}_{0/k}^T \;\; \hat{\boldsymbol{p}}_{cin/k}'^T \right] \quad \text{with } \hat{\boldsymbol{\vartheta}}_k \in \Re^{N_\vartheta} \tag{22}$$

However, it is well known that increasing the size of the vector of parameters to identify may lead to some numerical troubles such as local minima during the optimization procedure. Moreover, without going into further details in non linear system observability (and more specifically, the uniform observability (Gauthier and Kupka 1994)) there always exists a minimum number of measured states which is necessary to guarantee the system observability. This minimum number is depending on the specific non linear model being used. Since in bioprocesses the number of physical sensors is restricted, the number of measured state becomes rapidly not sufficient to insure the system observability especially when the state vector is augmented with unknown parameters. In order to circumvent these problems the proposed adaptive algorithm aims to exploit the information provided by the AO. Indeed, like in OBE with partial state measurement, an estimation of the state which is not measured by hardware sensors ($\boldsymbol{\xi}_2$) is provided by the AO and subsequently considered as a new measurement. With such an additional information one could expect the optimization problem to be easier. Moreover, as it will be illustrated in section 5, the additional information from the AO increases the number of "measured" states allowing an extension of the state estimate while keeping the system observability. A complete state vector provided by measurements and the AO is therefore available for the adaptive algorithm which is then used to provide a continuous state estimation of the complete state vector together with the unknown kinetic parameter(s). The principle of this adaptive observer is presented in figure (1).

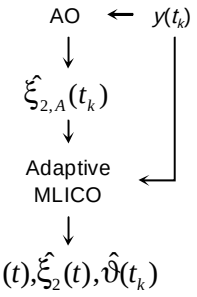

Fig. 1. Principle of the adaptive MLICO

Like in the classical MLICO, the prediction step between two measurement times is insured by integrating the process model. The prediction equations of the observer correspond therefore to (note that for the simplicity of notations, only the time and estimated parameters dependencies will be explicitly written):

$$\frac{d\hat{\boldsymbol{\xi}}(t)}{dt} = \boldsymbol{K}\boldsymbol{\varphi}(\hat{\boldsymbol{\vartheta}}_k, t) - D(t)\hat{\boldsymbol{\xi}}(t) + \boldsymbol{u}(t) \tag{23}$$

$$\hat{\boldsymbol{\xi}}(t) = \boldsymbol{g}(\hat{\boldsymbol{\vartheta}}_k, t) \qquad \forall t \in [t_k, t_{k+1}] \tag{24}$$

where $\boldsymbol{g}(\hat{\boldsymbol{\vartheta}}_k, t)$ is the prediction of $\boldsymbol{\xi}(t)$ on the time interval $[t_k, t_{k+1}]$ deduced from the integration of (23) with the corrected likely initial conditions $\hat{\boldsymbol{\xi}}_{0/k}$ and kinetic parameters $\hat{\boldsymbol{p}}'_{cin/k}$. As for the correction step, two modifications are introduced. Firstly, the vector of parameters to identify contains the initial conditions and the uncertain kinetic parameter(s) (see 22). Secondly the non measured state estimation provided by the AO ($\hat{\boldsymbol{\xi}}_{2\mathcal{A}}$) is introduced in the cost function (20). The optimization problem used for the correction procedure corresponds therefore to:

$$\hat{\boldsymbol{\vartheta}}_k = \frac{1}{2} \underset{\xi_0}{Argmin} \sum_{i=1}^{k} \boldsymbol{\delta}^T(t_i)^T \boldsymbol{Q}_{\mathcal{A}}^{-1}(t_i)\boldsymbol{\delta}(t_i) \tag{25}$$

where

$\boldsymbol{\delta}(t_i) = \begin{bmatrix} \boldsymbol{y}(t_i) - \hat{\boldsymbol{g}}_1(t_i) \\ \hat{\boldsymbol{\xi}}_{2\mathcal{A}}(t_i) - \hat{\boldsymbol{g}}_2(t_i) \end{bmatrix}$ with $\hat{\boldsymbol{g}}_1(t_i)$ and $\hat{\boldsymbol{g}}_2(t_i)$ are the partitions of the vector $\hat{\boldsymbol{g}}$ into the measured and the non measured state trajectories. $\boldsymbol{Q}_{\mathcal{A}}$ is the covariance matrix of the "measurement" errors (i.e. the real measurement errors together with the estimation errors provided by the AO) developed in (Hulhoven and Bogaerts 2004).

5. CASE STUDIES

5.1 Simple microbial growth

In a first example, the adaptive observer is illustrated on a simple microbial culture. Consider a

fed-batch bacterial fermentation taking place in a perfectly stirred bioreactor. Consider the following reaction scheme :

$$\nu_S S \xrightarrow{\varphi} \overset{\curvearrowleft}{X} \tag{26}$$

where S denotes the substrate concentration, X the biomass concentration, φ the reaction rate and ν_S the yield coefficient. $\overset{\curvearrowleft}{X}$ denotes an autocatalytic reaction. The mass balances corresponding to this reaction scheme are :

$$\frac{dS}{dt} = -\nu_S \varphi - DS + DS^{in} \tag{27}$$

$$\frac{dX}{dt} = \varphi - DX \tag{28}$$

where D is the dilution rate and S^{in} the substrate concentration in the feed medium. The reaction rate φ will be described using the Monod law:

$$\varphi = X \frac{\mu_m S}{K_m + S} \tag{29}$$

The numerical values used for the simulation are : $\nu_S = 5[\,\mathrm{g}(10^{11} cell)^{-1}]$; $K_m = 12[\,\mathrm{g\,l^{-1}}]$; $\mu_m = 1.4[\,\mathrm{h^{-1}}]$; $S(0) = 12[\,\mathrm{g\,l^{-1}}]$; $X(0) = 0.14[10^{11} cell\ \mathrm{l^{-1}}]$; $S^{in} = 20[\,\mathrm{g\,l^{-1}}]$; $D = \frac{0.3}{t_f} t[\,\mathrm{h^{-1}}]$ where t_f is the final time of the culture.

In order to illustrate the performances of the adaptive observer the simulation results are considered as the real process, the substrate is assumed to be measured every hour with a white noise of zero mean $E[\varepsilon] = 0$ and standard deviation $\sigma = 0.5\,\mathrm{g\,l^{-1}}$. For this system, a classical MLICO may be used to identify the biomass. However, suppose now that the parameter μ_m is uncertain and has to be estimated in the observer algorithm. The unique substrate measurement is not sufficient to allow both estimations (biomass and μ_m). Using the biomass state estimation provided by the AO as an extra information allows to circumvent this problem. Figure (2) shows the results obtained by applying the adaptive observer to the process when an initial guess error on μ_m is introduced.As it can be observed from these results, the adaptive observer allows to provide a continuous and converging state estimation of the biomass and the substrate. Moreover, it jointly provides an estimation of the uncertain parameter μ_m which converges to the true value.

5.2 Animal cell culture

Another case study is inspired from the simulated example of an animal cell culture presented in (Perrier *et al.* 2000). The process model is based on the following reaction scheme

$$k_1 S \xrightarrow{\varphi_1} \overset{\curvearrowleft}{X} \tag{30}$$

$$k_2 S \xrightarrow{\varphi_2} \overset{\curvearrowleft}{X} + k_3 L \tag{31}$$

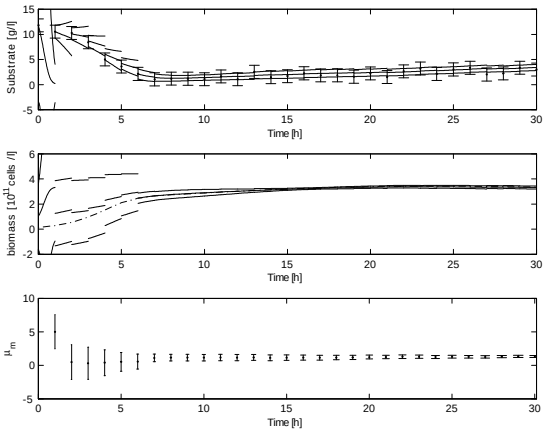

Fig. 2. Simple microbial growth adaptive observation $\hat{X}(0) = 10^{11} cells/\,\mathrm{l}$, $\hat{\mu}_{\max}(0) = 5\,\mathrm{h^{-1}}$, — : estimated signal with confidence intervals : 99% and - - - : real signal.

The mass balances corresponding to this reaction scheme are :

$$\frac{dS}{dt} = -k_1 \varphi_1 - k_2 \varphi_2 - DS + DS^{in} \tag{32}$$

$$\frac{dX}{dt} = \varphi_1 + \varphi_2 - DX \tag{33}$$

$$\frac{dL}{dt} = k_3 \varphi_2 - DL \tag{34}$$

The reaction rates φ_1 and φ_2 are described using the following models:

$$\varphi_1 = X \frac{\mu_{m,1} S}{K_R + S} \frac{K_L}{K_L + L} \tag{35}$$

$$\varphi_2 = X \frac{\mu_{m,2} S}{K_F + S} \tag{36}$$

The numerical values used for the simulation are identical to those used in (Perrier *et al.* 2000). Only, the feeding profile is modified :$D = \frac{F^{in}}{V}$, $F^{in} = 1\,\mathrm{l\,h^{-1}}$, $S^{in} = 0.05\,\mathrm{mol\,l^{-1}}$.

As in the previous example the simulated process will be considered as the real process. The lactate and substrate are supposed to be measured every 3 hours with a white noise of zero mean $E[\varepsilon] = 0$ and standard deviation $\sigma = 0.3mM$. Since in this case two state measurements are available, introducing the biomass state estimation from the AO allows to use the adaptive observer to estimate two kinetic parameters and to provide a state estimation from the corrected model and initial conditions. Figure (3) and (4) show these results when an error is introduced on the initial estimates of $\mu_{m,1}$ and $\mu_{m,2}$.

6. CONCLUSION

This contribution proposes an adaptive observer used to estimate bioprocess state variables with

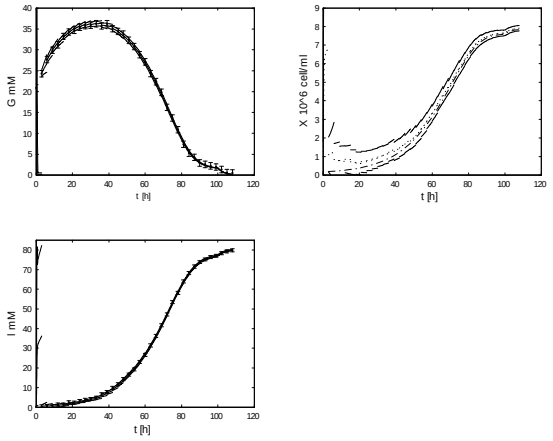

Fig. 3. Animal cell culture adaptive state observation. $\hat{X}(0) = 10^6 cells/\,\mathrm{ml}$, $\hat{\mu}_{m,1}(0) = 5\,\mathrm{h}^{-1}$ and $\hat{\mu}_{m,2}(0) = 2\,\mathrm{h}^{-1}$, — : adaptive observation with confidence intervals : 99%. - - - : real signal.

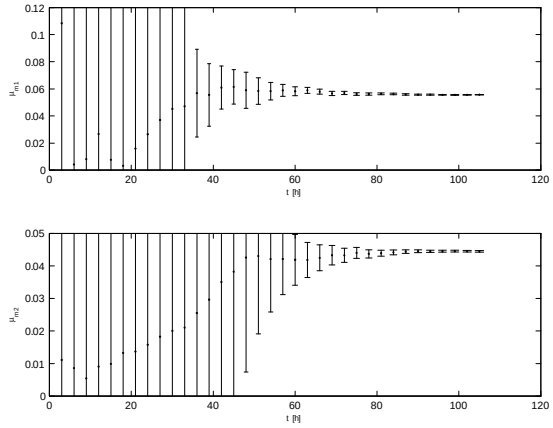

Fig. 4. Parameter estimation with confidence intervals : 99%. $\hat{\mu}_{m,1}(0) = 5\,\mathrm{h}^{-1}$ and $\hat{\mu}_{m,2}(0) = 2\,\mathrm{h}^{-1}$

estimated and corrected kinetic parameters. The theory of this adaptive observer is based on the full horizon observer. The unknown or uncertain kinetic parameters are estimated jointly with the initial conditions using a maximum likelihood estimator. In order to allow this parameter extension, the information from the non-measured state provided by an AO is introduced in the cost function. The initial conditions and the uncertain kinetic parameters are therefore identified on the basis of the measurements and the state observation from an AO. The corrected model is then integrated in order to provide a complete and continuous state estimation. Since this observer is based on the full horizon observer theory it exploits its main advantages i.e.: a true non linear stochastic approach, directly designed from the process model, involving a technique previously developed for bioprocess model parameter estimation and provides a continuous state estimation from discrete and rare measurements which is particularly realistic in the field of bioprocesses. Finally, this observer was illustrated on two simulated examples in order to show its ability to provide biomass observation and up to two parameters estimation from only two measurements.

REFERENCES

Bastin, G. and D. Dochain (1986). On-line estimation of microbial growth rates. *Automatica* **22**, 705–711.

Bastin, G. and D. Dochain (1990). *On-line estimation and adaptive control of bioreactors*. Elsevier. Amsterdam. coucou.

Bogaerts, Ph. ((1999)). *Contribution à la modélisation mathématique pour la simulation et l'observation d'état des bioprocédés, PhD thesis*. Université libre de Bruxelles.

Bogaerts, Ph. and R. Hanus (2000). *Macroscopic modelling of bioprocesses with a view to engineering applications. In : Focus on Biotechnologyv (Ph. Thonart and M. Ed. Hofman)*. Vol. **5** (Engineering and Manufacturing for biotechnology). Kluwer Academic Publishers.

Bogaerts, Ph. and R. Hanus (2001). On-line estimation of bioprocesses with full horizon observers. *Mathematics and Computers in Simulation* **56**, 425–441.

Dochain, D. (2003). State and parameter estimation in chemical and biochemical processes: a tutorial. *Journal of Process Control* **13**, 801–818.

Farza, M., H. Hammouri, S. Othman and K. Busawon (1997). Nonlinear observers for paramter estimation in bioprocesses. *Chemical Engineering Science* **52**(23), 4251–4267.

Gauthier, J;-P. and I. Kupka (1994). Observability and observers for nonlinear systems.. *SIAM Journal on Control and Optimization* **32**, 975–994.

Hulhoven, X. and Ph. Bogaerts (2004). stochastic full horizon-asymptotic hybrid observer applied to a simulated cell culture. In: *Proceedings of the Computer Application in Biotechnology Conference (Cab'9)*. IFAC. Nancy.

Oliveira, R., E. C. Ferreira and S. Feyo de Azevedo (2002). Stability dynamics of convergence and tuning of observer-based kinetics estimators. *Journal of Process Control* **12**, 311–323.

Oliveira, R., E. C. Ferreira, F. Oliveira and S. Feyo de Azevedo (1996). A study on the convergence of observer-based kinetics estimators in stirred tank bioreactors. *Journal of Process Control* **6**, 367–371.

Perrier, M., S. Feyo de Azevedo, E. C. Ferreira and D. Dochain (2000). Tuning of observer-based estimators: theory and application to the on-line estimation of kinetic parameters. *Control Engineering Practice* **8**, 377–388.