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PARAMETER IDENTIFICATION FOR STATE ESTIMATION OF A FED-BATCH BIOREACTOR: ANALYSIS THROUGH MODEL FALSIFICATION

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Abstract: State observers require a process model and some available measurements for reconstructing on-line unmeasured states. To enhance the sensitivity of these unmeasured states with respect to the measured ones, and in turn, to improve the quality of the estimates, a new cost function is discussed in this paper, which combines a maximum likelihood criterion with a sensitivity measure. Minimization of this combined cost function produces a model dedicated to state estimation purposes. A thorough analysis of the procedure is presented in the context of fed-batch bioreactor modeling, including parameter identification, model validation and design of extended Kalman filters and full horizon observers.

Keywords: parameter identification, state estimation, Kalman filters, observers, biotechnology.

1. INTRODUCTION

From a process model and some available measurements, state observers (or software sensors) allow unmeasured state variables to be reconstructed on-line. State estimation techniques are particularly important in biotechnology, where hardware sensors are extremely costly and have stringent operating conditions (sterilization, long processing times, etc.).

When a process model is established, the unknown parameters are usually estimated by minimizing a least squares or maximum likelihood criterion. These conventional criteria however do not express the condition that the unmeasured states – as reproduced by the process model – should be sensitive to the measured ones. Otherwise, even if the system is observable, the software sensor might produce poor estimates of the unmeasured states.

In previous studies (Bogaerts and Vande Wouwer, 2000a, b), the authors suggested a new parameter identification procedure yielding a model dedicated to state estimation purposes. Based on the concept of U-uniform observability of a nonlinear system (Gauthier and Kupka, 1994), a "measure of observability" is derived, which quantifies the ability to detect in the output trajectories any differences in the initial states. State estimation sensitivity is enforced by minimizing a cost function combining a conventional maximum likelihood criterion with this observability measure. The effectiveness of this parameter identification procedure was demonstrated with the design of a full horizon observer reconstructing biomass and glucose concentrations in batch CHO animal cell cultures from experimental measurements of glutamine and lactate concentrations.

Even though this real-case application was very successful, one might raise the objection that our observations were influenced by several specific choices and/or experimental conditions:

(a) the selection of a model structure and parametrization (i.e., the general kinetic model structure proposed in (Bogaerts and Hanus, 2000b)),
(b) the quantity and quality of the measurement data (i.e., rare and asynchronous measurements of biomass, glutamine, glucose and lactate

concentrations),

(c) the implementation of a specific state estimation algorithm (i.e., a full horizon observer (Bogaerts and Hanus, 2000a) reconstructing on-line the most likely initial conditions).

Of course, (a) and (b) could influence parameter identifiability, while (c) could have an effect on the quantification of the importance of the model sensitivity on the quality of the state estimates.

The objective of the present study is to alleviate these potential problems and to demonstrate the usefulness of the "identification for state estimation" concept even in the situation where the exact model structure is known and large sets of good-quality measurement data are available (of course, this ideal situation can only be investigated in simulation). Further, this study aims at showing that, whatever the state observer structure, model sensitivity significantly influences the quality of the unmeasured state estimates.

This paper is organized as follows. A fed-batch bioprocess, which will be used as a test-example throughout the complete study, is described in Section 2. In Section 3, the experimental conditions are detailed and the kinetic parameters are estimated by minimizing a classical maximum likelihood criterion. Section 4 briefly discusses the design of an extended Kalman filter and a full horizon observer and shows that, even a high-quality model (in the classical sense), can lead to poor estimates of unmeasured states. Section 5 presents a new cost function, which enforces state estimation sensitivity, and highlights the influence of the parameter identification step on the performance of both state estimators. Finally, Section 6 is devoted to concluding remarks.

2. PROCESS DESCRIPTION AND MODELING

Consider a simple macroscopic reaction scheme

$$\text{growth:} \qquad \nu_{S_2} S_2 \xrightarrow{\varphi_g} \wedge X \qquad (1)$$

$$\text{maintenance:} \qquad S_1 + \nu_X X \xrightarrow{\varphi_m} \nu_X X + \nu_P P \qquad (2)$$

where X, S_1, S_2 and P represent biomass, substrate 1-2 and product, respectively, and ν_{S_2}, ν_X and ν_P are pseudo-stoechiometric coefficients. The symbol "$\rightarrow\wedge$" means that the growth reaction is auto-catalyzed by X and the presence of "ν_X X" in both sides of the maintenance reaction means that X catalyzes this latter reaction.

The growth rate φ_g and the maintenance rate φ_m are described by classical Monod laws and inhibition factors

$$\varphi_g = \mu^g_{max} \frac{S_2}{K^g_M + S_2} \frac{K^g_i}{K^g_i + S_1} X \qquad (3)$$

$$\varphi_m = \mu^m_{max} \frac{S_1}{K^m_M + S_1} \frac{K^m_i}{K^m_i + X} X \qquad (4)$$

These growth and maintenance reactions take place in a bioreactor operated in fed-batch mode with a time-varying inlet flow rate $F_{in}(t)$ and constant inlet substrate concentrations $S_{1,in}$ and $S_{2,in}$. Simple mass balances allow the following dynamic model to be derived :

$$\frac{dX}{dt} = \varphi_g - DX \qquad (5)$$

$$\frac{dS_1}{dt} = -\varphi_m + D(S_{1,in} - S_1) \qquad (6)$$

$$\frac{dS_2}{dt} = -\nu_{S_2}\varphi_g + D(S_{2,in} - S_2) \qquad (7)$$

$$\frac{dP}{dt} = \nu_P \varphi_m - DP \qquad (8)$$

$$\frac{dV}{dt} = F_{in} \qquad (9)$$

where X, S_1, S_2, P now denotes the respective component concentrations, V is the culture volume and $D(t)=F_{in}(t)/V(t)$ is the dilution rate.

The model equations (5-9) together with the numerical values of the several parameters listed in Table 1 define the reference system, which is investigated in simulation in the continuation of this study.

Table 1. Model parameters

ν_{S_2}	$= 0.2$ mM/(10^5 cell/ml)	K^g_i	$= 70$ mM
ν_P	$= 1.7$	μ^m_{max}	$= 0.1$ h^{-1}
μ^g_{max}	$= 0.05$ h^{-1}	K^m_M	$= 0.2$ mM
K^g_M	$= 0.1$ mM	K^m_i	$= 3\ 10^5$ cell/ml

3. CLASSICAL PARAMETER IDENTIFICATION

For identification purposes, it is assumed that the component concentrations can be measured off-line at regular time intervals (e.g., every 5 hours). These measurements are spoiled by normally distributed, white noises with zero mean and variance matrix Q. Constant relative errors are considered (e.g., $\varepsilon^{rel}_X = 0.1$, $\varepsilon^{rel}_{S_1} = 0.05$, $\varepsilon^{rel}_{S_2} = 0.05$, $\varepsilon^{rel}_P = 0.05$). We also assume that the pseudo-stoechiometric coefficients as well as the model initial conditions (initial concentrations and volume) are known exactly and that only the values of the kinetic parameters must be inferred from measurement data. At this stage, note that a systematic identification procedure has been proposed in (Bogaerts and Hanus, 2000b), which allows the pseudo-stoechiometric coefficients to be estimated independently of the kinetic coefficients (Bastin and Dochain, 1990) by minimizing a

maximum likelihood criterion. This procedure also considers the estimation of the most likely initial conditions (since the concentration measurements are corrupted by noise at each sampling time, including the initial one).

From the previous discussion, the model and measurement equations can be rewritten in short form

$$\frac{dx}{dt} = f(x(t), u, \vartheta) \tag{10}$$

$$y(t_k) = x(t_k) + \varepsilon(t_k) \tag{11}$$

where x(t) is the state vector (concentrations and volume), u(t) is the input (dilution rate), ϑ is the unknown parameter vector (kinetic parameters), $y(t_k)$ and $\varepsilon(t_k)$ are the measurement and noise vector, respectively.

The maximum likelihood estimation $\hat{\vartheta}$ of ϑ is given by

$$\hat{\vartheta} = \underset{\vartheta}{\text{Arg min}}\ F_{ml}(\vartheta), \qquad \text{with} \tag{12}$$

$$F_{ml}(\vartheta) = \frac{1}{2}\sum_{k=1}^{N}(y(t_k) - \hat{x}(t_k))^T Q(t_k)^{-1}(y(t_k) - \hat{x}(t_k))$$

where $\hat{x}(t)$ is the state estimate obtained by integration of the model equations (10) with the parameters $\vartheta = \hat{\vartheta}$.

In this study, the kinetic parameters are estimated using measurement data taken from two experimental runs. A third experiment (whose data are not used for parameter estimation) provides a cross-validation test. These experiments differ in their initial conditions, inlet substrate concentrations and flow rate (taken, for instance, in the form $F_{in}(t) = 0$, for $t < t_b$ and $F_{in}(t) = \alpha(t - t_b)$, for $t_b \le t \le t_{fb}$, with $\alpha = 5\times10^{-4}\,l/h^2$) according to Table2.

Table 2. Experimental conditions

	Exp 1	Exp 2	Exp 3
X(0) (10^5 cell/ml)	1	1	1
S_1(0) (mM)	10	10	8
S_2(0) (mM)	8	1	5
P(0) (mM)	1	1	1
V(0) (l)	0.5	0.5	0.5
$S_{1,in}$ (mM)	5	5	10
$S_{2,in}$ (mM)	1	1	3
t_b (h)	30	35	50
t_{bf} (h)	80	80	90

To avoid any convergence problem of the optimization algorithm ("lsqnonlin" from the MATLAB 5.3 optimization toolbox), which would obscure the conclusions of our analysis, we start from the exact kinetic parameters given in Table 1. The main effect of (12) is therefore to slightly adjust the parameters to a specific realization of the measurement noise. Figure 1 shows the cross-validation test performed with experiment 3. In this graph, the circled points are the measured data and the bars represent the 99% confidence intervals. The solid lines are the concentration trajectories predicted by the identified model.

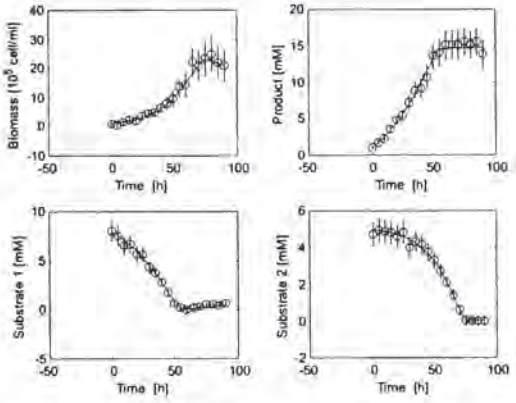

Fig. 1. Model cross-validation (experiment 3)

4. STATE ESTIMATOR DESIGN

Based on the model identified in the previous section, two different state estimators are designed, e.g., a continuous-discrete extended Kalman filter (Gelb, 1974) and a full horizon observer (Allgöwer *et al.*, 1999; Bogaerts and Hanus, 2000a.). The objective in this study is to obtain a continuous-time estimation of biomass and substrate 1 concentrations from discrete-time measurements of substrate 2 and product concentrations. As only two component concentrations are measured on-line, it is necessary to introduce a measurement matrix C in (11), i.e.,

$$y(t_k) = Cx(t_k) + \varepsilon(t_k) \tag{13}$$

where $\varepsilon(t_k)$ is a measurement noise vector with variance matrix $Q = \text{diag}(\sigma_{S2}^2, \sigma_P^2)$.

4.1 Continuous-discrete extended Kalman filter

The continuous-discrete extended Kalman filter is the generalization of the Kalman filter to nonlinear systems described by continuous-time state equations (10) and discrete-time measurement equations (13).

Prediction step (between samples):

$$\frac{d\hat{x}}{dt} = f(\hat{x}, u), \qquad t_k < t < t_{k+1} \tag{14}$$

$$\frac{dP}{dt} = A(\hat{x})P + PA(\hat{x})^T \tag{15}$$

Correction step (at sampling times):

$$K(t_k) = P(t_k^-)C^T\left[CP(t_k^-)C^T + Q(t_k)\right]^{-1} \tag{16}$$

$$\hat{x}(t_k^+) = \hat{x}(t_k^-) + K(t_k)\left(y(t_k) - C\hat{x}(t_k^-)\right) \quad (17)$$

$$P(t_k^+) = P(t_k^-) - K(t_k)CP(t_k^-) \quad (18)$$

The extended Kalman filter requires the on-line numerical integration of the state equation (14) and the Ricatti equation (15). The latter involves the matrix $A(\hat{x}) = (\partial f/\partial x)_{\hat{x}}$ resulting from the model linearization along the predicted state trajectory.

These equations are solved starting with the initial conditions $\hat{x}(0) = x_0$ and $P(0) = P_0$. For substrate 2 and product, these values are best taken from the measured concentrations and the measurement error variances at the initial time, respectively. For the unmeasured component concentrations, these initial values can only be guessed based on common sense and process knowledge. They represent the tuning parameters of the Kalman filter, which are taken here as, e.g., $X(0) = 3\times10^5$ cell/ml, $S_1(0) = 5$ mM, $P_0(1,1) = \sigma_X^2 = 10^6$ (cell/ml)^2 and $P_0(2,2) = \sigma_{S1}^2 = 10^6$ mM^2.

Figure 2 shows that the Kalman filter accurately estimates the measured substrate 2 and product concentrations as well as the unmeasured biomass concentration. However, it produces poor estimates of the unmeasured substrate 1 concentration, with the exception of the final times where substrate 1 disappears and growth limitation occurs. This latter observation will become clear in Section 5.

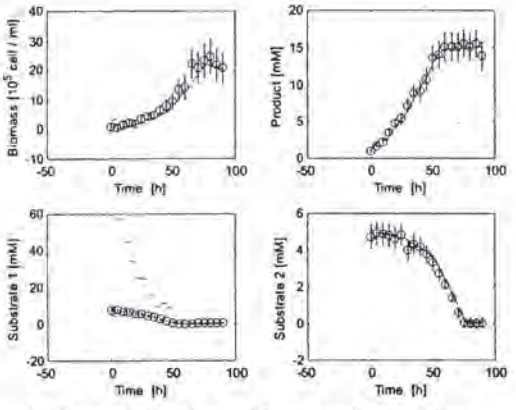

Fig. 2. State estimation with a continuous-discrete extended Kalman filter (experiment 3)

4.2 Full horizon observer

Between two measurement times, numerical integration of the state equations (10) from an estimate of the most likely initial conditions allows a prediction of the state vector to be computed on-line. At the next sample time, a new estimate of the initial conditions can be obtained by minimizing a maximum likelihood criterion based on all the measurements available up to this time. The procedure is repeated from sample to sample and can be summarized as follows:

Prediction step (between samples $t_k < t < t_{k+1}$).

$$\frac{d\hat{x}}{dt} = f(\hat{x}, u), \qquad 0 \le t < t_{k+1} \quad (19)$$

$$\hat{x}(0) = \hat{x}_{0/k}$$

Correction step (at sampling times):

$$\hat{x}_{0/k} = \underset{x_0}{\text{Arg min}}\, J_k(x_0) \qquad \text{with} \quad (20)$$

$$J_k(x_0) = \frac{1}{2}\sum_{j=1}^{k}(y(t_j) - C\hat{x}(t_j))^T Q(t_j)^{-1}(y(t_j) - C\hat{x}(t_j))$$

For the first time interval, the state equations (19) are solved starting with initial conditions determined in the same way as for the Kalman filter (i.e., $S_2(0)$ and $P(0)$ are the measured values while $X(0) = 3\times10^5$ cell/ml and $S_1(0) = 5$ mM are a priori initial guesses). The minimization of (20) is performed repeatedly with "lsqnonlin". The tuning parameters of the observer are the lower and upper bounds on x_0, which are taken here as, e.g., $0 < X_0 < 5$, $0 < S_{1,0} < 20$, $0 < S_{2,0} < 10$, $0 < P_0 < 5$.

The observations are basically the same as with the extended Kalman filter, i.e., the unmeasured substrate 1 concentration is poorly estimated (see fig. 3).

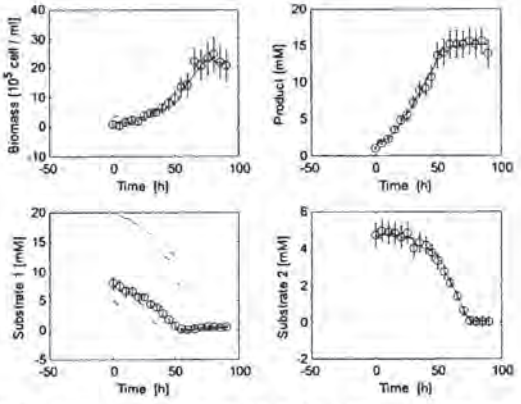

Fig. 3. State estimation with a full-horizon observer (experiment 3)

5. IDENTIFICATION FOR STATE ESTIMATION

It is now clear that, even with a high-quality model (in the classical sense of Section 3), state observers might fail to produce reliable estimates of unmeasured state variables. The objective of this section is to introduce a new cost function enforcing a higher sensitivity of the unmeasured part of the state with respect to the measured one. Complete developments can be found in (Bogaerts and Vande Wouwer, 2000a, b).

Consider the nonlinear model equations

$$\frac{dx}{dt} = f(x, u), \qquad x(0) = x_0 \quad (21)$$

$$y = Cx \qquad (22)$$

Let y(t,x(0),u(t)) denote the output trajectory corresponding to the initial condition x(0) and the input u(t). The system (21-22) is observable if, for any couple of different initial conditions x(0) and x'(0), there exists an input u(t) and a time $0 < t < \infty$ for which the outputs y(t,x(0),u(t)) and y(t,x'(0),u(t)) are different. Uniform observable systems have the particularity that each admissible input allows any couple of different initial states to be distinguished. Locally *U*-uniform observability in x(0) is restricted to a neighborhood V(x(0)) of x(0) and to inputs in an admissible domain U. Any locally U-uniformly observable multiple input - multiple output system can written in a pseudo-canonical form

$$\frac{d}{dt}\left[x_1^T \ldots x_i^T \ldots x_q^T\right] = \left[f_1^T(x_1, x_2, u) \ldots f_i^T(x_1, \ldots, x_{i+1}, u) \ldots f_{q-1}^T(x_1, \ldots, x_q, u)\, f_q^T(x_1, \ldots, x_q, u)\right]$$

$$y(t) = x_1 \qquad (23)$$

where

$$\forall i \in \{1,\ldots,q\},\ x_i \in \Re^{n_i},\ n_1 \geq n_2 \geq \ldots \geq n_q \text{ and } \sum_{1 \leq i \leq q} n_i = n$$

and

$$\forall i \in \{1,\ldots,q-1\},\ \forall (x,u) \in \Re^n \times U : \operatorname{rank} M_i(x,u) = n_{i+1}$$

with

$$M_i(x,u) = \left(\frac{\partial f_i(x,u)}{\partial x_{i+1}}\right)^T \left(\frac{\partial f_i(x,u)}{\partial x_{i+1}}\right) \in \Re^{n_{i+1} \times n_{i+1}}$$

If some matrices $M_i(x,u)$ are full-rank but ill conditioned, the system is theoretically observable but a difference in the initial states might be extremely difficult to detect in the output trajectories. This observation leads to the definition of an "observability measure" F_{obs} quantifying this ability to detect, in the output trajectories, any differences in the initial states. Assuming that the state trajectory x(t) is measured at discrete times t_k, (k=1,...,N), a candidate scalar measure is given by

$$F_{obs} = \sum_{k=1}^{N} \sum_{i=1}^{q-1} \sqrt{\operatorname{cond}(M_i(x(t_k), u(t_k))} \qquad (24)$$

where "cond" represents the condition number of the matrix, i.e., the ratio of its largest to its smallest eigenvalue.

Based on these results, a new form of the cost function combining the conventional maximum likelihood criterion F_{ml} (12) with the observability measure F_{obs} (24) can be defined as

$$\hat{\vartheta} = \operatorname*{Arg\,min}_{\vartheta} F(\vartheta) = \operatorname*{Arg\,min}_{\vartheta} \left\{F_{ml}(\vartheta) + \lambda F_{obs}(\vartheta)\right\} \qquad (25)$$

where λ is a weighting factor.

A straightforward way to select this weighting factor is to plot the separate components F_{ml} and F_{obs} as functions of λ (see fig. 4). For increasing λ, F_{obs} decreases while F_{ml} increases. A compromise solution must therefore be considered, e.g., $\lambda = 0.1$ (for this value, F_{ml} doubles whereas F_{obs} is reduced by a factor 2.6; for larger values of λ, F_{ml} increases significantly whereas F_{obs} only slightly decreases).

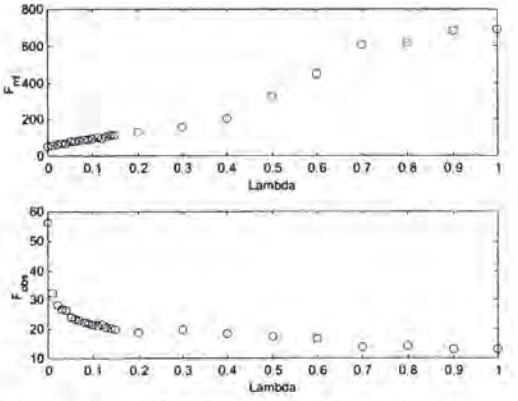

Fig. 4. Evolution of $F_{ml}(\lambda)$ (top) and $F_{obs}(\lambda)$ (bottom)

With this trade-off value for λ, the combined cost function (25) is minimized using again "lsqnonlin". The identified kinetic parameters are listed in Table 3. In comparison with Table 1, the values of some parameters, particularly K_i^g and K_M^m, have changed significantly.

Table 3. Identified parameters (cost function (25))

$\mu_{max}^g = 0.062\ h^{-1}$	$\mu_{max}^m = 0.11\ h^{-1}$
$K_M^g = 0.046$ mM	$K_M^m = 0.31$ mM
$K_i^g = 14$ mM	$K_i^m = 2.8\ 10^5$ cell/ml

If these new model parameters are used in the extended Kalman filter designed in Section 4, the results graphed in figure 5 are obtained. Significant improvements in the estimation of the unmeasured substrate 1 concentration can be observed. Indeed, the norm of the estimation error (defined as the sum of the squares of the estimation errors at each sampling time weighted by the corresponding measurement variances) has been reduced by a factor 30 ! Similar results can be obtained with the full-horizon observer as illustrated in figure 6.

A closer look at the expressions of the reaction rates (3-4) and a comparison of the numerical values of the kinetic parameters in Tables 1-2 allows a physical interpretation of these results. As K_i^g is much larger than S_1, the inhibition factor in (3) reduces to 1. On the contrary, S_1 is much larger than K_M^m, at least at the beginning of the experiments 1-2, so that the Monod factor in (4) also tends to unity. As a

consequence, the system is intrinsically insensitive to the variations of substrate 1 ! It is apparent from Table 2 that the minimization of the combined cost function (25) attempts to compensate these effects by significantly reducing the value of K_1^g and by increasing the value of K_M^m.

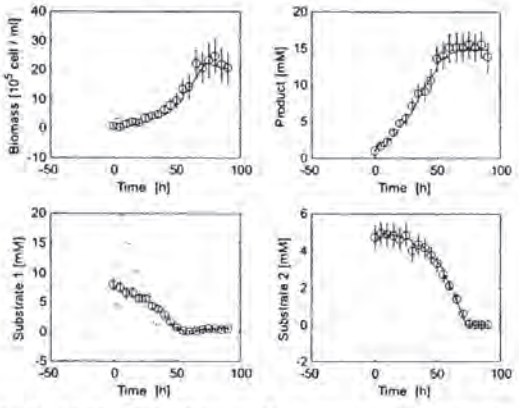

Fig. 5. State estimation with a continuous-discrete extended Kalman filter using a model identified with the combined criterion (25)

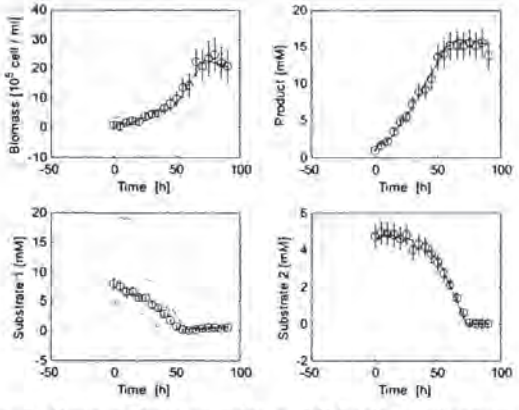

Fig. 3. State estimation with a full-horizon observer using a model identified with the combined criterion (25)

6. CONCLUSIONS

In this study, a new parameter identification procedure yielding a model dedicated to state estimation purposes is thoroughly analyzed. Basically, a new cost function is proposed which combines a classical maximum likelihood criterion with a "measure of observability". Based on the concept of U-uniform observability of a nonlinear system, this measure quantifies the ability to detect in the output trajectories any differences in the initial states. Minimizing the combined cost function enforces higher model sensitivities and in turn, a better transfer of information from measured to unmeasured variables. The simulation studies described in this paper show that this goal is achieved by modifying the numerical values of some model parameters in order to compensate an intrinsic lack of sensitivity with respect to some of the state variables. This model "falsification" allows significant improvements in the quality of state estimates provided by software sensors.

These results confirm previous authors' observations made in the context of batch animal cell cultures (Bogaerts and Vande Wouwer, 2000a, b). In these experimental applications, the degrees of freedom available at the modeling stage, e.g., the selection of the model structure and parametrization, can be used to enhance the sensitivity of the unmeasured states with respect to the measured ones.

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