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An Adaptive Cascade Structure for the Estimation and Control of Perfusion Animal Cell Cultures

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Abstract: This paper presents an effective and robust structure for the estimation and control of perfusion cell cultures, in which the cells and glucose concentrations are simultaneously controlled by manipulating the dilution and bleed rates. Firstly, a partially linearizing feedback controller is designed to ensure an approximately linear decoupled dynamics between the controlled outputs and the manipulated inputs. Then the model of the inner loop is used to design an extended Kalman filter, which estimates all the system states used in the implementation of the linearizing feedback control law from the (possibly noisy) measurements of cells and glucose concentrations. Two PI controllers are used in the outer loop for a good tracking performance, which are tuned using a receding horizon optimization procedure. The proposed structure shows good performance and robustness with respect to parameter uncertainties, non-cancelled nonlinearities and measurement noise.

Keywords: biotechnology, control system design, multivariable feedback control, cascade control, feedback linearization, Kalman filters, PI controllers, robustness

1. INTRODUCTION

Valuable bioproducts such as vaccines, recombinant proteins and antibodies, are industrially produced by the cultivation of cells that are programmed to synthesize them. These cells can grow in suspension in stirred tank reactors (Jain and Kumar, 2008). The efforts for increasing the culture productivity in these systems focus on elaborating specific culture media, and in optimal feeding policies. The most popular operating modes in cell cultures are batch, fed-batch and perfusion modes (Jain and Kumar, 2008; Komolpis et al., 2010). Batch and fed-batch modes do not offer many options for control, except for the feed rate in the latter, and the cells growth can be inhibited by the accumulation of toxic metabolites, which cannot be removed. In perfusion mode, fresh medium is fed to replenish the consumed nutrients, while an equal volume of spent medium is continuously withdrawn, allowing for the removal of inhibitory components. Cells are retained or recycled back to the reactor by some type of retention device (for instance an acoustic filter). Higher cell concentrations and higher productivity can be achieved in perfusion cultures than in conventional batch cultures (Komolpis et al., 2010). Hence perfusion processes provide consistent culture conditions, high productivity and low product residence times, but they require tight control of the perfusion rate. Too low perfusion rates may result in nutrient limitation, accumulation of inhibitory metabolites and retardation in cell growth rate. Too high perfusion rates may result in wash out of the cells in systems with partial cell retention. However, as emphasized by (Banik and Heath, 1995; Ozturk et al., 1997; Dalm et al., 2004), the removal of a small amount of cells from the reactor through the cell-containing flow (the bleed) is necessary for maintaining the viability of the culture, as well as for reaching steady state operation.

In spite of providing increased productivity of the culture, perfusion operation with partial cell retention is hardly used at industrial scale because of the complexity raised by the multivariable nature of the process. Hence, most of the published control studies focus only on manipulating the perfusion rate (Ozturk et al., 1997; Dowd et al., 2001). Among the existing control strategies for bioprocesses, the most encountered ones for cell culture control are the robust and predictive techniques (Dowd et al., 2001; Aehle et al., 2012). Robustness is needed to cope with the culture variability and sensitivity to environmental conditions or to alleviate the negative effect of the incomplete understanding of the intricate relationship between process parameters and outputs. On the other hand, predictive control is one of the few advanced techniques which is widely accepted in industry and deals with the optimization of cell growth processes in a straightforward manner.

Recently, the potential of using the bleed flow in multivariable control structures has been investigated in several simulation studies in view of a prospective practical implementation: Deschênes et al. (2006a,b) have developed an adaptive backstepping strategy for a simple model to simultaneously control the cell and metabolite concentrations, while Sbarciog et al. (2013b) have designed a multivariable nonlinear predictive control strategy based on a more realistic model, for accelerating the growth of cells and controlling the substrate concentration in the effluent. This approach has been further simplified and robustified in (Sbarciog et al., 2013a), where a cascade control structure has been proposed, that combines a partial feedback linearizing controller in the inner loop with linear predictive

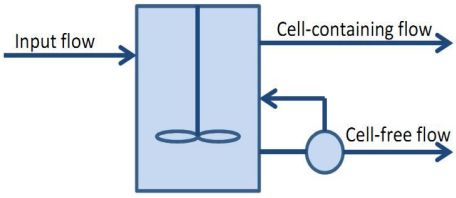

Fig. 1. Schematic representation of the perfusion culture

controllers in the outer loop. The cascade structure is an easy-to-implement solution with remarkable performance and robustness with respect to parametric uncertainty.

In this paper, we propose also a multivariable cascade control structure to simultaneously control the cells and glucose concentrations. This control structure consists of the partially linearizing feedback controller, which ensures an approximately linear decoupled dynamics between the controlled outputs and the manipulated inputs, and two PI controllers, which ensure the reference tracking performance. It is assumed that only measurements of the cells and glucose concentrations are available, therefore an extended Kalman filter is designed to estimate the states required for the implementation of the feedback controller. A receding horizon optimization based tuning algorithm is employed to periodically adjust the parameters of the two PI controllers. It is shown that the proposed control and estimation structure is efficient and robust with respect to parametric uncertainties and measurement noise.

The paper is organized as follows: Section 2 presents the animal cell growth process, while Section 3 introduces the design of the control structure. The simulation results are shown and discussed in Section 4. The conclusions and future research perspectives are highlighted in the last Section.

2. PROCESS MODEL

The animal cell culture considered in this study is described by a model, which expresses that the cells growth is activated by the presence of glucose and glutamine and their death is governed by lactate, ammonia and glutamine concentrations. A schematic representation of the perfusion culture is given in Figure 1. Medium containing glucose and glutamine is continuously supplied to the reactor. Components leave the reactor at the same rate. The amount of cells in the effluent is determined by the filtration device.

The mathematical model of the system illustrated in Figure 1 is given by:

$$\dot{\xi}_1 = -bl \cdot D\xi_1 + r_1(\xi) - r_2(\xi) \quad (1)$$

$$\dot{\xi}_2 = D(\xi_{in_2} - \xi_2) - ar_1(\xi) - r_3(\xi) \quad (2)$$

$$\dot{\xi}_3 = D(\xi_{in_3} - \xi_3) - br_1(\xi) \quad (3)$$

$$\dot{\xi}_4 = -D\xi_4 + cr_1(\xi) + dr_3(\xi) \quad (4)$$

$$\dot{\xi}_5 = -D\xi_5 + er_1(\xi) \quad (5)$$

where

- $\xi_1, \xi_2, \xi_3, \xi_4, \xi_5$ respectively represent the concentrations of viable cells, glucose, glutamine, lactate and ammonia. ξ_{in_2} and ξ_{in_3} are the concentrations of glucose and glutamine in the influent;
- $D = F/V$ is the dilution/perfusion rate and $bl \in [0, 1]$ is the bleed ratio;
- $r_i(\xi)$, $i = 1, 2, 3$ are reaction rates, given by:

$$r_1(\xi) = \mu_{max} \cdot \frac{\xi_2}{K_{Glc} + \xi_2} \cdot \frac{\xi_3}{K_{Gln} + \xi_3} \cdot \xi_1 = \mu_1(\xi) \cdot \xi_1 \quad (6)$$

$$r_2(\xi) = \frac{k_{d_{max}}}{(\mu_{max} - k_{d_{Lac}}\xi_4)(\mu_{max} - k_{d_{Amm}}\xi_5)} \cdot \frac{k_{d_{Gln}}}{k_{d_{Gln}} + \xi_3} \cdot \xi_1 = \mu_2(\xi) \cdot \xi_1 \quad (7)$$

$$r_3(\xi) = m_{Glc} \cdot \frac{\xi_2}{k_{m_{Glc}} + \xi_2} \cdot \xi_1 = \mu_3(\xi) \cdot \xi_1 \quad (8)$$

- $a, b, c, d, e > 0$ are the stoichiometric coefficients, defined as: $a = \frac{1}{Y_{X_v/Glc}}$, $b = \frac{1}{Y_{X_v/Gln}}$, $c = \frac{Y_{Lac/Glc}}{Y_{X_v/Glc}}$, $d = Y_{Lac/Glc}$, $e = \frac{Y_{Amm/Gln}}{Y_{X_v/Gln}}$.

This model has been developed from batch and fed-batch hybridoma culture results (de Tremblay et al., 1992), with the model parameters given in Table 1.

Table 1. Numerical values of the animal cell culture (as in de Tremblay et al. (1992))

$Y_{X_v/Glc}$	$1.09 \cdot 10^2$	10^6 cells/mmol
$Y_{X_v/Gln}$	$3.8 \cdot 10^2$	10^6 cells/mmol
$Y_{Lac/Glc}$	1.8	mmol/mmol
$Y_{Amm/Gln}$	0.85	mmol/mmol
μ_{max}	1.09	d^{-1}
$k_{d_{max}}$	0.69	d^{-1}
V	0.8	L
K_{Glc}	1	mmol/L
K_{Gln}	0.3	mmol/L
$k_{d_{Lac}}$	0.01	d^{-1}(mmol/L)$^{-1}$
$k_{d_{Amm}}$	0.06	d^{-1}(mmol/L)$^{-1}$
$k_{d_{Gln}}$	0.02	mmol/L
m_{Glc}	$1.68 \cdot 10^{-4}$	mmol(10^6 cells)$^{-1}d^{-1}$
$k_{m_{Glc}}$	19	mmol

3. CONTROL STRUCTURE

The main objective in controlling a cell culture is to achieve and maintain a high cell density in the reactor. High amounts of substrates supplied to the culture do not lead to a better and faster growth of the cells (Jain and Kumar, 2008). On contrary, the medium is spent inefficiently, as large amounts of expensive nutrients are lost via the effluent. Moreover, toxic byproducts causing cell death are produced. Therefore, many control implementations consider the regulation of the main nutrient glucose at a reasonable low level to minimize the formation of toxic metabolites (Dowd et al., 2001; Ozturk et al., 1997; Yang et al., 2000). In this paper we design the control structure to achieve a similar goal, i.e., the regulation of cell and glucose concentrations at specified setpoints by manipulating the dilution rate D and the bleed ratio bl.

The multivariable control structure used to simultaneously control the cells and the glucose concentrations is illustrated in Figure 2. It consists of i) a partially linearizing feedback controller, designed such as the inner loop has an approximately decoupled linear dynamics and ii) two PI controllers which compute the inputs of the nonlinear controller $\bar{\xi}_1$ and $\bar{\xi}_2$. An extended Kalman filter, which uses the model of the inner loop, provides estimates of the states required by the feedback linearizing controller. The autotuner determines the optimal parameters of

each PI controller by minimizing a predictive control criterion via receding horizon optimization.

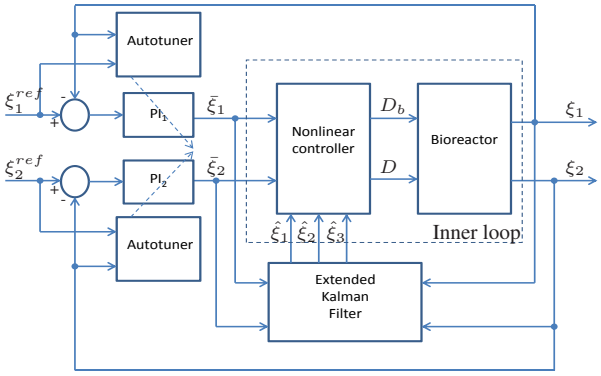

Fig. 2. Control structure

3.1 Partial feedback linearizing control

The inner loop controller is a partial feedback linearizing controller, which assumes the availability of biomass, glucose and glutamine concentrations (ξ_1, ξ_2 and ξ_3). The control law is given by:

$$D_b = bl \cdot D = \frac{1}{\xi_1}\left(r_1(\xi) - \lambda_1(\bar{\xi}_1 - \xi_1)\right) \quad (9)$$

$$D = \frac{1}{(\xi_{in_2} - \xi_2)}\left(a \cdot r_1(\xi) + r_3(\xi) + \lambda_2(\bar{\xi}_2 - \xi_2)\right) \quad (10)$$

where $\lambda_1, \lambda_2 > 0$ are the controller parameters (to be tuned) and $\bar{\xi}_1$, $\bar{\xi}_2$ are the controller inputs. Note that (9), (10) show a singularity in the control law, when $\xi_1 = 0$ and $\xi_2 = \xi_{in_2}$. However, defining a control for such a situation is meaningless, as $\xi_1 = 0$, $\xi_2 = \xi_{in_2}$ represent the condition of wash out, from which the system cannot be recovered.

For designing the controller parameters λ_1 and λ_2, we consider that the model (1)-(5) is not perfectly known. In this paper, it is assumed that μ_{max} may vary $\pm$ 20 % with respect to its nominal value, that is:

$$\mu_{max} = \mu_{max}(\delta) = \bar{\mu}_{max}(1 + 0.2\delta)\ ,\ \delta \in [-1, 1]\ , \quad (11)$$

where $\bar{\mu}_{max}$ stands for the nominal value of μ_{max} and δ is an uncertain parameter lying in the interval $[-1, 1]$. Notice that we cannot straightforwardly implement the control law given in (9)-(10), because μ_{max} is uncertain. To overcome this problem, we estimate the value of $r_1(\xi) = r_1(\xi, \delta)$ based on the nominal value of μ_{max} leading to

$$D_b = \frac{1}{\xi_1}\left(\hat{r}_1(\xi) - \lambda_1(\bar{\xi}_1 - \xi_1)\right) \quad (12)$$

$$D = \frac{1}{(\xi_{in_2} - \xi_2)}\left(a \cdot \hat{r}_1(\xi) + r_3(\xi) + \lambda_2(\bar{\xi}_2 - \xi_2)\right) \quad (13)$$

where

$$\hat{r}_1(\xi) = \bar{\mu}_{max} \cdot \frac{\xi_2}{K_{Glc} + \xi_2} \cdot \frac{\xi_3}{K_{Gln} + \xi_3} \cdot \xi_1 \quad (14)$$

Using (12) and (13) in the model (1)-(5) and defining

$$\chi_1 = \bar{\xi}_1 - \xi_1\ ,\ \chi_2 = \bar{\xi}_2 - \xi_2\ ,$$

the following dynamics for the controlled outputs is obtained:

$$\dot{\chi}_1 = -\lambda_1\chi_1 - \left(r_1(\xi, \delta) - \hat{r}_1(\xi)\right) + r_2(\xi) \quad (15)$$

$$\dot{\chi}_2 = -\lambda_2\chi_2 + a\left(r_1(\xi, \delta) - \hat{r}_1(\xi)\right) \quad (16)$$

Notice that the above dynamics is not linear since the term $r_1(\xi, \delta) - \hat{r}_1(\xi)$ is not cancelled due to parameter uncertainty and $r_2(\xi)$ is not considered in the feedback linearizing based controller to avoid additional measurements.

Thus, we design λ_1 and λ_2 to minimize the effects of the non-canceled nonlinearities in (15)-(16) on the state vector $\chi := [\chi_1\ \chi_2]^T$ in the $\mathcal{H}_\infty$ sense. To this end, we embed (15)-(16) into the following *quasi*-LPV representation (Leith and Leithead, 2000):

$$\mathcal{G}_{wz} : \left\{ \dot{\chi} = \begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix} \chi + \begin{bmatrix} 0.2\delta & 1 \\ 0.2a\delta & 0 \end{bmatrix} w\ ,\ z = \chi \right. \quad (17)$$

where the disturbance input w models the non-cancelled dynamics, that is:

$$w := \begin{bmatrix} \dfrac{\bar{\mu}_{max}\xi_1\xi_2\xi_3}{(K_{Glc} + \xi_2)(K_{Gln} + \xi_3)} \\ r_2(\xi) \end{bmatrix}$$

Then, the parameters λ_1 and λ_2 are designed to minimize an upper-bound on $\|\mathcal{G}_{wz}\|_\infty$ for all $\delta \in [-1, 1]$ using similar steps as in the approach proposed in (Dewasme et al., 2011).

Notice that the overall feedback system aims at operating in set point regions such that the death rate $r_2(\xi)$ is close to zero. In addition, if $\|\mathcal{G}_{wz}\|_\infty$ is relatively small, we may also assume that $\Delta r_1 := r_1(\xi, \delta) - \hat{r}_1(\xi) \simeq 0$. Hence, the following simplified dynamics is considered as the model of the inner loop:

$$\dot{\xi}_1 = \lambda_1\left(\bar{\xi}_1 - \xi_1\right) \quad (18)$$

$$\dot{\xi}_2 = \lambda_2\left(\bar{\xi}_2 - \xi_2\right) \quad (19)$$

$$\dot{\xi}_3 = D(\xi_{in_3} - \xi_3) - b\hat{r}_1(\xi) \quad (20)$$

$$\dot{\xi}_4 = -D\xi_4 + c\hat{r}_1(\xi) + dr_3(\xi) \quad (21)$$

$$\dot{\xi}_5 = -D\xi_5 + e\hat{r}_1(\xi) \quad (22)$$

where D is given by (13).

3.2 Extended Kalman filter

The nonlinear controller requires the knowledge of the cells, glucose and glutamine concentrations. Cells and glucose concentrations are measured on-line, however they may be affected by measurement noise which can destabilize the control loop. Glutamine concentration cannot be measured on-line, thus it needs to be estimated. Therefore an extended Kalman filter (EKF) is designed, which uses the model of the inner loop (18)-(22) to provide estimates of the system states from the noisy measurements of cells and glucose concentrations.

Figure 3 shows the estimates provided by the EKF, when constant inputs are applied to the inner loop. The estimation is carried out in the realistic scenario of a 20% increase in μ_{max} with respect to the nominal value $\bar{\mu}_{max}$. Noise free estimates of the cells and glucose concentrations are obtained. A small estimation error may be noticed for the cells and glutamine concentrations due to the difference between the real inner loop model and the simplified version assumed by the EKF, which supposes that the cells death rate is negligible. Obviously, this estimation error is influenced by the uncertainty on μ_{max}, as the cells death rate (7) depends on this parameter. Nevertheless, the overall performance will not be affected in steady state (assuming that μ_{max} is time-invariant) by a constant estimation error as the integral action of the outer loop will reject in steady state constant errors.

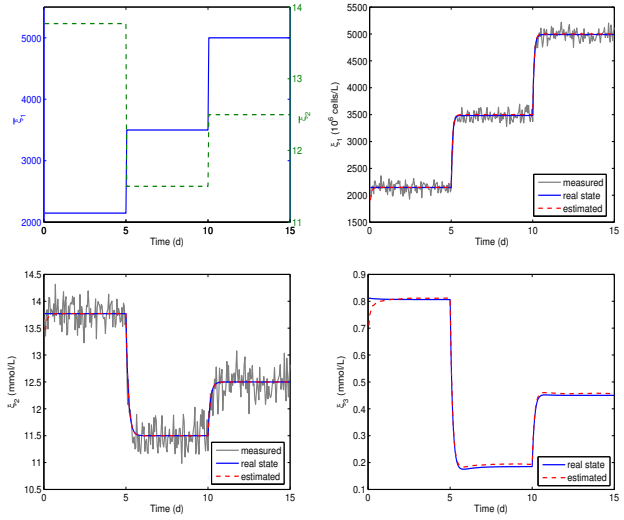

Fig. 3. Estimation of biomass, glucose and glutamine concentrations provided by the EKF

3.3 Receding horizon optimization based tuning of PI controllers

Classical PI controllers are used in the outer loop to ensure the tracking of the setpoint changes and to reject the disturbances acting on the process:

$$\bar{\xi}_j(t) = K_{p_j}\left(e_j(t) + \frac{1}{T_{i_j}}\int_0^t e_j(t)dt\right) \qquad j = 1,2 \quad (23)$$

where K_{p_j}, T_{i_j} respectively represent the proportional gains and the integral time constants. The control errors are defined as

$$e_j(t) = \xi_j^{ref}(t) - \xi_j(t) \qquad j = 1,2 \quad (24)$$

and the incremental discrete time representation of (23) is given by

$$\bar{\xi}_j(k) = \bar{\xi}_j(k-1) + K_{p_j}\left(e_j(k) - e_j(k-1)\right) + \frac{K_{p_j}}{T_{i_j}}T_s e_j(k) \quad (25)$$

where T_s represents the sampling period and k is the discrete time index ($t = kT_s$). Conventionally, the parameters K_{p_j}, T_{i_j} are time-invariant. However, to cope with the nonlinear nature of the process we adapt the controller parameters periodically. To this end, we use a discrete time representation of the inner loop

$$\xi_j^m(k) = \frac{B_j\left(q^{-1}\right)}{A_j\left(q^{-1}\right)}\bar{\xi}_j(k) + \frac{1}{1-q^{-1}}\upsilon(k), \quad j = 1,2 \quad (26)$$

where ξ_1^m, ξ_2^m are the models outputs; $A_j\left(q^{-1}\right)$, $B_j\left(q^{-1}\right)$ are polynomials in the shift operator q found by discretizing respectively the dynamics (18), (19) with a sampling period T_s; $\upsilon(t)$ is uncorrelated noise with zero mean value. Using (25), (26) iteratively, predictions of the process outputs over a horizon N_p ($\xi_j^m(k+l)$, $l = 1 \ldots N_p$) are computed. Then the parameters of the PI controllers are found by minimizing

$$J_i = \sum_{l=1}^{N_p}\left(\xi_i^{ref}(k+l) - \xi_i^m(k+l)\right)^2 + \alpha_i\left(\Delta\bar{\xi}_i(k+l-1)\right)^2 \quad (27)$$

with $\Delta\bar{\xi}_i(k) = \bar{\xi}_i(k) - \bar{\xi}_i(k-1)$, $i = 1,2$. In principle the tuning of the PI controllers may be done at every sampling instant. However, this leads to unnecessary computational effort. Therefore, the tuning is triggered periodically (every N_a samples, with $N_a \leq N_p$) as long as the control errors are higher than some thresholds.

A coarse (due to the presence of non-cancelled nonlinearities, estimation and measurement errors) evaluation of the closed loop stability can be done by computing the closed loop dynamics from (18), (19), (23) (second order dynamics) and requiring that the poles have negative real parts. The stability is ensured as long as $K_{p_i} > -1$, $i = 1,2$.

4. SIMULATION RESULTS AND DISCUSSION

Simulation results of the proposed control and estimation loop are presented in Figures 4, 5 and 6. The parameters of the partially linearizing feedback controller are set to $\lambda_1 = 11.8319$, $\lambda_2 = 6.9534$. A sampling period of 0.05d is used. For the tuning algorithm of the PI controllers the prediction horizon N_p has been set to 15 samples and the penalty coefficients α_1, α_2 in the cost functions (27) have been chosen equal to 10. The PI parameters are adapted every $N_a = 10$ samples as long as the control errors are higher than the imposed thresholds (i.e. $|e_1(t)| > 50$, $|e_2(t)| > 1$), otherwise no adaptation occurs. The optimization problems are solved using the Nelder-Mead algorithm. Constraints on the dilution and bleed rates are imposed: $0 \leq D \leq 3.75\text{d}^{-1}$, $0 \leq D_b \leq D$ (which is equivalent to $0 \leq bl \leq 1$). To cope with inputs saturation, an anti-windup mechanism is included in the PI-controllers implementation.

All the investigations are carried out in the presence of measurement noise, which largely affects the glucose concentration and less the cells concentration. The outputs of the partially linearizing feedback controller are computed based on the estimates of cells, glucose and glutamine concentrations provided by the EKF. The simulation results presented here include: the controlled outputs ξ_1, ξ_2 and their respective setpoints; the inputs of the inner loop controller computed by the two PI controllers represented with continuous line and the inputs which are admissible (to comply with the physical constraints on the flow rates) represented with dashed line; the process inputs calculated by the partially linearizing feedback controller: the dilution and the bleed rates; the parameters of the PI controllers.

Figures 4 and 5 present the closed loop response for the same setpoint profiles, but different magnitudes of the maximum growth μ_{max}. The responses are almost identical, which shows that the proposed control structure is highly robust with respect to parametric uncertainty. The PI controllers are periodically tuned, however more adaptation of the controller gains occur after a change in the setpoint. When the system approaches the steady state, the optimization for recomputing the controller parameters is triggered by the magnitude of the measurement noise rather than the process dynamics.

Figure 6 presents the closed loop response for a particular setpoint, where the glucose concentration is controlled at a low constant level while the cells concentration is periodically increased. This is one of the most representative setpoint changes for animal cells culture, as one of the control goals is to achieve a high cells density in the reactor without wasting high amounts of nutrients via the effluent. The results displayed in Figure 6 demonstrate the effectiveness of the cascade control structure in achieving one of the most important goals in cells culture control, in spite of parametric uncertainty, lack of full state measurement and presence of measurement noise.

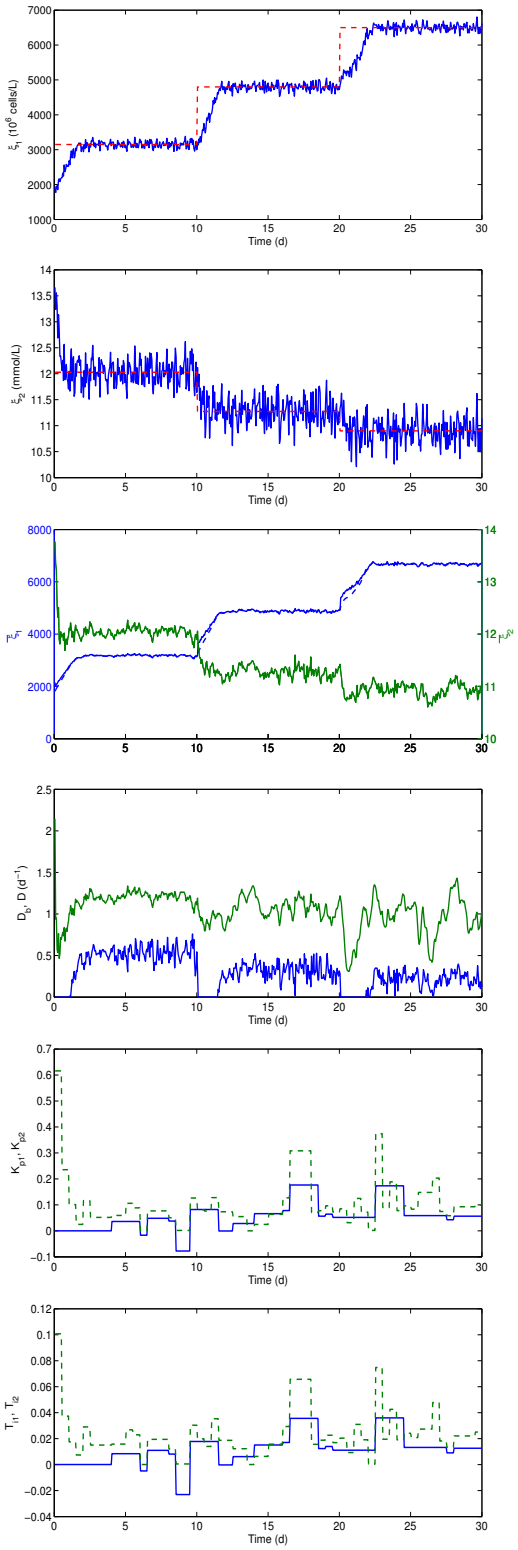

Fig. 4. Closed loop response for a 20% decrease in the maximum specific growth μ_{max}

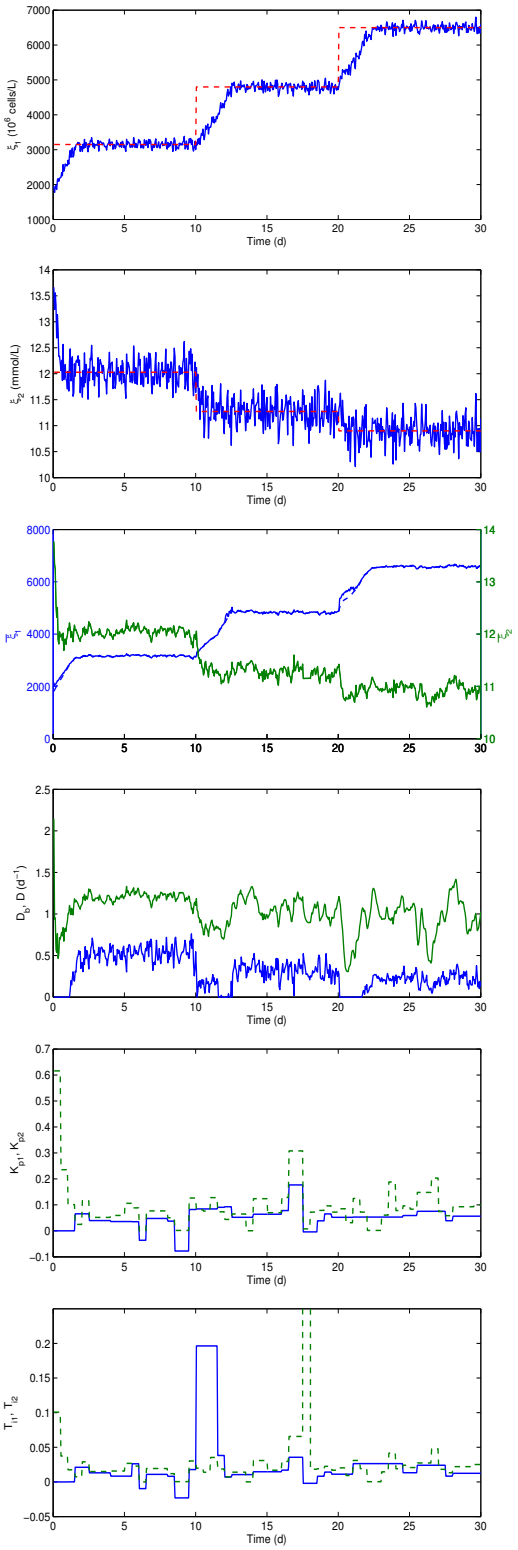

Fig. 5. Closed loop response for a 20% increase in the maximum specific growth μ_{max}

5. CONCLUSIONS AND PERSPECTIVES

In this paper an estimation and multivariable control structure has been presented for an animal cell culture. The proposed strategy i) is effective in simultaneously controlling the cells and glucose concentrations; ii) is robust with respect to parametric uncertainties, non-cancelled nonlinearities and measurement noise; iii) is easy to implement as it combines principles and tools widely used in control engineering practice: the EKF, the partially linearizing feedback controller and the PI controllers. Additionally, the computational effort is low, due to the partial linear inner loop model used in the implementation of the EKF and the periodic optimization of the PI parameters. However, in some cases the real-life implementation of the cascade control loop may be hampered by the assumption of the kinetics structure knowledge that has been used in this design.

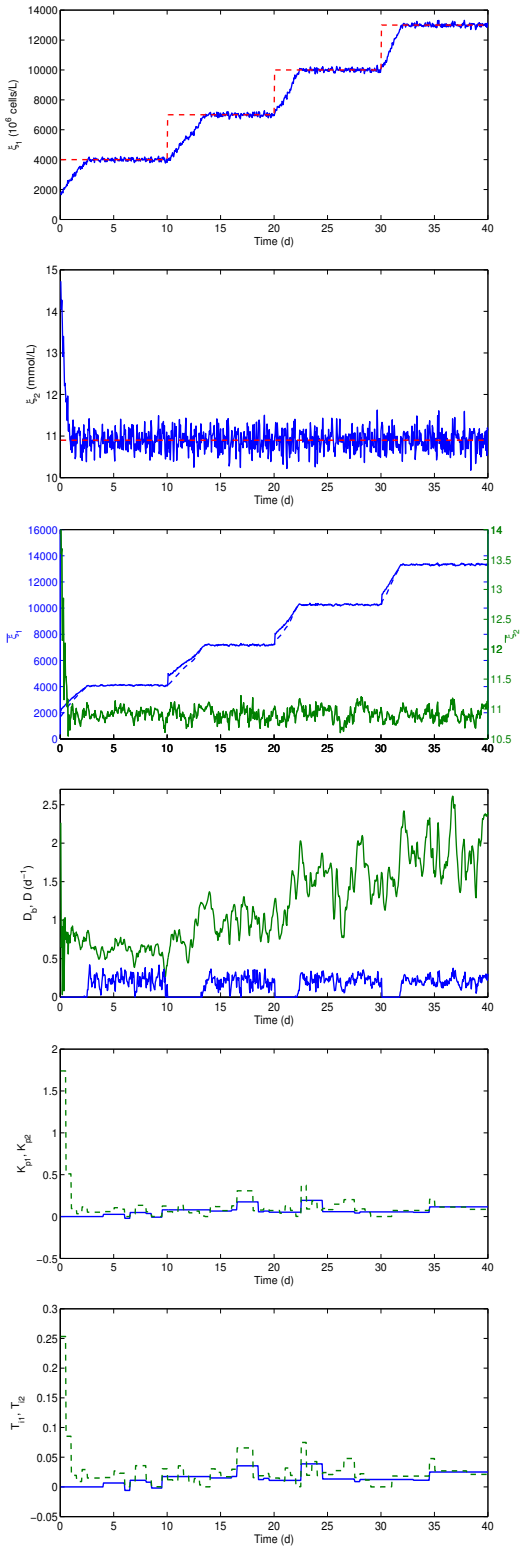

Fig. 6. Closed loop response for a low glucose setpoint with a 20% decrease in the maximum specific growth μ_{max}

Therefore our future developments aim at further robustifying the cascade control structure, such that it becomes independent of the process kinetics.

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