DESIGN OF INTERVAL OBSERVERS FOR AN *E. COLI* FED-BATCH FERMENTATION WITH UNCERTAIN INPUTS

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Abstract

In bioreactors, the measurement of variables that play a key role in the quality and productivity of fermentations, is of major importance. However, their direct measurement is often expensive or even impossible considering the current sensor technology. Therefore, on-line estimation of unmeasured variables in bioreactors can be an interesting approach.

The objective of this work is to introduce an alternative solution for the state observation of bioprocesses in cases where the kinetic model is unclear and the concentration of the influent substrates is badly known, a situation that is common in many practical applications.

The high-cell density fed-batch fermentation of *Escherichia coli* is studied in terms of applicability of a simple interval observer for the estimation of relevant variables of the process, when uncertainties of the process inputs exist.

The simple interval observer is designed on the basis of the cooperativity properties of the observer error dynamics (Rapaport and Dochain, 2005). Further assumptions are the knowledge of the (lower and upper) bounds of the influent substrate concentration. Furthermore, an appropriate state transformation and conditions that guarantee system cooperativity have been introduced for that purpose.

The performance of the interval observer is illustrated through numerical simulation.

1 Introduction

It is well known that industries are interested in decreasing the production costs and increasing the process yield, keeping the quality of the metabolic products. Thus, the ability to accurately and automatically control bioprocesses at their optimal state is of great importance, since it can contribute to achieve that goal. However, the lack of on-line instruments has limited the application of control theory to these processes. Therefore, the development of state observers, also called software sensors (Dochain, 2003) can be an attractive alternative since a large amount of additional information can be obtained, using a model together with a limited set of state variable measurements (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, the Kalman, and the non-linear observers, which are based on the perfect knowledge of both model structure and parameters. However, the uncertainty in the model parameters can generate a large bias in the estimation of unmeasured state(s). The asymptotic observers (Bastin and Dochain, 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).

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In this work it is intended to study an alternative solution for the state observation of a high-cell density fed-batch fermentation of *E. coli*, supposing that the kinetics model structure is unknown and the concentration of influent substrates is badly known, a situation that happens often in real applications, for example when using complex substrates..

The approach used is based on an interval analysis. The objective is to reconstruct intervals for the missing state variables, for which the state is certain to lie, based on a given interval of variation of the uncertain variable(s).

In this study, the design of the interval observers is based on the assumption that measurements of acetate, dissolved oxygen and carbon dioxide concentrations are available. This choice is due to the fact that, nowadays, the sensors for these state variables are more developed and thus, more reliable. Therefore, the purpose is to estimate the intervals of variation of the biomass and substrate concentrations.

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):

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

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

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 can be represented as follows:

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

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 of the culture medium weight with the time is given by:

$$\frac{dW}{dt} = F \tag{3}$$

where F includes 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.

A typical observation question is the estimation of biomass and glucose concentrations from on-line measurements of acetate, dissolved oxygen and carbon dioxide concentrations. It is assumed that the kinetics are unknown, the dilution rate D and the yield coefficients (matrix K) are known, and that the influent glucose concentration S_{in} is uncertain but bounded between known lower and upper bounds:

$$S_{in}^{-} \leq S_{in}(t) \leq S_{in}^{+} \tag{4}$$

3. Design of the Interval Observer

The motivation of interval observers is to generate state estimates with bounds that are related to the uncertainty of the model or of the measurements (Gouzé *et al.*, 2000).

The design is based on the cooperativity properties of the observer error dynamics. Cooperative systems are dynamical systems for which the non-diagonal terms of the Jacobian matrix are positive (Rapaport and Dochain, 2005). Considering the following non-linear state space model:

$$\frac{dx}{dt} = f(t, x) \tag{5}$$

cooperation means that for any (t, x):

$$\frac{\partial f_i}{\partial x_j}(t, x) \geq 0, \qquad \text{for } i \neq j \tag{6}$$

Considering the cooperative system (5 and 6) and being f^{-} and f^{+} two vector fields such that:

$$f^{-}(x) \leq f(t, x) \leq f^{+}(x), \quad \forall (t, x) \tag{7}$$

and the initial conditions x_0^{-}, x_0, x_0^{+} (such that $x_0^{-} \leq x_0 \leq x_0^{+}$) the solution of the dynamical system is:

$$\frac{d}{dt}\begin{bmatrix} x^{-} \\ x \\ x^{+} \end{bmatrix} = \begin{bmatrix} f^{-}(x^{-}) \\ f(t, x) \\ f^{+}(x^{+}) \end{bmatrix}, \quad \begin{bmatrix} x^{-}(0) \\ x(0) \\ x^{+}(0) \end{bmatrix} = \begin{bmatrix} x_0^{-} \\ x_0 \\ x_0^{+} \end{bmatrix} \tag{8}$$

and fulfills the following property:

$$x^{-}(t) \leq x(t) \leq x^{+}(t), \quad t \geq 0 \tag{9}$$

Therefore, two estimates can be computed, an upper one and a lower one, that bounds the unmeasured variables. Since the best final estimate is aimed, the interval [$x^{-}(t)$, $x^{+}(t)$] should become smaller (or ideally tends to {$x(t)$}) when the time t increases (Gouzé *et al.*, 2000).

It should be noticed that interval observer $(\hat{X}, \hat{S})$ cannot be designed directly from an observer of the dynamical model given by eq. (2). In fact, the off-diagonal term of the Jacobian matrix (eq. (10)) of the observer (with $\bar{g}_1$, $\bar{g}_2$, $\bar{g}_3$, $\bar{g}_4$ and $\bar{g}_5$ the observer gains) does not fulfill the condition of eq. (6), as the off-diagonal terms $(-K\mu)$ and $\left(-K\frac{\partial \mu}{\partial S}X\right)$ are negatives:

$$J(\hat{X}, \hat{S}, \hat{A}, \hat{O}, \hat{C}) = \begin{bmatrix} (\mu - D) & \left(\frac{\partial \mu}{\partial S}X\right) & \left(\frac{\partial \mu}{\partial A}X + \bar{g}_1\right) & \bar{g}_1 & \bar{g}_1 \\ (-K\mu) & \left(-K\frac{\partial \mu}{\partial S}X - D\right) & \left(-K\frac{\partial \mu}{\partial A}X + \bar{g}_2\right) & \bar{g}_2 & \bar{g}_2 \\ (K\mu) & \left(K\frac{\partial \mu}{\partial S}X\right) & \left(K\frac{\partial \mu}{\partial A}X - D + \bar{g}_3\right) & \bar{g}_3 & \bar{g}_3 \\ (-K\mu) & \left(-K\frac{\partial \mu}{\partial S}X\right) & \left(-K\frac{\partial \mu}{\partial A}X + \bar{g}_4\right) & -D + OTR + \bar{g}_4 & \bar{g}_4 \\ (K\mu) & \left(K\frac{\partial \mu}{\partial S}X\right) & \left(K\frac{\partial \mu}{\partial A}X + \bar{g}_5\right) & \bar{g}_5 & -D - CTR + \bar{g}_5 \end{bmatrix} \tag{10}$$

Nevertheless, it should be notice that the notion of cooperativity is coordinates dependent, and therefore an approach to achieve this property is to consider a partition in the state variables vector ξ

induced by the measured and unmeasured variables, ξ_1 and ξ_2, respectively, the dynamical model given by eq. (2) can be re-written as follows:

$$\frac{d\xi_1}{dt} = K_1 r(\xi,t) - D\xi_1 + F_1 - Q_1 \tag{11a}$$

$$\frac{d\xi_2}{dt} = K_2 r(\xi,t) - D\xi_2 + F_2 - Q_2 \tag{11b}$$

The following transformation can be established:

$$Z \equiv \xi_2 - K_2 K_1^{-1} \xi_1 \tag{12}$$

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

The dynamics of Z are independent of the reaction rate $r(\xi,t)$ and the following equivalent state representation for the process dynamics can be written:

$$\frac{dZ}{dt} = -DZ - K_2 K_1^{-1}\left(F_1 - Q_1\right) + \left(F_2 - Q_2\right) \tag{13}$$

and the following standard observer equations can be also derived:

$$\frac{d\hat{Z}}{dt} = -D\hat{Z} - K_2 K_1^{-1}\left(F_1 - Q_1\right) + \left(F_2 - Q_2\right) \tag{14a}$$

$$\hat{\xi}_2 = \hat{Z} + K_2 K_1^{-1} \xi_1 \tag{14b}$$

If the measured variables are A, O and C the matrix used in the state transformation of eq. (12) will be:

$$K_2 K_1^{-1} = \begin{bmatrix} 1 & 1 & 1 \\ -k_1 & -k_2 & 0 \end{bmatrix} \begin{bmatrix} 0 & k_3 & -k_4 \\ -k_5 & -k_6 & -k_7 \\ k_8 & k_9 & k_{10} \end{bmatrix}^{-1} = \begin{bmatrix} \alpha_1 & \alpha_2 & \alpha_3 \\ \alpha_4 & \alpha_5 & \alpha_6 \end{bmatrix} \tag{15}$$

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

$$\frac{d}{dt}\begin{bmatrix} \hat{Z}_1 \\ \hat{Z}_2 \end{bmatrix} = -D\begin{bmatrix} \hat{Z}_1 \\ \hat{Z}_2 \end{bmatrix} - \begin{bmatrix} \alpha_1 & \alpha_2 & \alpha_3 \\ \alpha_4 & \alpha_5 & \alpha_6 \end{bmatrix} \begin{bmatrix} 0 \\ OTR \\ -CTR \end{bmatrix} + \begin{bmatrix} 0 \\ \frac{F_{in}}{W} S_{in} \end{bmatrix} \tag{16a}$$

$$\begin{bmatrix} \hat{X} \\ \hat{S} \end{bmatrix} = \begin{bmatrix} \hat{Z}_1 \\ \hat{Z}_2 \end{bmatrix} + \begin{bmatrix} \alpha_1 & \alpha_2 & \alpha_3 \\ \alpha_4 & \alpha_5 & \alpha_6 \end{bmatrix} \begin{bmatrix} A \\ O \\ C \end{bmatrix} \tag{16b}$$

The cooperative properties of this observer can then be checked. If the observer errors are defined by:

$$e_{Z_1} = \hat{Z}_1 - Z_1 \text{ and } e_{Z_2} = \hat{Z}_2 - Z_2 \tag{17}$$

Their dynamics are given by the following equation:

$$\frac{d}{dt}\begin{bmatrix} e_{Z_1} \\ e_{Z_2} \end{bmatrix} = -D\begin{bmatrix} e_{Z_1} \\ e_{Z_2} \end{bmatrix} \tag{18}$$

with the following Jacobian matrix:

$$J\left(e_{Z_1}, e_{Z_2}\right) = \begin{bmatrix} -D & 0 \\ 0 & -D \end{bmatrix} \tag{19}$$

It can easily be seen that the error system is cooperative and thus it is possible to build an interval observer.

Considering the lower and upper bounds for the initial value of the estimate of biomass and substrate concentrations:

$$X_0^- \le X_0 \le X_0^+, \qquad S_0^- \le S_0 \le S_0^+ \tag{20}$$

the following set of interval observer equations can be defined:

$$\frac{d}{dt}\begin{bmatrix}\hat{Z}_1^+\\ \hat{Z}_2^+\end{bmatrix} = -D\begin{bmatrix}\hat{Z}_1^+\\ \hat{Z}_2^+\end{bmatrix} - \begin{bmatrix}\alpha_1 & \alpha_2 & \alpha_3\\ \alpha_4 & \alpha_5 & \alpha_6\end{bmatrix}\begin{bmatrix}0\\ OTR\\ -CTR\end{bmatrix} + \begin{bmatrix}0\\ \frac{F_{in}}{W}S_{in}^+\end{bmatrix} \tag{21a}$$

$$\begin{bmatrix}\hat{X}^+\\ \hat{S}^+\end{bmatrix} = \begin{bmatrix}\hat{Z}_1^+\\ \hat{Z}_2^+\end{bmatrix} + \begin{bmatrix}\alpha_1 & \alpha_2 & \alpha_3\\ \alpha_4 & \alpha_5 & \alpha_6\end{bmatrix}\begin{bmatrix}A\\ O\\ C\end{bmatrix} \tag{21b}$$

$$\frac{d}{dt}\begin{bmatrix}\hat{Z}_1^-\\ \hat{Z}_2^-\end{bmatrix} = -D\begin{bmatrix}\hat{Z}_1^-\\ \hat{Z}_2^-\end{bmatrix} - \begin{bmatrix}\alpha_1 & \alpha_2 & \alpha_3\\ \alpha_4 & \alpha_5 & \alpha_6\end{bmatrix}\begin{bmatrix}0\\ OTR\\ -CTR\end{bmatrix} + \begin{bmatrix}0\\ \frac{F_{in}}{W}S_{in}^-\end{bmatrix} \tag{21c}$$

$$\begin{bmatrix}\hat{X}^-\\ \hat{S}^-\end{bmatrix} = \begin{bmatrix}\hat{Z}_1^-\\ \hat{Z}_2^-\end{bmatrix} + \begin{bmatrix}\alpha_1 & \alpha_2 & \alpha_3\\ \alpha_4 & \alpha_5 & \alpha_6\end{bmatrix}\begin{bmatrix}A\\ O\\ C\end{bmatrix} \tag{21d}$$

This simple interval observer will then give estimates for the upper and lower bounds of both biomass and glucose concentrations X and S, respectively: X^+, X^- and S^+, S^-.

4. Simulation results and Discussion

The performance of the interval observer has been tested by numerical simulations. Simulated "experimental" values of the state variables were obtained by integration of the differential equations of eq. (2), using the MATLAB version 6 subroutine ODE23s. The implementation of the observer using both "experimental" and estimated data was conducted using the Euler integration method. It is assumed that glucose concentration in the feed S_{in} is unknown, but it is assumed that its time varying bounds are known ($S_{in}^-(t) \le S_{in}(t) \le S_{in}^+(t), \quad \forall t \ge 0$). It is also assumed that a priori bounds on initial values of X_0 and S_0 are known.

Therefore, the simulation results have been performed by considering that uncertainty is concentrated on the influent glucose concentration with 10% variation around its nominal value (S_{in} = 250 g/kg). The results obtained are presented in Figure 1. As it can be seen, the "experimental" values for biomass and glucose are always between the lower and the upper bounds estimated using the interval observer.

5. Conclusions

In this work, an interval observer is presented in order to handle the uncertainties on the influent glucose concentration. A key issue associated with interval observer is the cooperativity of the observer error dynamics. An appropriate state transformation and conditions that guarantee system cooperativity have been introduced for that purpose.

Nevertheless the good results obtained, experimental validation of this work is needed and is under investigation.

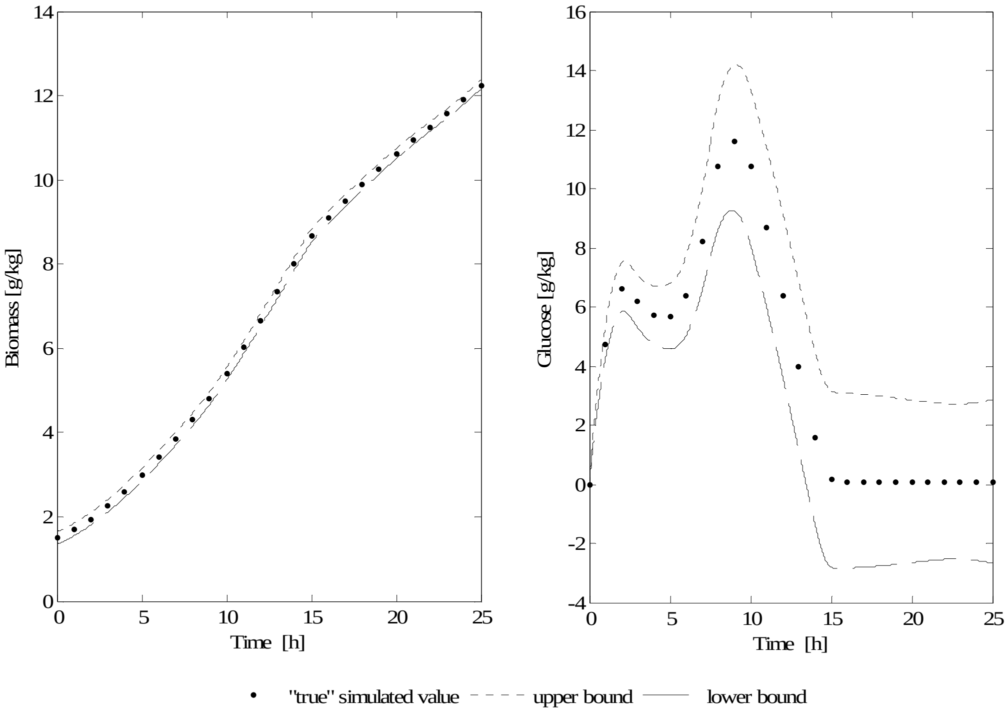

Figure 1. Interval observer simulation in presence of uncertainty on the influent glucose concentration.

Moreover, as pointed out by Rapaport and Dochain (2005) the interval observers principles used can also be applied in order to account for the uncertainties in the yield coefficients as well as for bounded noise on the outputs. Further studies taking into account the above-mentioned approaches are undergoing examination.

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