CONTINUOUS-DISCRETE OBSERVER DESIGN FOR A CHO-K1 CELL CULTURE IN SUSPENSION

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Abstract: Bioprocess models are often uncertain due to the lack of experimental data for structure selection and parameter estimation. Maximum-likelihood parameter estimation techniques, which take the measurement errors into account, allow confidence intervals in the identified parameters to be evaluated. In turn, this information can be advantageously exploited in the design of (more) robust state estimators (or software sensors) for process monitoring and control. In this paper, the formulation of continuous-discrete Kalman filters and receding horizon observers are extended to include *a posteriori* knowledge on model parameter uncertainties. These extended observers/filters are then successfully applied to a real-case study, i.e., animal cell cultures in perfusion mode.

Keywords: state estimation, parameter estimation, Kalman filter, receding-horizon observer, biotechnology

1. INTRODUCTION

State estimation techniques are particularly important for monitoring bioprocess operation. Indeed, biomass and component concentrations are difficult to measure on-line and measurements are often corrupted by relatively large errors. As on-line measurements are collected at discrete times only, and with relatively low sampling rates (a few hours to 1-2 days), continuous (model)–discrete (measurements) software sensors taking the stochastic nature of the measurement signals into account are the first choices in bioprocess applications (Bogaerts and Vande Wouwer, 2003).

Stochastic continuous-discrete software sensors include the Kalman filter (Gelb, 1989) and the receding horizon observer (Allgöwer *et al.*, 1999; Bogaerts and Hanus, 2001). In the former, uncertainties are introduced in the form of uncorrelated white noises corrupting the state and measurement equations. The selection of the covariance matrices of these noises is often based on some *a priori* knowledge and some form of tuning. In the latter, measurement errors are introduced in a maximum-likelihood or Gauss-Markov cost function, which is repeatedly minimized in order to estimate most-likely model initial conditions.

The main objective of this paper is to show how *a posteriori* information on model parameter uncertainties, which results from the model parameter identification procedure, can be incorporated in the observer/filter design. Indeed, maximum-likelihood parameter identification yields estimates of the parameter covariance matrices which are very useful for model analysis and validation purposes, but which are seldom exploited when considering the state estimation problem.

As a result, extended forms of the Kalman filter and receding-horizon observer are developed. These software sensors are then applied to a real-case study, i.e., CHO-K1 cell cultures in a bioreactor operated in perfusion mode.

This paper is organized as follows. The next section deals with the extended Kalman filter whereas section 3 presents the full- or receding-horizon observer.

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Section 4 describes the experimental set-up and shows results of the application of both state estimation techniques. Finally, section 5 is devoted to some concluding remarks.

2. EXTENDED KALMAN FILTER

The Kalman Filter is one of the most popular state estimation algorithms, which takes the stochastic nature of the process and measurement noises into account.

Assume that the state equation for $x \in \mathbb{R}n_x$ and the output $y \in \mathbb{R}n_y$ are corrupted by normally distributed white noise with zero mean according to

$$\dot{x}(t) = f(x(t),u(t),t) + w(t); \quad w(t) \sim \mathcal{N}(0,Q(t)) \quad (1)$$
$$y_i \quad = h(x(t_i)) + v_i; \qquad v_i \quad \sim \mathcal{N}(0,R_i), \quad (2)$$

where $Q \in \mathbb{R}^{n_x \times n_x}$ and $R \in \mathbb{R}^{n_y \times n_y}$ are the state and output error covariance matrices, respectively.

The continuous-discrete extended Kalman Filter (Gelb, 1989) makes use of a linearisation along the state estimate trajectory and corresponds to continuous-time prediction and discrete-time measurements:

- initialization ($i = 0,1,2,\ldots$)

$$\hat{x}(t_i) = \hat{x}_i \quad (3)$$
$$P(t_i) = P_i \quad (4)$$

- continuous prediction step for $t_i \le t < t_{i+1}$

$$\dot{\hat{x}}(t) = f(\hat{x}(t),u(t),t) \quad (5)$$
$$\dot{P}(t) = F(\hat{x}(t),u(t),t)P(t) + P(t)F^{\mathrm{T}}(\hat{x}(t),u(t),t) + Q(t) \quad (6)$$

- discrete correction step at $t = t_i$ ($i = 1,2,\ldots$)

$$\hat{x}_i = \hat{x}(t_i^-) + K_i\left(y_i - h_i(\hat{x}(t_i^-))\right) \quad (7)$$
$$P_i = \left(I - K_i H_i(\hat{x}(t_i^-))\right) P(t_i^-) \quad (8)$$
$$K_i = P(t_i^-)H_i^{\mathrm{T}}(\hat{x}(t_i^-)) \cdot \left(H_i(\hat{x}(t_i^-))P(t_i^-)H_i^{\mathrm{T}}(\hat{x}(t_i^-)) + R_i\right)^{-1} \quad (9)$$

- linearisations

$$F(\hat{x}(t),u(t),t) = \left.\frac{\partial f(x(t),u(t),t)}{\partial x^{\mathrm{T}}}\right|_{x(t)=\hat{x}(t)} \quad (10)$$
$$H_i(\hat{x}(t \to t_i)) = \left.\frac{\partial h(x(t))}{\partial x^{\mathrm{T}}}\right|_{x(t)=\hat{x}(t \to t_i)} \quad (11)$$

The matrices P_0, Q and R_i play the following roles:

- The initial state covariance matrix P_0 represents the confidence paid to the initial guess for the state vector $\hat{x}_0$. If the latter is arbitrarily chosen, the elements of P_0 should be set to high values (so that these estimates are almost replaced by the measurements at the first correction instant, where possible).
- The state covariance matrix Q is often used to keep a certain level of uncertainty on the state estimates preventing that the correction matrix K_i vanishes for $i \to \infty$, which would result in the disappearance of the correction and therefore in a pure simulation.
- The measurement covariance matrix R contains information on the measurement noise. Large values for the diagonal elements are equivalent to significant measurement noise in the output signal and non-zero off-diagonal elements indicate correlations between the respective signals. Less confidence is therefore paid to the measurements in the correction step.

A further extension of the Kalman filter is derived in the following, taking the information about the modelling errors into account. This information can be quantified with the error covariance matrices obtained at the model identification stage.

Consider the system

$$\dot{x}(t) = f(x(t),u(t),p); \quad x(t_0) = x_0 \quad (12)$$
$$y_i \quad = h(x(t_i)) + v_i; \qquad v_i \sim \mathcal{N}(0,R_i). \quad (13)$$

Compared to (1), the disturbance of the state equation does not appear explicitly, and the model parameter vector $p \in \mathbb{R}^{n_p}$ is considered instead.

Consider an analogous system for the state estimate,

$$\dot{\hat{x}}(t) = f(\hat{x}(t),u(t),\hat{p}); \quad \hat{x}(t_0) = \hat{x}_0 \quad (14)$$

with the estimated initial state $\hat{x}_0$ and the estimated parameter vector $\hat{p}$. Their error covariance matrices are denoted P_0 and Q_p, respectively, and are assumed to be known, e.g., from a preliminary parameter estimation.

The estimation error $\tilde{x} = \hat{x} - x$ is due to the errors on the initial state and the parameters, i.e. in first-order approximation

$$\tilde{x} \approx S_p(x,u,p)\tilde{p} + S_0(x,u,p)\tilde{x}_0 \quad (15)$$

where the sensitivity matrices are defined as

$$S_p = \frac{\partial x}{\partial p}; \quad S_0 = \frac{\partial x}{\partial x_0} \quad (16)$$

The covariance matrix of the estimation error is then written

$$P = S_p Q_p S_p^{\mathrm{T}} + S_0 P_0 S_0^{\mathrm{T}} \quad (17)$$

The equations for the sensitivities of the state with respect to the parameter vector and the initial state are obtained through differentiation of the state equation with respect to p and x_0, respectively:

$$\dot{S}_p = F_x(x,u,p)S_p + F_p(x,u,p); \quad S_p(t_0) = 0 \quad (18)$$
$$\dot{S}_0 = F_x(x,u,p)S_0; \qquad S_0(t_0) = I \quad (19)$$

with the Jacobian matrices

$$F_x(x,u,p) = \frac{\partial f(x,u,p)}{\partial x} \quad (20)$$
$$F_p(x,u,p) = \frac{\partial f(x,u,p)}{\partial p}. \quad (21)$$

The dynamic equation for the propagation of P is derived by differentiation of (17) with respect to time:

$$\dot{P} = \dot{S}_p Q_p S_p^{\mathrm{T}} + S_p Q_p \dot{S}_p^{\mathrm{T}} + \dot{S}_0 P_0 S_0^{\mathrm{T}} + S_0 P_0 \dot{S}_0^{\mathrm{T}}; \quad P(t_0) = P_0 \quad (22)$$

Substitution of the time derivatives $\dot{S}_p$ and $\dot{S}_0$ with (18) and (19), respectively,

$$\dot{P} = F_x S_p Q_p S_p^{\mathrm{T}} + F_p Q_p S_p^{\mathrm{T}} + S_p Q_p S_p^{\mathrm{T}} F_x^{\mathrm{T}} + S_p Q_p F_p^{\mathrm{T}} + F_x S_0 P_0 S_0^{\mathrm{T}} + S_0 P_0 S_0^{\mathrm{T}} F_x^{\mathrm{T}}; \quad P(t_0) = P_0 \quad (23)$$

leads with (17) to

$$\dot{P} = F_x P + P F_x^{\mathrm{T}} + F_p Q_p S_p^{\mathrm{T}} + S_p Q_p F_p^{\mathrm{T}}; \quad P(t_0) = P_0. \quad (24)$$

The difference bewtween equations (24) and (6) is the dynamic definition of the uncertainty in the state equation

$$\begin{aligned} Q(t) =& F_p(\hat{x}(t), u(t), \hat{p}) Q_p S_p^{\mathrm{T}}(t) \\ &+ S_p(t) Q_p F_p^{\mathrm{T}}(\hat{x}(t), u(t), \hat{p}) \quad (25) \\ \dot{S}_p(t) =& F_x(\hat{x}(t), u(t), \hat{p}) S_p(t) \\ &+ F_p(\hat{x}(t), u(t), \hat{p}); \quad S_p(t_0) = 0 \quad (26) \end{aligned}$$

3. FULL-HORIZON AND RECEDING-HORIZON OBSERVERS

The so-called Full-Horizon observer is very much alike an on-line parameter estimator, since it uses an optimization algorithm to determine the most likely initial conditions of the bioprocess model (Bogaerts, 1999; Bogaerts and Hanus, 2001), which is equivalent to a cross-validation of the model. If, in addition, some parameters are uncertain and re-estimated in the course of state observation, the solution of the estimation problem is equivalent to a direct model validation. A major difference lies the reduced number of on-line signals, as compared to a classical off-line parameter estimation procedure.

Consider the non-linear system (12, 13). The principle of the Full-Horizon observer is to estimate the best initial condition in a maximum-likelihood sense and to predict the current state through simulation of the system:

$$\dot{\hat{x}} = f(\hat{x}, u, \hat{p}); \quad \hat{x}(t_0) = \hat{x}_{0|i} \quad (27)$$

with

$$[\hat{x}_{0|i}, \hat{p}_i] = \arg\min_{x_0, p} j(x_0, p) \quad (28)$$

$$\begin{aligned} j(x_0, p) =& \sum_{k=0}^{i} \left(y_k - h(x(t_k, x_0, p))\right)^{\mathrm{T}} R_k^{-1} \\ &\cdot \left(y_k - h(x(t_k, x_0, p))\right) \\ &+ \left(x_0 - \hat{x}_{0|0}\right)^{\mathrm{T}} P_0^{-1} \left(x_0 - \hat{x}_{0|0}\right) \\ &+ \left(p - \hat{p}_0\right)^{\mathrm{T}} Q_p^{-1} \left(p - \hat{p}_0\right) \quad (29) \end{aligned}$$

The first term in the cost function (28) represents the classical sum of squared deviations between the experimental data y_k and model-predicted output $h(x(t_k))$, weighted by the measurement covariance matrix R_k. The last two terms are introduced here to take the *a priori* knowledge on the initial condition and the parameter vector with their respective covariance matrices P_0 and Q_p, e.g., calculated in a preliminary parameter estimation procedure, into account.

The current estimate of the initial condition serves as an initial guess for the following optimization step, since it is supposed to be the best estimate based on the available data.

The uncertainty associated with the estimated variable vector $\theta_i^{\mathrm{T}} = \left[x_{0|i}^{\mathrm{T}} \; p^{\mathrm{T}}\right]$, containing the initial condition and the re-estimated model parameter vector, which is obtained from data between t_0 and t_i, can be evaluated via the Fisher information matrix F_i:

$$\begin{aligned} & F_i(\hat{x}_{0|i}, \hat{p}_i) \\ &= \sum_{k=0}^{i} \left(\left. \frac{\partial h(x(t,\theta))}{\partial \theta} \right|_{t_k} \right)^{\mathrm{T}} R_k^{-1} \left(\left. \frac{\partial h(x(t,\theta))}{\partial \theta} \right|_{t_k} \right) \\ &= \sum_{k=0}^{i} \left(\left. \frac{\partial h}{\partial x} \right|_{\hat{x}(t_k)} \cdot \left[S_0(t_k) \;\; S_p(t_k)\right] \right)^{\mathrm{T}} R_k^{-1} \\ &\quad \cdot \left(\left. \frac{\partial h}{\partial x} \right|_{\hat{x}(t_k)} \cdot \left[S_0(t_k) \;\; S_p(t_k)\right] \right) \quad (30) \end{aligned}$$

with the sensitivity matrices S_0 and S_p defined in (16). In order to calculate the actual joint covariance matrix Q_i of the estimated initial condition $\hat{x}_{0|i}$ and the re-estimated parameters $\hat{p}_i$ the expression for the Cramer-Rao inequality is extended as follows:

$$Q_i(\hat{x}_{0|i}, \hat{p}_i) \geq \left(F_i(\hat{x}_{0|i}, \hat{p}_i) + \begin{bmatrix} P_0 & 0 \\ 0 & Q_p \end{bmatrix}^{-1} \right)^{-1} \quad (31)$$

assuming that the initial estimates $\hat{x}_{0|0}$ and $\hat{p}_0$ are not correlated. The current state covariance matrix $P(t)$ is obtained from equation (17)

$$P(t) = \left[S_0(t) \;\; S_p(t)\right] Q_i \left[S_0(t) \;\; S_p(t)\right]^{\mathrm{T}} \quad (32)$$

and the matrix differential equation system for the sensitivity matrices, respectively,

$$\dot{S}_0 = F_x(\hat{x}, u, \hat{p}) S_0; \quad S_0(t_0) = I \quad (33)$$

$$\dot{S}_p = F_x(\hat{x}, u, \hat{p}) S_p + F_p(\hat{x}, u, \hat{p}); \quad S_p(t_0) = 0 \quad (34)$$

with the Jacobian matrices (20,21).

Thus, the diagonal elements of $P(t)$ represent the variances of the current state estimates $\hat{x}(t)$, and their respective approximate 95% confidence intervals

$$\mathbb{X}(t) = \hat{x}(t) \pm \Delta x_{0.95}(t) \quad (35)$$

can be determined with:

$$\Delta x_{0.95}(t) \approx 1.96 \sqrt{P_{jj}(t)}; \quad j = 1, \ldots, n_x \quad (36)$$

When the system under consideration is operated in fed-batch or continuous mode and the influence of the

initial condition decreases as time evolves, a possible strategy is to define a limited-range horizon into the past, and to consider only the data within this time window. As time evolves, the horizon of the observer moves forward leading to a Receding-Horizon observer,

$$\dot{\hat{x}} = f(\hat{x}, u, \hat{p}_0); \quad \hat{x}(t_0) = \hat{x}_{i-l+1|i} \tag{37}$$

with the new initial condition of the considered time window obtained by

$$\hat{x}_{i-l+1|i} = \arg\min_{x_{i-l+1}} \Big\{ \sum_{k=i-l+1}^{i} (y_k - h(x(t_k)))^{\mathrm{T}} R_k^{-1} \cdot (y_k - h(x(t_k))) \Big\} \tag{38}$$

with a fixed horizon of l preceding output instants.

An adaptive version of the Receding-Horizon observer results, if the horizon l is adjusted such that a minimum confidence level in the state estimate can be guaranteed. Based on the current estimate $x_{i-l+1|i}$ resulting from the optimization (38), the confidence in this estimate is approximated by

$$P_{i-l+1|i} \geq \left(\sum_{k-l+1}^{i} \left(\left.\frac{\partial h}{\partial x}\right|_{\hat{x}(t_k)} \left.\frac{\partial \hat{x}}{\partial \hat{x}_0}\right|_{t_k} \right)^{\mathrm{T}} R_k^{-1} \cdot \left(\left.\frac{\partial h}{\partial x}\right|_{\hat{x}(t_k)} \left.\frac{\partial \hat{x}}{\partial \hat{x}_0}\right|_{t_k} \right) \right)^{-1}. \tag{39}$$

An approximation of the covariance matrices for horizons of different lengths, assuming that the trajectory remains the same, i.e. $\hat{x}_{k|i} \approx \hat{x}(t_k, \hat{x}_{i-l+1|i}, \hat{p}_i)$, is then written analogously:

$$P_{k|i} \approx \left(\sum_{\tilde{k}=k}^{i} \left(\left.\frac{\partial h}{\partial x}\right|_{\hat{x}(t_{\tilde{k}})} \left.\frac{\partial \hat{x}}{\partial \hat{x}_0}\right|_{t_{\tilde{k}}} \right)^{\mathrm{T}} R_{\tilde{k}}^{-1} \cdot \left(\left.\frac{\partial h}{\partial x}\right|_{\hat{x}(t_{\tilde{k}})} \left.\frac{\partial \hat{x}}{\partial \hat{x}_0}\right|_{t_{\tilde{k}}} \right) \right)^{-1}. \tag{40}$$

Since the matrices fulfil the inequality $P_{k_1|i} \leq P_{k_2|i}$, for $k_1 < k_2$, it is straightforward to determine the minimum horizon $l \in \{1, \ldots, i\}$ in $k = i - l + 1$ that fulfils the confidence condition $P_{k|i} \leq P_{\max}$, i.e.,

$$l_i = \min \left\{ \tilde{l} \mid P_{i-\tilde{l}+1|i} \leq P_{\max} \right\} \tag{41}$$

4. APPLICATION TO A CHO-K1 CELL CULTURE

Both state estimation techniques are now applied to cultures of animal cells, i.e., Chinese Hamster Ovary (CHO-K1) cells, in a bioreactor operated in perfusion mode. In this mode, the culture growth medium (glucose, amino-acids, and metabolites such as ammonium and lactate) is continuously replaced, whereas biomass is retained within the bioreactor thanks to a filtering device.

A dynamic model of this bioprocess has been developed by Haag *et al.* (2004), which consists of the following mass balance equations for the medium concentrations $c \in \mathbb{R}^{n_c}$:

$$\frac{\mathrm{d}c}{\mathrm{d}t} = \Upsilon r(c) + (c_{\mathrm{in}} - c)\frac{\dot{V}_{\mathrm{in}}}{V} + (c - c_{\mathrm{out}}(c))\frac{\dot{V}_{\mathrm{out}}}{V}; \quad c(t_0) = c_0, \tag{42}$$

where the first term on the right-hand side represents production or consumption according to the considered reaction scheme, the second term represents dilution due to the feed flow rate and the third term represents a correction term in the case where the harvesting concentration differs from the reactor concentration, i.e., due to the presence of a filtering device. The reaction term consists of the constant stoichiometric matrix $\Upsilon \in \mathbb{R}^{n_c \times n_r}$ containing the yield coefficients and a reaction rate vector $r \in \mathbb{R}^{n_r}$ non-linearly dependent on the medium concentrations c.

A specific structure of the reaction kinetics is assumed (Haag *et al.*, 2003). Therein, each component can have a modulating effect – from limitation to inhibition – in each considered reaction. The reaction rate of reaction j is therefore formulated as follows:

$$r_j(c) = r_{j,\max} \prod_{i=1}^{n_c} a_{ji} \tag{43}$$

with the modular functions $0 \leq a_{ji} \leq 1$ defined as

$$a_{ji} = \frac{c_i^{\operatorname{sign}\{K_{ji}^*\}}}{c_i^{\operatorname{sign}\{K_{ji}^*\}} + K_{ji}^{*2}} \tag{44}$$

$$= \begin{cases} \dfrac{c_i}{c_i + K_{ji}^{*2}} & \text{(limitation), if } K_{ji}^* > 0; \\ 1 & \text{(neutral), if } K_{ji}^* = 0; \\ \dfrac{K_{ji}^{*-2}}{c_i + K_{ji}^{*-2}} & \text{(inhibition), if } K_{ji}^* < 0. \end{cases} \tag{45}$$

Four experiments were carried out. Among them, three are used for parameter estimation and one is left for model cross-validation. The dynamical model involves 47 model parameters, which have been identified using a systematic procedure described by Haag *et al.* (2004). The resulting model is briefly summarized in Table 1 representing a pseudo-reaction scheme with the respective actual maximum specific reaction rates.

4.1 Extended Kalman Filter

An Extended Kalman Filter allows the information on the output and modelling errors to be taken into account with the covariance matrices R and Q_p, which are a direct result of the identification procedure. The measurement covariance matrix R is diagonal and of dimension 7×7 with the variances given in Table 2. The symmetric parameter covariance matrix is calculated from the 47×47 Fisher information matrix (19 estimated stoichiometric coefficients in Υ and 28

Table 1. Simplified reaction scheme obtained by model identification. Stoichiometric coefficients are normalized with respect to the viable biomass in units of $^{\text{mmoles}}/_{10^9\text{cells}}$.

$$
\begin{aligned}
25.0\,\text{Glc} + 4.0\,\text{Gln} + 1.6\,\text{Lac} &\xrightarrow{\max \mu_1 \approx 0.2\,{}^{1}/_{\text{d}}} \text{X}_\text{v} + 0.9\,\text{Glu} + 28.9\,\text{NH}_3 \\
7.8\,\text{Glc} + 0.1\,\text{Gln} + 2.7\,\text{Glu} &\xrightarrow{\max \mu_2 \approx 0.2\,{}^{1}/_{\text{d}}} \text{X}_\text{v} + 27.8\,\text{Lac} + 23.9\,\text{NH}_3 \\
5.6\,\text{Glc} + 1.0\,\text{Gln} + 4.5\,\text{NH}_3 &\xrightarrow{\max \mu_3 \approx 0.6\,{}^{1}/_{\text{d}}} \text{X}_\text{v} + 4.4\,\text{Lac} + 0.5\,\text{Glu} \\
\text{X}_\text{v} &\xrightarrow{\max k_\text{d} \approx 0.2\,{}^{1}/_{\text{d}}} \text{X}_\text{d}
\end{aligned}
$$

estimated modulation coefficients K_{ij}^*, $i = 1,\ldots,4$, $j = 1,\ldots,7$).

Table 2. Output errors estimated by model identification.

component	unit	$\hat{\sigma}_y$
viable cells	$10^6\,{}^{\text{cells}}/_{\text{ml}}$	2.62
non-viable cells	$10^6\,{}^{\text{cells}}/_{\text{ml}}$	0.96
glucose	$^{\text{g}}/_{\text{l}}$	0.30
lactate	$^{\text{g}}/_{\text{l}}$	0.14
glutamate	$^{\text{mg}}/_{\text{l}}$	20.1
glutamine	$^{\text{mg}}/_{\text{l}}$	41.5
ammonia	$^{\text{mg}}/_{\text{l}}$	14.4

The initial condition of the observer is chosen according to the mean value of the experimental data that have served for model identification, i.e., the measurements of Experiments 2 to 4. The confidence in this initial condition (expressed as the covariance matrix P_0) is given by the variances of these data.

Consider the case, where all states are measured at discrete sampling times. Figure 1 shows the graphical results of state estimation based on the measurement data of Experiment 1, which have not been used for identification. In this case, the benefit from state estimation is the continuous-time reconstruction and prediction of all the states.

The graphical results demonstrate the efficiency of the state estimation, which takes all available information on the parameter uncertainties in the model and the measurement errors into account. The measurements mostly remain within the estimated confidence intervals of the predicted states.

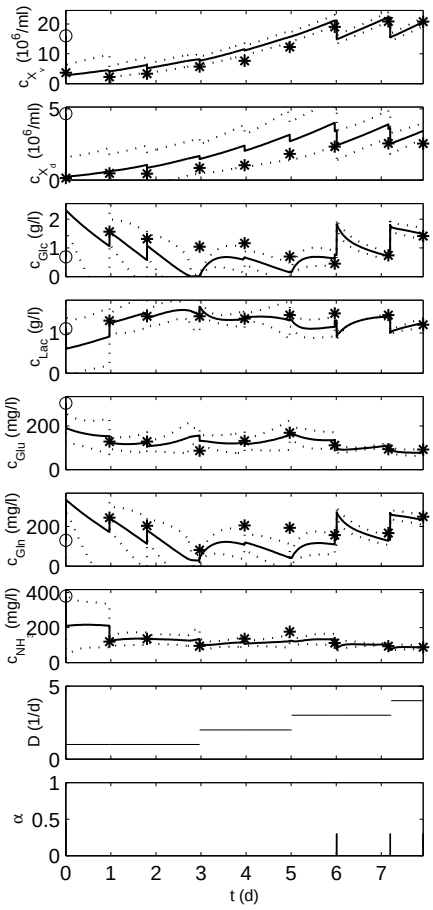

Fig. 1. Extended Kalman Filter for CHO-K1 cell culture applied on Experiment 1. Stars: Measurement outputs; solid line: continuous-time state estimation; dotted line: estimated 95% state confidence interval; circle: initial state estimate.

4.2 Full- and Receding-Horizon observer

The graphical results of the Full-Horizon observer are shown in Figure 2.

Apparently, these results are not as good as those of the Extended Kalman filter, especially towards the end of the observation time.

In fact, the problem lies in the estimation of the initial conditions of a system, which is continuously diluted. In some sense, the history of the process, and particularly the initial condition, is washed out, and the sensitivity of the current state with respect to this initial condition vanishes. Since the dilution rate $D = \dot{V}_{\text{in}}/V$ increases from $1\,{}^{1}/_{\text{d}}$ to $4\,{}^{1}/_{\text{d}}$ in the considered experiments, i.e., the bioreactor volume is exchanged several times a day, this washing out effect is increased.

An appealing alternative is therefore the Receding-Horizon observer, which considers a limited number of measurements into the past (in this specific case, the five most recent time instants) in order to estimate the initial condition of the current time window through a Gauss-Markov criterion.

The graphical results given in Figure 3 show indeed much better results than the Full-Horizon version and are comparable with the performance of the Extended Kalman Filter.

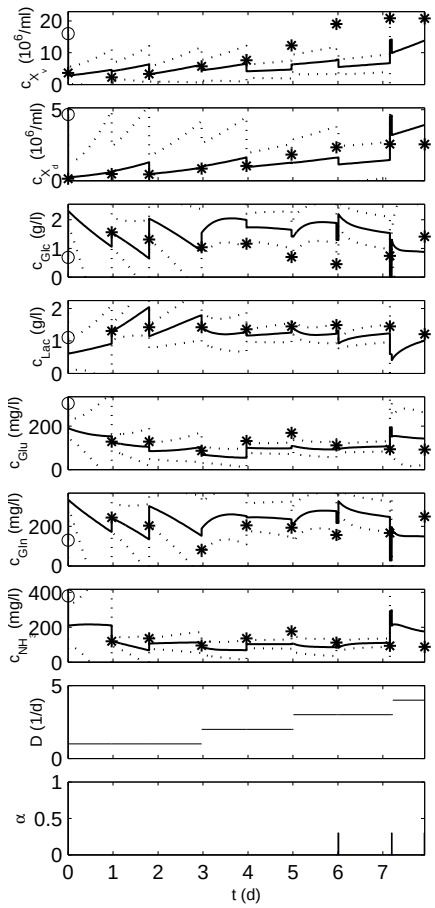

Fig. 2. Full-Horizon observer for CHO-K1 cell culture applied on Experiment 1. Stars: Measurement outputs; solid line: continuous-time state estimation; dotted line: estimated 95% state confidence interval; circle: initial state estimate.

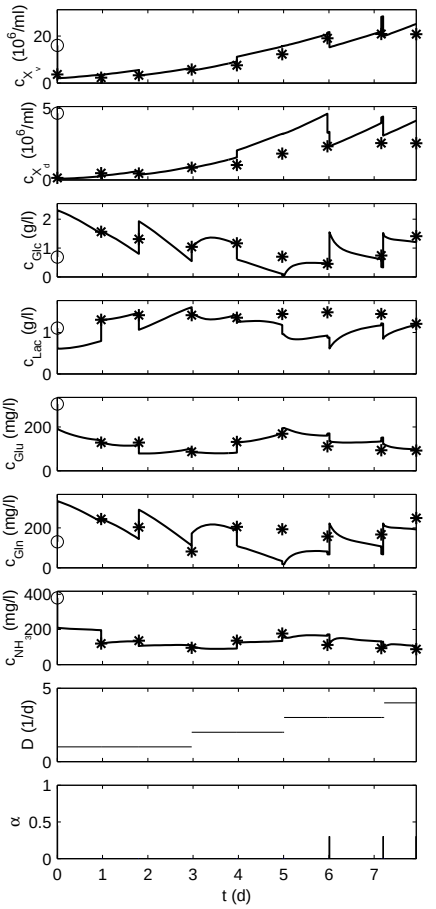

Fig. 3. Receding-Horizon observer for CHO-K1 cell culture applied on Experiment 1. Stars: Measurement outputs; solid line: continuous-time state estimation; circle: initial state estimate.

5. CONCLUSION

In this paper, extensions of the Kalman filter and receding-horizon observer are described, which take advantage of the information on the covariance matrices of the model parameter errors, the measurement errors and the initial state estimate errors, which results from the model parameter identification procedure. An application of both techniques is shown on a real experimental case study, namely animal cell cultures in a perfused continuous bioreactor.

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