DESIGN OF ON-LINE STATE ESTIMATORS FOR A RECOMBINANT *E. COLI* FED-BATCH FERMENTATION

Ana C. A. Veloso[1,2], I. Rocha[1] and E. C. Ferreira[1]

[1]*Centro de Engenharia Biológica, Universidade do Minho*
4710-057 Braga, PORTUGAL
[2]*Escola Superior Agrária de Bragança, Campus de Santa Apolónia, Apartado 172*
5301-855 Bragança, PORTUGAL

e-mail: {anaveloso, irocha, ecferreira}@deb.uminho.pt

Abstract: In recent years a remarkable effort has been made in the development of new sensors and process analytical technology. However, it is still difficult to find reliable and low cost commercial sensors for on-line measurements of important variables. Therefore, considerable attention has been focused on the development of on-line software sensors. Nevertheless, the application of those algorithms to complex biological processes is still very incipient. In this work two different state estimators have been studied regarding their applicability to the recombinant *Escherichia coli* fed-batch fermentation. Both algorithms showed the ability to estimate on-line biomass and acetate concentrations. However, the extended Kalman observer exhibited a better convergence in spite of being less flexible regarding the combination of the measured and estimated variables. *Copyright © 2005 IFAC*

Key-words: Soft sensors, Non-linear systems, State estimation, Fed-batch fermentation, *E. coli*, Extended Kalman Observer, Asymptotic Observer.

1. INTRODUCTION

Nowadays, the ability to accurately and automatically control bioprocesses at their optimal state is of paramount importance to many industries since it can contribute for decreasing the production costs and increase the yield, keeping the quality of the metabolic products. However, the main difficulties in the design of monitoring and control systems for biological processes lie in the lack of cheap and reliable sensors capable of providing direct and on-line measurements of the biological state variables, together with the significant model uncertainty and the non-linear and time-varying nature of the system.

State observers, also called software sensors (Dochain, 2003), have received in recent years an increased attention since they allow on-line monitoring of state variables that are not measurable in real time (Assis and Filho, 2000; Valdés *et al.*, 2003; Bernard and Gouzé, 2004, Bogaerts and Wouwer, 2004).

In the literature, two classes of state observers are usually found. The first class includes the classical observers, such as the Luenberger and Kalman observers, and the non-linear observers, which are based on the perfect knowledge of both model structure and parameters. On the other hand, the uncertainty in the model parameters can generate a large bias in the estimation of unmeasured state(s). The asymptotic observers (Bastin and Docahin, 1990), which constitute the second class of observers, do not require the knowledge of the process kinetics. Nevertheless, a potential problem concerning these observers is the dependence of the estimation

convergence rate on the operating conditions (Dochain, 2003).

However, in spite of the well-developed theory behind some state observers, not many documented examples exist where those algorithms are applied to complex bioprocesses, described by dynamical models containing several balance equations and with complex kinetics.

In this work, the high-cell density fed-batch fermentation of *Escherichia coli* is studied in terms of applicability of state observers for the on-line estimation of relevant variables of the process. The importance of this process for the biopharmaceutical industry is widely recognized as *E. coli* represents the organism of choice for the production of many recombinant proteins. However, several state variables are not easily measured on-line during this process, posing additional difficulties for the implementation of control algorithms. As an example, in spite of its important role for model predictive control, estimation of specific growth rates, prevention of acetate accumulation and optimization of the production of recombinant proteins (regarding both productivity and moment of induction), biomass concentration is still very difficult to measure on-line for this fermentation process.

On the other hand, Flow Injection Analysis (FIA) methods may provide on-line data for glucose, the carbon source, and acetate, the main by-product (Rocha and Ferreira, 2002). This on-line information can be used by the software sensors for the estimation of the remaining variables included in the mathematical model, in what can be regarded as one step towards the complete characterization of the process. Simultaneously, the self-developed modular supervisory system facilitates the integration of different measurements, the on-line estimation of variables and the application of those measurements in control algorithms.

2. PROCESS MODELLING

The dynamics of a reaction network in a stirred tank bioreactor can be described by the following mass balance equations written in matrix form as (Bastin and Dochain, 1990):

$$\frac{d\xi}{dt} = Kr(\xi,t) - D\xi + F - Q \qquad (1)$$

in which $\boldsymbol{\xi}$ is a vector representing the n state components concentrations ($\boldsymbol{\xi} \in \Re^n$), $\mathbf{r}$ is the growth rate vector corresponding to m reactions ($\mathbf{r} \in \Re^m$), $\mathbf{K}$ is the matrix of yield coefficients ($\mathbf{K} \in \Re^{n\times m}$), $\mathbf{F}$ is the vector of feed rates and $\mathbf{Q}$ is the vector of gaseous outflow rates ($\mathbf{F}$, $\mathbf{Q} \in \Re^n$), D is the dilution rate (being D^{-1} the residence time).

As previously presented (Rocha and Ferreira, 2004), during the aerobic growth of *E. coli* with glucose as the only added substrate, the microorganism can follow three main metabolic pathways: oxidative growth on glucose, fermentative growth on glucose, and oxidative growth on acetate, the corresponding dynamical model for fed-batch fermentation being represented as follows:

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ A \\ O \\ C \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 \\ -k_1 & -k_2 & 0 \\ 0 & k_3 & -k_4 \\ -k_5 & -k_6 & -k_7 \\ k_8 & k_9 & k_{10} \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ A \\ O \\ C \end{bmatrix} + \begin{bmatrix} 0 \\ \left(\frac{F_{in}}{W}\right) S_{in} \\ 0 \\ OTR \\ 0 \end{bmatrix} - \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ CTR \end{bmatrix} \qquad (2)$$

where X, S, A, O, and C represent biomass, glucose, acetate, dissolved oxygen, and dissolved carbon dioxide concentrations, respectively; μ_1, μ_2, and μ_3 are the specific growth rates; k_i are the yield (stoichiometric) coefficients; F_{in} and S_{in} are the substrate feed rate and the influent glucose concentration, respectively; W is the culture medium weight. CTR is the carbon dioxide transfer rate from liquid to gas phase, and OTR is the oxygen transfer rate from gas to liquid phase.
The variation with the time of the culture medium weight is given by:

$$\frac{dW}{dt} = F \qquad (3)$$

where F takes into account, weight variations due to the substrate feed rate, the amount of culture removed or added during sampling, base and acid additions, evaporation and mass taken from the reactor due to gas exchanges, that can not be considered negligible in small-scale high-cell density reactors.

However, the three metabolic pathways represented in the mathematical model do not occur simultaneously in the cell, originating four partial models corresponding to the oxidative and fermentative growth on glucose regimen (μ_1,μ_2,>0) to the oxidative growth on glucose and acetate regimen (μ_1, μ_3, >0), and to the oxidative growth on glucose (μ_1>0) or acetate (μ_3>0) regimens.

3. DERIVATION OF OBSERVERS

Two different observers for state estimation are studied: the Kalman and the asymptotic observers.

3.1. Extended Kalman Observer (EKO)

When high nonlinearities are included in the mathematical model of the process, the extended version of the Kalman observer should be used (Biagiola and Figueroa, 2004).

In order to obtain the EKO, the following assumptions are made: (i) a full knowledge of the model is available: the structure of the reaction kinetics **r(ξ,t)** is completely known; also the numerical values of all the coefficients involved in the model (yield and kinetic coefficients) are given; and (ii) **D**, **F** and **Q** are known on-line, together with a *q* subset of state variables.

This vector of state variables measured is denoted $\boldsymbol{\xi_1}$ and is related to the state of the system as follows:

$$\xi_1 = L\xi \tag{4}$$

where the $q \times n$ matrix **L** is an elementary matrix which selects the measured components of $\boldsymbol{\xi}$. On the other hand, the vector of unmeasured states is denoted $\boldsymbol{\xi_2}$, so that $(\boldsymbol{\xi_1},\boldsymbol{\xi_2})$ constitutes a partition of $\boldsymbol{\xi}$.

A general class of state observers for nonlinear systems of the form of eq. (1) is as follows:

$$\frac{d\hat{\xi}}{dt} = Kr\left(\hat{\xi},t\right) - D\hat{\xi} + F - Q + \Omega\left(\hat{\xi},t\right)\left[\xi_1 - \hat{\xi}_1\right] \tag{5}$$

where $\hat{\boldsymbol{\xi}}$ denotes the on-line estimate of $\boldsymbol{\xi}$, and $\boldsymbol{\Omega}\left(\hat{\boldsymbol{\xi}},\mathbf{t}\right)$ is an $n \times q$ gain matrix depending on $\hat{\boldsymbol{\xi}}$. The state observer design problem is then reduced to that of a reasonable choice of the gain matrix $\boldsymbol{\Omega}\left(\hat{\boldsymbol{\xi}},\mathbf{t}\right)$. To solve this problem, the observation error is introduced at this point, $e = \xi - \hat{\xi}$, and its dynamics deduced (Bastin and Dochain, 1990). Considering a linearized tangent approximation of the dynamical model of the observation error around *e=0* will give:

$$\frac{de}{dt} = \left[M\left(\hat{\xi}\right) - \Omega\left(\hat{\xi}\right)L\right]e \tag{6}$$

with:

$$M\left(\hat{\xi}\right) \equiv K\left[\frac{\partial r\left(\xi,t\right)}{\partial \xi}\right]_{\xi=\hat{\xi}} - DI_N \tag{7}$$

where $\mathbf{I_N}$ is the $n \times n$ identity matrix.

Considering that the model of eq. (1) is exponentially observable, the design of the EKO is then reduced to the quadratic optimisation problem of finding the matrix $\boldsymbol{\Omega}\left(\hat{\boldsymbol{\xi}},\mathbf{t}\right)$ that minimises the mean square observation error taking into account the constraint of the linear tangent error model (eqs. 6 and 7). The solution of this optimisation problem is given by:

$$\Omega\left(\hat{\xi},t\right) = R\left(\hat{\xi},t\right)L^T \tag{8}$$

where the $n \times n$ square symmetric matrix $\mathbf{R}\left(\hat{\boldsymbol{\xi}},\mathbf{t}\right)$ is generated by the Riccati equation:

$$\frac{dR}{dt} = -RL^T LR + RM^T\left(\hat{\xi},t\right) + M\left(\hat{\xi},t\right)R \tag{9}$$

For the fed-batch *E. coli* fermentation considered in this work, the exponential observability condition (Bastin and Dochain, 1990) was studied for 9 different combinations of measured and estimated variables for checking the applicability of the EKO for this particular process. Each of those cases is classified as: observable for the situation when the full model (FM) described by eq. (2) is used, observable only under some of the regimens described in section 2 (described by a partial model – PM), or as not observable. Those results are illustrated in table 1, and it can be concluded that the EKO can be applied to *E. coli* fed-batch fermentation in a limited number of situations. However, it is clear that, with the developed on-line FIA system for the analysis of glucose and / or acetate, together with state-of-the-art sensors for measuring dissolved oxygen and carbon dioxide, it is possible to estimate on-line biomass and another state variable. Also, even in the absence of a FIA system and using only the mentioned commercial sensors for dissolved gases (case 4), it is possible to estimate 3 important state variables during the course of the fermentation, if the cells do not exhibit only the oxidative growth on acetate or the oxidative growth on glucose regimens.

Table 1 Observability of the model for different combinations of the measured and estimated variables using the EKO and the AO. *OTR*, *CTR*, *W* and *F* are measured on-line for all cases.

Case	Measured variables	Estimated variables	EKO	AO
1	A, O, C	X, S	FM	FM
2	S, A, O	X, C	Not Obs.	FM
3	S, O, C	X, A	FM	FM
4	O, C	X, S, A	PM	PM
5	A, O	X, S, C	Not Obs.	PM
6	S, O	X, A, C	Not Obs.	PM
7	S, C	X, A, O	Not Obs.	PM
8	S, A	X, O, C	Not Obs.	PM
9	A, C	X, S, O	Not Obs.	PM

Taking the example of measuring on-line the state variables *A*, *O* and *C* (case 1), the following state partition is chosen: $\xi_1^T = [A \quad O \quad C]$ and $\xi_2^T = [X \quad S]$. The matrix **L** is as follows:

$$L = \begin{bmatrix} 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \tag{10}$$

The matrix $M(\hat{\xi}) = M(\hat{X}, \hat{S}, \hat{A}, \hat{O}, \hat{C})$ is given by eq. (7), where:

$$\left[\frac{\partial r(\xi,t)}{\partial \xi}\right]_{\xi=\hat{\xi}} = \begin{bmatrix} \frac{\partial(\mu_1\hat{X})}{\partial\hat{X}} & \frac{\partial(\mu_1\hat{X})}{\partial\hat{S}} & \frac{\partial(\mu_1\hat{X})}{\partial\hat{A}} & \frac{\partial(\mu_1\hat{X})}{\partial\hat{O}} & \frac{\partial(\mu_1\hat{X})}{\partial\hat{C}} \\ \frac{\partial(\mu_2\hat{X})}{\partial\hat{X}} & \frac{\partial(\mu_2\hat{X})}{\partial\hat{S}} & \frac{\partial(\mu_2\hat{X})}{\partial\hat{A}} & \frac{\partial(\mu_2\hat{X})}{\partial\hat{O}} & \frac{\partial(\mu_2\hat{X})}{\partial\hat{C}} \\ \frac{\partial(\mu_3\hat{X})}{\partial\hat{X}} & \frac{\partial(\mu_3\hat{X})}{\partial\hat{S}} & \frac{\partial(\mu_3\hat{X})}{\partial\hat{A}} & \frac{\partial(\mu_3\hat{X})}{\partial\hat{O}} & \frac{\partial(\mu_3\hat{X})}{\partial\hat{C}} \end{bmatrix} \tag{11}$$

The observer is then written from eqs. (1) and (5) with the last term of eq. (5) defined as:

$$\Omega(\hat{\xi},t)\left[\xi_1 - \hat{\xi}_1\right] = \begin{bmatrix} \Omega_1 & \Omega_2 & \Omega_3 \\ \Omega_4 & \Omega_5 & \Omega_6 \\ \Omega_7 & \Omega_8 & \Omega_9 \\ \Omega_{10} & \Omega_{11} & \Omega_{12} \\ \Omega_{13} & \Omega_{14} & \Omega_{15} \end{bmatrix} \begin{bmatrix} A-\hat{A} \\ O-\hat{O} \\ C-\hat{C} \end{bmatrix} \tag{12}$$

The gain $\boldsymbol{\Omega}(\hat{X}, \hat{S}, \hat{A}, \hat{O}, \hat{C})$ is calculated from eq. (8) as follows:

$$\Omega(\hat{\xi}) = \begin{bmatrix} \Omega_1 & \Omega_2 & \Omega_3 \\ \Omega_4 & \Omega_5 & \Omega_6 \\ \Omega_7 & \Omega_8 & \Omega_9 \\ \Omega_{10} & \Omega_{11} & \Omega_{12} \\ \Omega_{13} & \Omega_{14} & \Omega_{15} \end{bmatrix} = \begin{bmatrix} R_7 & R_8 & R_9 \\ R_{10} & R_{11} & R_{12} \\ R_3 & R_{13} & R_{14} \\ R_{13} & R_4 & R_{15} \\ R_{14} & R_{15} & R_5 \end{bmatrix} \tag{13}$$

With the matrix **R** defined as:

$$R = \begin{bmatrix} R_1 & R_6 & R_7 & R_8 & R_9 \\ R_6 & R_2 & R_{10} & R_{11} & R_{12} \\ R_7 & R_{10} & R_3 & R_{13} & R_{14} \\ R_8 & R_{11} & R_{13} & R_4 & R_{15} \\ R_9 & R_{12} & R_{14} & R_{15} & R_5 \end{bmatrix} \tag{14}$$

The only tuning parameters for this observer are the initial values of the elements of this matrix, necessary for the numerical solution of eq. (9).

3.2. Asymptotic Observer (AO)

The AO allows reconstructing the missing states variables even when the process is not exponentially observable and the kinetics are unknown. The following additional assumptions should also be observed for the design of these observers: (i) the yield coefficients (matrix **K**) is known; and (ii) the number q of measured state variables is equal to or greater than the rank of the matrix **K**: $q=\dim(\xi)\geq p=\text{rank}(K)$ (Bastin and Dochain, 1990).

Considering a partition in the state variables vector $\boldsymbol{\xi}$ induced by the measured and unmeasured variables as in the previous case, the dynamical model can be re-written as follows:

$$\frac{d\xi_1}{dt} = K_1 r(\xi,t) - D\xi_1 + F_1 - Q_1 \tag{15a}$$

$$\frac{d\xi_2}{dt} = K_2 r(\xi,t) - D\xi_2 + F_2 - Q_2 \tag{15b}$$

The following state transformation can then be defined:

$$Z \equiv \xi_2 - K_2 K_1^{-1} \xi_1 \tag{16}$$

Where $\mathbf{K_1^{-1}}$ is the pseudo-inverse of the matrix $\mathbf{K_1}$, considering that $\mathbf{K_1}$ has full rank. $\mathbf{K_1}$ and $\mathbf{K_2}$ are obtained from the matrix **K** applying the induced partition.

The dynamics of **Z**, are independent of reaction rate **r(ξ,t)**:

$$\frac{dZ}{dt} = -DZ - K_2 K_1^{-1}(F_1 - Q_1) + (F_2 - Q_2) \tag{17}$$

Finally, the equation of the AO is given by:

$$\frac{d\hat{Z}}{dt} = -D\hat{Z} - K_2 K_1^{-1}(F_1 - Q_1) + (F_2 - Q_2) \tag{18a}$$

$$\hat{\xi}_2 = \hat{Z} + K_2 K_1^{-1} \xi_1 \tag{18b}$$

Unlike the EKO, the speed of convergence of the estimation is completely determined by the experiment condition through the value of the dilution rate, implying that $D(t)$ does not remain equal to zero for excessively long period of time (Bastin and Dochain, 1990).

For the *E. coli* model of eq. (2), if the three reactions are to be considered, and due to the limitation imposed by the condition $q\geq p=\text{rank}(K)$, the number of measured variables has to be equal or grater than 3. In this case, as opposed to the EKO, all the combinations of 3 measured variables are theoretically possible. The same occurs when a partial model is considered for the measurement of 2 state variables, as illustrated in table 1.

If the measured variables are, for example *A*, *O* and *C* as in the previous case, the matrix used in the state transformation of eq. (16) will be:

$$K_2 K_1^{-1} = \begin{bmatrix} 1 & 1 & 1 \\ -k_1 & -k_2 & 0 \end{bmatrix} \begin{bmatrix} 0 & k_3 & -k_4 \\ -k_5 & -k_6 & -k_7 \\ k_8 & k_9 & k_{10} \end{bmatrix}^{-1} = \begin{bmatrix} \alpha_1 & \alpha_2 & \alpha_3 \\ \alpha_4 & \alpha_5 & \alpha_6 \end{bmatrix} \quad (19)$$

The observer, in this case, is given by the following equations:

$$\frac{d}{dt}\begin{bmatrix} \hat{Z}_1 \\ \hat{Z}_2 \end{bmatrix} = -D\begin{bmatrix} \hat{Z}_1 \\ \hat{Z}_2 \end{bmatrix} + \begin{bmatrix} 0 \\ \frac{F_{in}}{W} S_{in} \end{bmatrix} - \begin{bmatrix} \alpha_1 & \alpha_2 & \alpha_3 \\ \alpha_4 & \alpha_5 & \alpha_6 \end{bmatrix} \begin{bmatrix} 0 \\ OTR \\ -CTR \end{bmatrix} \quad (20a)$$

$$\begin{bmatrix} \hat{X} \\ \hat{S} \end{bmatrix} = \begin{bmatrix} \hat{Z}_1 \\ \hat{Z}_2 \end{bmatrix} + \begin{bmatrix} \alpha_1 & \alpha_2 & \alpha_3 \\ \alpha_4 & \alpha_5 & \alpha_6 \end{bmatrix} \begin{bmatrix} A \\ O \\ C \end{bmatrix} \quad (20b)$$

One of the main advantages of this class of observers is that there are no tuning parameters like in the previous case, as the initial values of the *Z* variables can be directly obtained from eqs. (18b) or (20b) replacing the values of the estimated variables by their experimental initial values, usually known.

4. RESULTS AND DISCUSSION

The model simulations were performed by solving the differential equations of eq. (2) using the MATLAB version 6 subroutine ODE23s. The implementation of the observers using both "experimental" and simulated data was conducted using the Euler integration method. The observability of the model, together with most of the mathematical operations behind the design of the state observers was performed using the Symbolic Math toolbox running in MATLAB 6.

For validating the developed observer algorithms and for performance comparison between the EKO and the AO, the most relevant cases from table 1 were selected for simulation: case 1 and case 4. During the simulation, "real" values of the state variables were obtained by integration of the differential equation of eq. (2). These "real" values were then corrupted with white noise, according to the standard deviations typically found in this process at the authors' lab, originating "experimental" values. Then, the observer algorithms were used to obtain the "estimated" variables from the "experimental" data corresponding to the measured variables.

The performance of the observers was evaluated by calculating the quadratic difference between "experimental" and "estimated" data, according to the following equation:

$$dif_{\xi} = \sum_{j=1}^{np} \left(\frac{\xi_{exp,j} - \xi_{est,j}}{\overline{\xi}_{exp,j}} \right)^2 \quad (21)$$

where *np* is the number of experimental points and ξ_{exp} and ξ_{est} are "experimental" and "estimated" values of the state variable ξ..

The results of the performance index for both state observers in cases 1 and 4 are shown in table 2. While, as mentioned before, the EKO is much less flexible in terms different combinations of measured and estimated variables, its performance is superior to the AO under similar conditions. Additionally, the AO is less robust regarding experimental errors, being the performance improved when the "experimental" data are affected by lower noise. However, it should be noticed that the EKO requires a much higher sampling rate than AO (0.3 and 3 minutes, respectively), which could be a problem regarding experimental implementation.

This fact is possibly due to the exponential convergence of the EKO, as opposed to the asymptotical convergence of the AO. Another issue that has to be considered is the low value of the dilution rate for the experiment studied, which can negatively influence the performance of the AO.

In spite of this lower performance, the AO can still be used with confidence for the estimation of both biomass and acetate concentrations.

Table 2 Performance index for both EKO and AO for cases 1 and 4 of table 1

Observer	Case	difX	difS	difA
EKO	1	6.70	8.23	---
	4	6.93	7.93	33.7
AO	1	63.9	1.21E7	---
	4	53.5	1.05E7	50.7

In Figure 1 the fermentation used for calculating the performance indexes is characterized in terms of "experimental" and "estimated" variables. It can be seen that, although the errors associated with some of the measured variables is significantly high, the EKO performs well in the estimation of the variables that are not measured on-line.

5. CONCLUSIONS

During a fed-batch *E. coli* fermentation process, variables such as biomass concentration are determined using off-line laboratory analysis, making them of limited use for control purposes. However, these variables can be on-line estimated using software sensors.

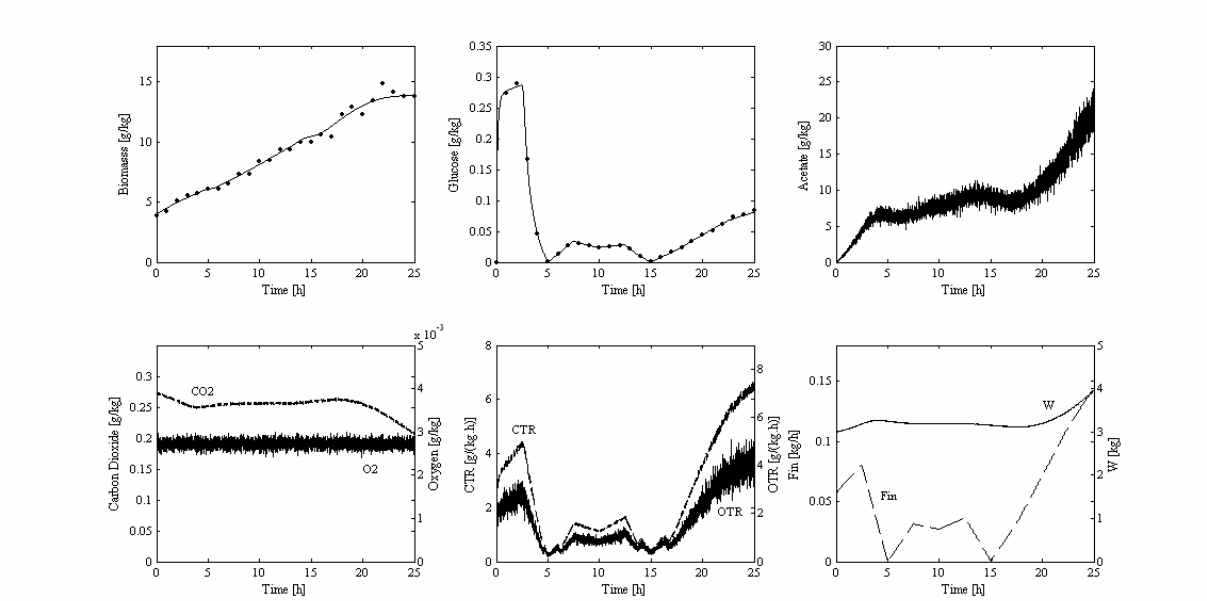

Figure 1 Performance of the EKO regarding the time evolution of relevant variables in a fed-batch fermentation of *E. coli*. Measured variables are A, O, and C, while the estimated variables are X and S. For the estimated variables, the points correspond to experimental data and the lines are the estimated values. The other variables used for state estimation are also shown: CTR, OTR, F_{in} and W.

In this work, two state observer algorithms were applied to the estimation of several non-measured state variables, and their performance and flexibility were compared. From simulation studies, it can be concluded that the extended Kalman observer is less flexible regarding the choice of the measured variables, but its performance is superior to the asymptotic observer. Nevertheless, this algorithm can still be applied to the on-line estimation of biomass and acetate. Experimental validation of these results is under progress.

ACKNOWLEDGEMENTS

A.C.A. Veloso and I. Rocha are most grateful for the financial support provided by PRODEP and FCT, respectively.

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