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Parameter Identification for State Estimation: Design of an Extended Kalman Filter for Hybridoma Cell Fed-Batch Cultures

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Abstract: The monitoring and optimization of hybridoma cell fed-batch cultures depend on the availability of appropriate on-line sensors for the main culture components. A simple and efficient approach to maintain hybridoma cultures in the optimal operating conditions is to regulate the substrate concentrations at the critical values ($G=G_{crit}$ and/or $Gn=Gn_{crit}$) such as to control the hybridoma cells at the critical metabolism state. However, reliable glucose and glutamine probes are currently rare and/or very expensive on the market and it is necessary to design software sensors which are at same time cheap and reliable and that can be used for online measurement. In this study, the overflow metabolism model is used to develop an extended Kalman filter for online estimation of glucose and glutamine in hybridoma cell fed-batch cultures based on the considered available measurements (biomasses (on-line), lactate and ammonia (on-line or off-line)). The observability conditions are examined, and the performances are analysed with simulations of hybridoma cell fed-batch cultures. Glutamine estimation sensitivity is enforced by minimizing a cost function combining a usual least-squares criterion with a state estimation sensitivity criterion.

Keywords: hybridoma cultures, bioprocess optimization, software sensors, extended Kalman filter, parameter identification for state estimation.

1. INTRODUCTION

Mammalian cell cultures are widely used for production of many recombinant proteins with diagnostic and therapeutic applications (Rodrigues *et al*., 2010; Wurm *et al*., 2004). The high demand for these biopharmaceuticals has led to the development of large-scale manufacturing processes.

Industrial recombinant proteins production is usually achieved using fed-batch cultures of mammalian cells. From an operational point of view, the main goal is to maximize the recombinant protein production and, consequently, the biomass production, all in a minimum of time (i.e., to maximize the biomass productivity).

The main problem encountered comes from the metabolic changes of such strains in the presence of feeding overflow. This "overflow metabolism" is a metabolic phenomenon that is induced when the rate of substrate consumption exceeds a critical value, leading to a by-product formation which inhibits the oxidative capacity and the cell growth. It occurs for instance in *Saccharomyces cerevisiae* cultures with aerobic ethanol formation, in *Pichia pastoris* with aerobic methanol formation, in *Escherichia coli* cultures with aerobic acetate formation or in mammalian cell cultures with the aerobic lactate and ammonia formation. To avoid this undesirable effect, controlling cells at the edge of overflow metabolism is firstly recommended. Therefore, the substrate concentrations must be maintained at the critical level ($G=G_{crit}$ and $Gn=Gn_{crit}$) such as to control the cells at the critical metabolism state (Amribt *et al*., 2013b). These control schemes all require the on-line measurement of glucose and glutamine concentrations, implying the availability of glucose and glutamine probes or the use of alternative strategies based on more basic measurement signals, or software sensors reconstructing glucose and glutamine from the measurements of basic signals as designed in (Hitzmann *et al*., 2000; Arndt *et al*., 2004; Arndt *et al*., 2005; Veloso *et al*., 2009; Dewasme *et al*., 2012).

In fact there are few sensors which are at the same time cheap and reliable and that can be used for online measurement of substrates in mammalian cell cultures. The challenge is to estimate glucose and glutamine when only a few measurements are available.

In this work, the focus is placed on the development of an extended Kalman filter for online estimation of glucose and glutamine in hybridoma cell fed-batch cultures based on the overflow metabolism model (Amribt *et al*., 2013a). In order to design the software sensor, biomass concentration is assumed to be measured on-line, while lactate and ammonia are assumed to be measured either on-line or off-line.

The quality of glutamine estimation provided by the extended Kalman filter is improved by modifying the numerical values of some model parameters based on the minimization of a cost function combining a usual least-squares criterion with a state estimation sensitivity criterion (Bogaerts and Vande

wouwer, 2004). This procedure corresponds to parameter identification for state estimation.

Previous published studies were restricted to the development of state observers for reconstructing byproduct in *Saccharomyces cerevisiae* cultures with aerobic ethanol formation (Hitzmann *et al*., 2000; Arndt *et al*., 2005) and in *Escherichia coli* cultures with aerobic acetate formation (Arndt *et al*., 2004; Veloso *et al*., 2009; Dewasme *et al*., 2012). The originality of this paper consists in designing a state observer for online estimation of glucose and glutamine in mammalian cell cultures with phenomena of overflow metabolism, based on parameter identification for state estimation.

The paper is organized as follows. The overflow metabolism model of hybridoma cell fed-batch cultures is briefly presented in Section 2. The observability condition of the system is analyzed in Section 3. The extended Kalman filter is developed and its performances are tested in simulation in Section 4, while Section 5 presents the parameter identification for state estimation procedure and its performances. Final conclusions and future work directions are pointed out in Section 6.

2. OVERFLOW METABOLISM MODEL

The metabolism network is described by the following macroscopic reactions linking cells (*X*), glucose (*G*), glutamine (*Gn*), lactate (*L*) and ammonia (*N*):

Glucose consumption: $G \xrightarrow{\varphi_G} a \times X + b \times L$ (1)

Glutamine consumption: $Gn \xrightarrow{\varphi_{Gn}} c \times X + d \times N$ (2)

Glucose overflow metabolism: $G \xrightarrow{\varphi_{Over-G}} 2 \times L$ (3)

Glutamine overflow metabolism: $Gn \xrightarrow{\varphi_{Over-Gn}} N + (1/2) \times L$ (4)

where *a, b, c* and *d* are the stoichiometric coefficients, and φ_G, φ_{Gn}, $\varphi_{over\text{-}G}$ and $\varphi_{over\text{-}Gn}$ are the nonlinear growth rates given by:

$$\varphi_G = \min(\varphi_{G1}, \varphi_{G\,max}) \quad (5)$$

$$\varphi_{Gn} = \min(\varphi_{Gn1}, \varphi_{Gn\,max}) \quad (6)$$

$$\varphi_{Over-G} = \max(0, \varphi_{G1} - \varphi_{G\,max}) \quad (7)$$

$$\varphi_{Over-Gn} = \max(0, \varphi_{Gn1} - \varphi_{Gn\,max}) \quad (8)$$

The kinetic models associated with the global glucose consumption φ_{G1}, the global glutamine consumption φ_{Gn1}, the maximum respiratory capacity for glucose φ_{Gmax} and the maximum respiratory capacity for glutamine φ_{Gnmax} are given by:

$$\varphi_{G1} = \mu_{G\,max1} \frac{G}{K_G + G} \frac{Gn}{K_{Gn1} + Gn} X_V \quad (9)$$

$$\varphi_{Gn1} = \mu_{Gn\,max1} \frac{Gn}{K_{Gn} + Gn} \frac{K_N}{K_N + N} X_V \quad (10)$$

$$\varphi_{G\,max} = \mu_{G\,max2} X_V \quad (11)$$

$$\varphi_{Gn\,max} = \mu_{Gn\,max2} X_V \quad (12)$$

During a culture, the cells are likely to change their metabolism because of their limited respiratory capacity. At low substrate uptake rates ($\varphi_{G1} < \varphi_{Gmax}$ and $\varphi_{Gn1} < \varphi_{Gnmax}$), glucose and glutamine are consumed with biomass growth and metabolites (lactate and ammonia) production without overflow metabolism, which is defined as **respiratory metabolism**. At high substrate uptake rates ($\varphi_{G1} > \varphi_{Gmax}$ and/or $\varphi_{Gn1} > \varphi_{Gnmax}$), there is a limitation of respiratory capacity, resulting in **overflow metabolism** towards excess metabolites production. The state at which overflow metabolism is initiated ($\varphi_{G1}=\varphi_{Gmax}$ and $\varphi_{Gn1}=\varphi_{Gnmax}$) is referred to as **critical metabolism.**

The mass balance equations for the system in fed-batch mode are:

$$\frac{dX_V}{dt} = a\varphi_G + c\varphi_{Gn} - \mu_d X_V - \frac{F}{V} X_V \quad (13)$$

$$\frac{dX_d}{dt} = \mu_d X_V - \frac{F}{V} X_d \quad (14)$$

$$\frac{dG}{dt} = -\varphi_G - m_G X_V - \varphi_{G-Over} + \frac{F}{V}(G_{in} - G) \quad (15)$$

$$\frac{dGn}{dt} = -\varphi_{Gn} - \varphi_{Gn-Over} + \frac{F}{V}(Gn_{in} - Gn) \quad (16)$$

$$\frac{dL}{dt} = b\varphi_G + 2\varphi_{G-Over} + \frac{1}{2}\varphi_{Gn-Over} - \frac{F}{V} L \quad (17)$$

$$\frac{dN}{dt} = d\varphi_{Gn} + \varphi_{Gn-Over} - \frac{F}{V} N \quad (18)$$

$$\frac{dV}{dt} = F \quad (19)$$

where X_v is the viable biomass, X_d is the dead biomass, m_G is the maintenance coefficients of glucose, V (L) is the reactor volume, F (L/h) the volumetric feed rate, G_{in} and G_{nin}, are the concentrations of glucose and glutamine in the feed stream. μ_d is the specific death rate given by:

$$\mu_d = \mu_{d\,max} \frac{K_{Gd}}{K_{Gd} + G} \frac{K_{Gnd}}{K_{Gnd} + Gn} \quad (20)$$

Additionally, an indicator of overflow is proposed for each substrate (glucose and glutamine) as follows:

$$\text{Indi.over.}\xi = \frac{\varphi_{\xi 1} - \varphi_{\xi\,max}}{\varphi_{\xi\,max}} \quad \text{with} \quad \xi = G, Gn \quad (21)$$

These two indicators of metabolism overflow are positive if culture is operated at the state of overflow metabolism.

The model parameters values and initial conditions of state variables are listed in Table 1(Amribt *et al*., 2013a).

3. SYSTEM OBSERVABILITY

A fundamental question that arises is to know whether it is possible to estimate the state of a system on the basis of a specific mathematical model, the knowledge about its inputs and some physical measurements. Answering this question calls upon the analysis of system observability, which can be quite intricate for nonlinear systems. However, this analysis can be assessed using canonical forms (Gauthier and Kupka, 1994; Zeitz, 1984):

Table 1. Numerical values of model parameters and initial conditions of state variables

Parameter	Value	Parameter	Value	Initial conditions
μ_{Gmax1}	1.0006 mmol/(10^9 cells.h)	K_{Gn1}	0.0005 mM	X_{v0}=2.90×105 cells/mL
μ_{Gmax2}	0.0283 mmol/(10^9 cells.h)	a	1.1462 10^9 cells/mmol	X_{d0}=0.1×10^5 cells/mL
μ_{Gnmax1}	0.1992 mmol/(10^9 cells.h)	b	1.2939 mmol/mmol	G_0=17.17 mM
μ_{Gnmax2}	0.0203 mmol/(10^9 cells.h)	c	0.1186 10^9 cells/mmol	Gn_0=3.02 mM
μ_{dmax}	0.0111 1/h	d	0.3000 mmol/mmol	L_0=1.12 mM
K_G	23.2350 mM	m_G	0.0367 mmol/(10^9 cells.h)	N_0=0.29 mM
K_{Gn}	0.0004 mM	K_{Gd}	2.1862 mM	V_0=0.35L
K_N	0.9931 mM	K_{Gnd}	0.0020 mM	G_{in}=15mM, Gn_{in}=9.3mM

$$\forall i \in \{1,\ldots q\}, \xi_i \in \Re^{n_i}, n_1 \geq n_2 \geq \ldots \geq n_q, \quad \sum_{1\leq i\leq q} n_i = N = dim\,\xi \tag{22}$$

$$\frac{d\xi}{dt} = \begin{bmatrix} \dot{\xi}_1 \\ \dot{\xi}_2 \\ \vdots \\ \dot{\xi}_{q-1} \\ \dot{\xi}_q \end{bmatrix} = \begin{bmatrix} f_1(\xi_1,\xi_2) \\ f_2(\xi_1,\xi_2,\xi_3) \\ \vdots \\ f_{q-1}(\xi_1,..,\xi_q) \\ f_q(\xi_1,..,\xi_q) \end{bmatrix} \tag{23}$$

$$y = \xi_1$$

where ξ is the state vector, y the vector of measured states, f_i a partition of the nonlinear state equations, q the number of partitions.

To evaluate if the system is observable, one first checks if the bioprocess model can be put in the form of (23) by defining an appropriate partition, and then the following condition is evaluated:

$$rank \frac{\partial f_i}{\partial \xi_{i+1}} = n_{i+1} \quad \forall i \in \{1,....,q-1\} \tag{24}$$

The objective is to obtain a continuous-time estimation of glucose and glutamine from measurements of biomass and metabolites (lactate and ammonia). The viable biomass, X_v can be measured on-line using a capacitance probe considering the cells as dipoles and providing permittivity measurements correlated with biomass concentration. Off-line lactate and ammonia measurements can be performed by enzymatic test kit methods, while on-line predictions can be performed by a Near Infra-Red (NIR) probe by correlating frequency spectrums with off-line measurements of these metabolites.

The model equations {(13), (15), (16), (17), (18)} can be put in the canonical observability form:

$$\dot{\xi} = \begin{bmatrix} \dot{\xi}_1 \\ \dot{\xi}_2 \end{bmatrix} = \begin{bmatrix} f_1(\xi_1,\xi_2) \\ f_2(\xi_1,\xi_2) \end{bmatrix}, \quad y = \xi_1 \tag{25}$$

with

$$\xi_1 = [X_V \;\; L \;\; N]^{\mathrm{T}} \qquad \xi_2 = [G \;\; Gn]^{\mathrm{T}} \tag{26}$$

$$f_1(\xi_1,\xi_2) = \begin{bmatrix} a\varphi_G + c\varphi_{Gn} - \mu_d X_V - \frac{F}{V} X_V \\ b\varphi_G + 2\varphi_{G-Over} + \frac{1}{2}\varphi_{Gn-Over} - \frac{F}{V} L \\ d\varphi_{Gn} + \varphi_{Gn-Over} - \frac{F}{V} N \end{bmatrix} \tag{27}$$

$$f_2(\xi_1,\xi_2) = \begin{bmatrix} -\varphi_{G1} + \frac{F}{V}(G_{in} - G) \\ -\varphi_{Gn1} + \frac{F}{V}(Gn_{in} - Gn) \end{bmatrix} \tag{28}$$

Note that the differential equation of dead biomass is not considered, as X_d does not influence the other states and does not need to be estimated.

The global observability condition is:

$$rank \frac{\partial f_1}{\partial \xi_2} = \begin{bmatrix} \frac{\partial \dot{X}_V}{\partial G} & \frac{\partial \dot{X}_V}{\partial Gn} \\ \frac{\partial \dot{L}}{\partial G} & \frac{\partial \dot{L}}{\partial Gn} \\ \frac{\partial \dot{N}}{\partial G} & \frac{\partial \dot{N}}{\partial Gn} \end{bmatrix} = n_2 = 2 \tag{29}$$

Which, given the model proposed in section 2, is verified if G, Gn and N do not vanish.

4. THE EXTENDED KALMAN FILTER

The Kalman filter, which is by far the most popular state estimation technique used for bioprocess monitoring, is an exponential observer that minimizes the variance of the estimation error. If the process model is nonlinear then the filter is called extended Kalman filter. Furthermore, it is called continuous discrete, if the process model is continuous and the measurements are collected at discrete time intervals (which is most often the case in bioprocess applications). The algorithm proceeds in two steps: a prediction step (corresponding to the time period between two measurement times) and a correction step occurring each time a new measurement is available.

Prediction step (between samples):

$$\frac{dx}{dt} = f(x,u), \quad t_k < t < t_{k+1} \tag{30}$$

$$\frac{dP}{dt} = A(x(t))P(t) + P(t)A(x(t))^{\mathrm{T}} \tag{31}$$

Correction step (at sampling times):

$$K(t_k) = P(t_k^-)C^{\mathrm{T}}\left[CP(t_k^-)C^{\mathrm{T}} + Q(t_k)\right]^{-1} \tag{32}$$

$$x(t_k^+) = x(t_k^-) + K(t_k)(y(t_k) - Cx(t_k^-)) \tag{33}$$

$$P(t_k^+) = P(t_k^-) - K(t_k)CP(t_k^-) \tag{34}$$

where x is the vector of concentrations of the macroscopic components, C the measurements matrix, K the correction gain, P the covariance matrix of the state estimation errors, Q the covariance matrix of the measurement noise, t_k^+ and t_k^- the time instants characterizing respectively the values before and after correction.

The extended Kalman filter requires the on-line numerical integration of the state equation (30) and the Ricatti equation (31). The latter involves the matrix $A(x) = (\partial f/\partial x)_x$ resulting from the model linearization along the predicted state trajectory.

These equations are solved starting with the initial conditions $x(0)=x_0$ and $P(0)=P_0$. For biomass, lactate and ammonia these values are best taken from the measured concentrations and the measurement error variances at the initial time. For the unmeasured component concentrations, these initial values can only be guessed based on common sense and process knowledge. The nominal values of the unmeasured part of x_0 are chosen as the initial conditions of culture 3 in (Amribt *et al.*, 2013a) (see Table 1) with P_0=diag([0.3x_0]^2). The noise standard deviation is chosen as in Amribt *et al.* (2013a) (assuming the variation coefficients of 10% for the biomass and 5% for lactate and ammonia).

The state estimation obtained with 50 runs of an extended Kalman filter when varying initial conditions randomly around ±50% of nominal values and using the measurements of biomass, lactate and ammonia (sampling period of 30min) are shown in Fig.1.

It can be observed that in the majority of runs the extended Kalman filter estimates accurately the unmeasured glucose concentration as well as the measured biomass, lactate and ammonia concentrations. However, the unmeasured glutamine concentration is poorly estimated, and the Kalman filter only converges locally when the glutamine is depleted.

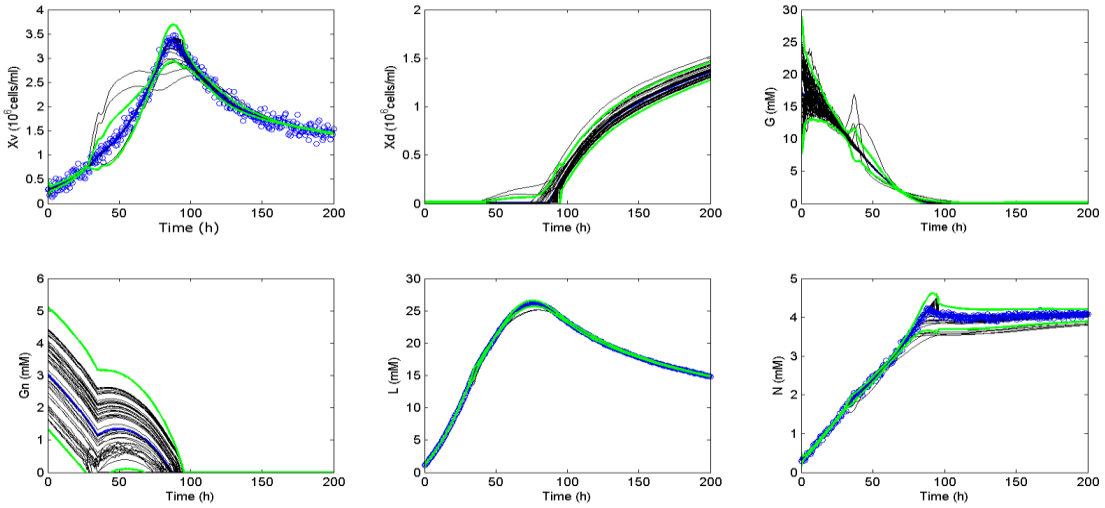

Fig. 1. Estimation of glucose and glutamine using the measurements (blue circles) of biomass, lactate and ammonia. In black: 50 runs of extended Kalman filter when varying initial conditions randomly around ±50% of nominal values. In blue: model evolution. In green: confidence intervals at 95%.

5. PARAMETER IDENTIFICATION FOR STATE ESTIMATION

To compensate for the lack of sensitivity and convergence of the extended Kalman filter with respect to glutamine, the numerical values of model parameters are modified based on the minimization of a cost function combining the identification criterion $J(\theta)$ (sum of squared differences between model predictions and experimental measurements as in Amribt *et al.* (2013a)) with a state estimation sensitivity measure criterion $F_{obs}(\theta)$ (Bogaerts and Vande wouwer, 2004).

The new cost function can be defined as:

$$F(\theta) = J(\theta) + \lambda F_{obs}(\theta) \tag{35}$$

with

$$F_{obs}(\theta) = \sum_{j=1}^{q}\sum_{i=1}^{p}\sqrt{\text{cond}\left[\left(\frac{\partial f_1}{\partial \xi_2}\right)_{ij}^{\mathrm{T}}\left(\frac{\partial f_1}{\partial \xi_2}\right)_{ij}\right]} \tag{36}$$

where "cond" represents the condition number of the matrix (the ratio of its largest to its smallest eigenvalue), λ is a weighting factor, p the number of measurements and q the number of experiments.

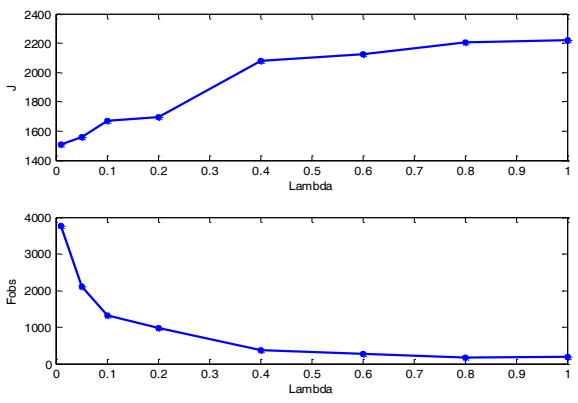

Fig. 2. Evolution of $J(\theta)$ and $F_{obs}(\theta)$ as functions of λ.

A problem that arises when using combined cost functions is the choice of the weighting factor λ. In our case, based on the evolution of the $J(\theta)$ and $F_{obs}(\theta)$ as functions of λ (see Figure 2) the value of 0.1 is retained (significant decrease of F_{obs} with no significant increase of J).

With this value of λ the cost function (35) is minimized (function fminsearch in Matlab) and the identified parameters are listed in Table 2. The modified model has been validated in the same way as the original model; see Amribt *et al.* (2013a). In comparison with Table 1, the values of some parameters, particularly $\mu_{dmax,}$ K_{Gn} and K_{Gn1}, have changed significantly.

Table 2. Modified values of model parameters

Parameter	Value	Parameter	Value
μ_{Gmax1}	1.5265	K_{Gn1}	0.2006
μ_{Gmax2}	0.0371	a	0.8757
μ_{Gnmax1}	0.1447	b	1.1806
μ_{Gnmax2}	0.0222	c	0.0805
μ_{dmax}	0.4753	d	0.4099
K_G	42.7822	m_G	0.0352
K_{Gn}	0.2770	K_{Gd}	1.7429
K_N	3.5332	K_{Gnd}	0.0020

Estimations of glucose and glutamine with an extended Kalman filter using the modified model parameters are shown in Figure 3. Significant improvement in the estimation of glutamine can be observed. Therefore, the performances of the extended Kalman filter with respect to the variation of initial conditions are improved.

Additionally, Root Mean Square Error (RMSE) (37) of the estimations of glucose and glutamine when varying initial conditions randomly around ±50% of nominal values are calculated for an extended Kalman filter using the nominal model parameters and the one using the modified model parameters, and results are shown in Table 4.

$$\mathrm{RMSE}_k = \sqrt{\frac{\sum_{j=1}^{N}\sum_{i=1}^{n}(Xobs_{k,ij} - Xmod_{k,ij})^2}{n \times N}} \tag{37}$$

where $Xobs_{k,ij}$ is the observed values of variable k (k = glucose or glutamine) at the i^{th} time instant in the j^{th} run, $Xmod_{k,ij}$ is the modeled values of variable k at the i^{th} time instant in the j^{th} run.

The glutamine RMSE obtained with the extended Kalman filter using the modified model parameters has been reduced by a factor 2 in comparison with the extended Kalman filter using the nominal model parameters, while, the glucose RMSE remains similar.

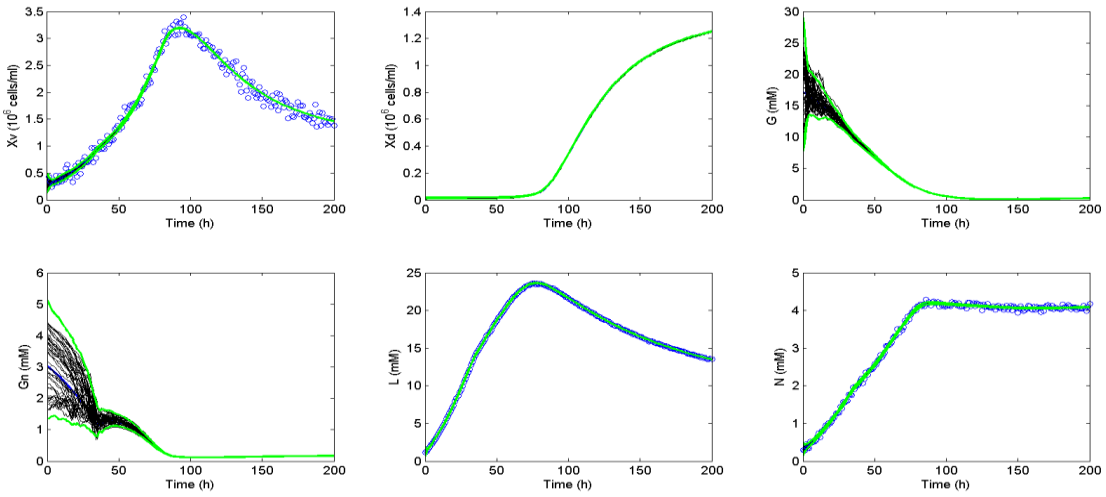

Fig. 3. Estimation of glucose and glutamine based on modified model parameters and using the measurements (blue circles) of biomass, lactate and ammonia. In black: 50 runs of extended Kalman filter when varying initial conditions randomly around ±50% of nominal values. In blue: model evolution. In green: confidence intervals at 95%.

Table 3. RMSE of glucose and glutamine obtained for different extended Kalman filter configurations

	Glucose RMSE	Glutamine RMSE
EKF using nominal model parameters	0.7844	0.5423
EKF using modified model parameters	0.7025	0.3036
EKF using modified model parameters (L and N (off-line))	0.9177	0.3069

As reliable probes for on-line measurement of lactate and ammonia are rare and more expensive than the one of biomass, an extended Kalman filter using the on-line measurement of biomass (sampling period of 30 min) and off-line measurements of lactate and ammonia (sampling period of 12 hour) is developed, and results are shown in Figure 4.

It can be observed that the estimations of glucose and glutamine obtained whit an extended Kalman filter using the off-line measurements of lactate and ammonia are satisfactory and the RMSE are similar to the ones obtained with the extended Kalman filter using the on-line measurements of lactate and ammonia.

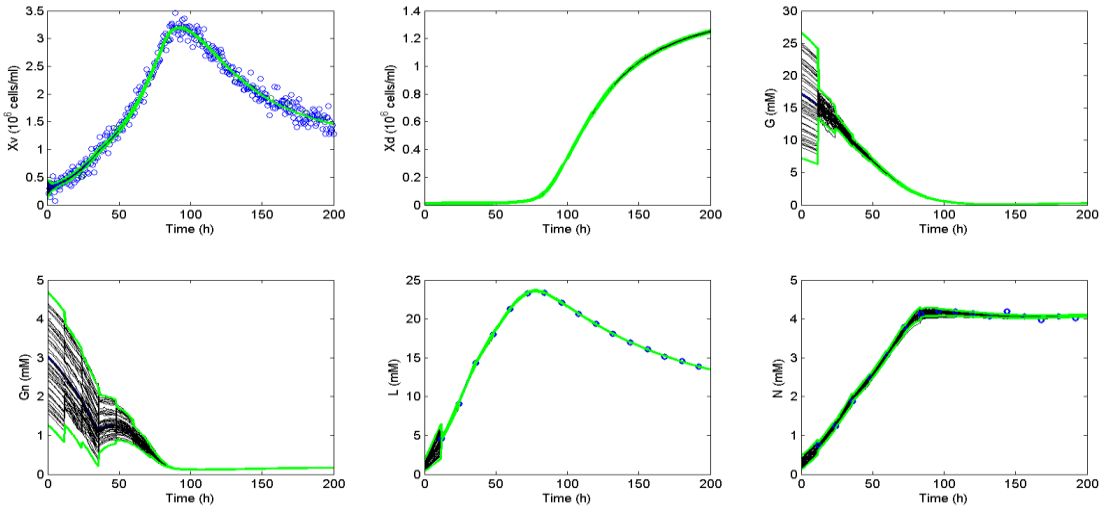

Fig. 4. Estimation of glucose and glutamine based on modified model parameters and using the measurements (blue circles) of biomass (on-line), lactate and ammonia (off-line). In black: 50 runs of extended Kalman filter when varying initial conditions randomly around ±50% of nominal values. In blue: model evolution. In green: confidence intervals at 95%.

6. CONCLUSIONS

One of the difficulties encountered in control and optimization of bioprocesses is the lack of reliable on-line sensors, which can measure the key process state variables. In this paper the design and implementation of an extended Kalman filter using the measurements of biomass (on-line), lactate and ammonia (off-line) for continuous glucose and glutamine estimation in hybridoma cell fed-batch cultures has been presented. The observability analysis indicates that the sensor configuration is observable.

The resulting observer efficiently estimates the unmeasured glucose concentration, but the glutamine concentration is poorly estimated due to a lack of sensitivity of the measured output with respect to the unmeasured glutamine. To circumvent that problem, the model parameters are identified by minimizing a cost function combining the identification criterion with a state estimation sensitivity criterion.

A perspective is to discuss the estimation performances and robustness of the developed extended Kalman filter with respect to model uncertainties and measurement noise. This will be the subject of further investigation.

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REFERENCES

Amribt, Z., Niu, H. and Bogaerts, Ph. (2013a). Macroscopic modelling of overflow metabolism and model based optimization of hybridoma cell fed-batch cultures. *Biochemical Engineering Journal*, 70, 196-209.

Amribt, Z., Dewasme, L., Vande Wouwer, A. and Bogaerts, Ph. (2013b). Optimal operation of hybridoma cell fed-batch cultures using the overflow metabolism model: numerical and analytical approach. *Accepted for the 12th IFAC Symposium on Computer Applications in Biotechnology* (CAB2013), Mumbai (India), *Dec 16-18.*

Arndt, M., Kleist, S., Miksch, G., Friehs, K., Flaschel ,E., Trierweiler, J., and Hitzmann, B. (2005). A feedforward-feedback substrate controller based on a kalman filter for a fed-batch cultivation of escherichia coli producing phytase. *Computers and Chemical Engineering*, 29, 1113-1120

Arndt, M. and Hitzmann, B. (2004). Kalman Filter Based Glucose Control at Small Set Points during Fed-Batch Cultivation of *Saccharomyces cerevisiae*. *Biotechnology Progress*, *20,* 377-383.

Bogaerts, P. and Vande Wouwer, A. (2004). Parameter identification for state estimation – application to bioprocess software sensors. *Chemical Engineering Science*, 59(12), 2465-2476

Dewasme, L., Goffaux, G., Hantson, A.-L. and Vande Wouwer, A. (2013). Experimental validation of an Extended Kalman Filter estimating acetate concentration in *E. coli* cultures. *Journal of Process Control,* 23, 148-157.

Gauthier, J.-P. and Kupka I. (1994). Observability and observers for nonlinear systems. *SIAM Journal Control and Optimization*, 32 (4), 975-994.

Hitzmann, B., Broxtermann, O., Cha, Y.-L., Sobieh, O., Stärk, E. and Scheper, T. (2000). The control of glucose concentration during yeast fed-batch cultivation using a fast measurement complemented by an extended Kalman filter. *Bioprocess Engineering*, 23, 337-341.

Rodrigues, M. E., Costa, A. R., Henriques, M., Azeredo, J. and Oliveira, R. (2010). Technological progresses in monoclonal antibody production systems. *Biotechnology Progress*, 26, 332-351.

Veloso, A. C. A., Rocha, I. and Ferreira, E. C. (2009). Monitoring of fed-batch e. coli fermentations with software sensors. *Bioprocess Biosystems Engineering*, 32,381–388.

Wurm, F.M. (2004). Production of recombinant protein therapeutics in cultivated mammalian cells. *Nature Biotechnology*, 22, 1393-1398.

Zeitz, M. (1984). Observability canonical (phase-variable) form for nonlinear time-variable systems. *International Journal of System Science*, 15(9), 949–958.