Preprints of the
9th International Symposium on Advanced Control of Chemical Processes
The International Federation of Automatic Control
June 7-10, 2015, Whistler, British Columbia, Canada

An Observer-based Robust Control Strategy for Overflow Metabolism Cultures in Fed-Batch Bioreactors

G. Araujo Pimentel * **M. Benavides** * **L. Dewasme** *
D. Coutinho ** **A. Vande Wouwer** *

* *University of Mons - Automatic Control Laboratory - Biosys, Boulevard Dolez 31, 7000 Mons, Belgium.* <guilherme.araujopimentel, micaela.benavides, laurent.dewasme, alain.vandewouwer>@umons.ac.be
** *Federal University of Santa Catarina, Department of Automation and Systems, Florianópolis - SC, Brazil.* daniel.coutinho@ufsc.br

Abstract: An observer-based robust control strategy is proposed for controlling overflow metabolism cultures operated in fed-batch mode. In order to maximize the biomass productivity, the controller is designed to regulate the inhibitory by-product concentration at small levels keeping the substrate concentration close to its critical level. To this end, a reduced order nonlinear model of the bioprocess dynamics is obtained and a partial feedback linearizing strategy is applied. The resulting free linear dynamics is designed by means of a convex optimization problem aiming at mitigating the effects of non canceled nonlinearities and model uncertainties. An adaptive extended Luenberger observer is also designed for estimating the by-product concentration from the measurements of biomass and substrate concentrations. Realistic numerical simulations demonstrate that the proposed observer based robust control strategy is able to maximize the biomass concentration despite large disturbances on the measurements (30 % for substrate and 15 % for biomass concentrations).

Keywords: overflow metabolism, fed-batch bioreactor, robust control, robust observer.

1. INTRODUCTION

Fed-batch bioreactors are common processes in the pharmaceutical industry producing recombinant proteins from genetically manipulated host microorganisms. The current objective of a fed-batch culture is to maximize the biomass productivity through the manipulation of the feed rate profile, despite the possible presence of inhibitory by-products. This study considers cultures of microorganisms subject to "overflow metabolism" also called "short-term Crabtree effect" which involves the generation of inhibitory by-products as a response to an excess of feeding (Deken, 1966; Crabtree, 1929). This phenomenon is observed, for instance, in *Saccharomyces Cerevisiae*, *Escherichia Coli*, *Pichia Pastoris* and *Mammalian cell* cultures with the production of ethanol, acetate, methanol and lactate, respectively (Sonnleitner and Käppeli, 1986; Rocha, 2003; Amribt et al., 2013).

Several feedback strategies have been proposed in the specialized literature to control fed-batch bioreactors, such as PID controllers (Axelsson, 1989), adaptive control (Dewasme et al., 2011; Axelsson, 1988; Chen et al., 1995) and nonlinear model predictive control (NMPC) (Hafidi et al., 2008; Santos et al., 2012). An usual challenge is the availability of a number of measurement signals, a problem that has often been handled using software sensor techniques (Bastin and Dochain, 1990; Dewasme et al., 2013), since hardware sensors are either expensive, unreliable or non-existent.

This paper aims at designing a feedback strategy to maximize the biomass productivity of *E. Coli* cultures. The proposed strategy consists in a simple partial linearizing robust controller coupled to an extended adaptive Luenberger observer. As in practice it is quite difficult to measure the substrate critical concentration, the proposed strategy regulates the by-product concentration at small levels in order to improve cell respirative capacity. In this setting, a reduced-order model of the by-product dynamics is derived and a partial feedback linearizing control law is designed. The resulting free linear dynamics is computed in order to mitigate the effects of model uncertainty and of the non-canceled dynamics by means of the linear matrix inequality (LMI) framework. A robust extended Luenberger observer having two operating modes (respirative and respiro-fermentative regimes) is designed to estimate the by-product concentration. The observer gains are numerically computed in the H_∞ setting considering a linear parameter varying (LPV) model of the bioreactor and a common quadratic Lyapunov function to allow arbitrary switching.

This paper is organized as follows: Section 2 presents the bioreactor model detailing the overflow metabolism of *E.Coli* cultures and the main operating regimes; Section 3 introduces the feedback control strategy consisting in the robust control and the switching observer designs; The overall behavior of the controller-observer is demonstrated via realistic numerical simulations in Section 4, while Section 5 draws some conclusions.

2. BIOREACTOR DYNAMIC MODEL

The overflow metabolism of different strains (yeasts, bacteria, animal cells, etc.), which the fermentative pathway follows a comparable mechanism from a macroscopic point of view, have been extended and experimentally proved by Rocha (2003); Amribt et al. (2013) and can be summarized by the following three main catabolic reactions:

- Substrate Oxidation
$$k_1 S + k_5 O \xrightarrow{\varphi_1} X + k_8 C \tag{1}$$
- Substrate Fermentation
$$k_2 S + k_6 O \xrightarrow{\varphi_2} X + k_9 C + k_3 A \tag{2}$$
- By-product Oxidation
$$k_4 A + k_7 O \xrightarrow{\varphi_3} X + k_{10} C \tag{3}$$

In the above reactions, in the particular case of *E. Coli* cultures, the variables X, S, A, O and C denote respectively the biomass, substrate, acetate, dissolved oxygen and carbon dioxide concentrations, while the yield coefficients are represented by k_i. The nonlinear growth rates φ_i, $i = 1, 2, 3$, are expressed as follows:

$$\varphi_i = \mu_i X \ , \ i = 1, 2, 3 \tag{4}$$

It is assumed that specific growth rates μ_i depend on the operating regime (Rocha, 2003):

$$\mu_1 = \frac{\min(q_S, q_{Scrit})}{k_1} \tag{5}$$

$$\mu_2 = \frac{\max(0, q_S - q_{Scrit})}{k_2} \tag{6}$$

$$\mu_3 = \begin{cases} \dfrac{\max(0, q_A)}{k_4} & q_S k_{OS} + q_A k_{OA} \leq q_O, \\ & \text{if} \\ \dfrac{\max(0, (q_O - q_S k_{OS}/k_{OA})}{k_4} & \text{otherwise} \end{cases} \tag{7}$$

The substrate consumption q_S, the critical substrate consumption $q_{S,crit}$, and the product oxidative rate q_A are modeled as follows:

$$q_S = \mu_{S,max} \frac{S}{S + K_S} \tag{8}$$

$$q_{S,crit} = \frac{q_O}{k_{OS}} = \frac{\mu_{O,max}}{k_{OS}} \frac{O}{K_O + O} \frac{K_{iO}}{K_{iO} + A} \tag{9}$$

$$q_A = \mu_{A,max} \frac{A}{K_A + A} \frac{K_{iA}}{K_{iA} + A} \tag{10}$$

where K_S, K_O and K_A are the half saturation parameters linked to each state and K_{iA} and K_{iO} are the inhibition constants. k_{OS} represents the yield coefficient between oxygen and substrate consumptions and k_{OA} between oxygen and by-product consumptions. Smooth saturation factors ruled by Monod laws are composed of $\mu_{S,max}$, $\mu_{O,max}$ and $\mu_{A,max}$ as maximal values of specific growth rates and the inhibitory by-product is represented by A.

Sonnleitner and Käppeli (1986) have introduced the bottleneck assumption to explain the metabolic switches of microorganisms in relation to their limited oxidative capacity. The changes can be explained by the substrate inlet flux and two operation regimes. The respiro-fermentative (RF) regime occurs when the by-product is produced, as a consequence of overfeeding (the concentration of substrate is larger than the critical concentration S_{crit}). The respirative (R) regime takes place when both substrate and by-product are oxidized (see Figure 1).

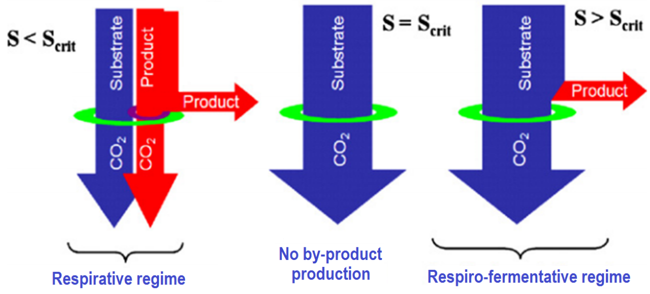

Fig. 1. Bottleneck assumption for cell limited respiratory capacity (adapted from Sonnleitner and Käppeli (1986)).

The fed-batch bioreactor dynamics are described with the following differential equations:

$$\begin{aligned} \frac{dX}{dt} &= (\mu_1 + \mu_2 + \mu_3)X - DX \\ \frac{dS}{dt} &= -(k_1\mu_1 + k_2\mu_2)X - D(S + S_{in}) \\ \frac{dA}{dt} &= (k_3\mu_2 - k_4\mu_3)X - DA \\ \frac{dO}{dt} &= -(k_5\mu_1 + k_6\mu_2 + k_7\mu_3)X - DO + k_La(O_{sat} - O) \\ \frac{dC}{dt} &= (k_8\mu_1 + k_9\mu_2 + k_{10}\mu_3)X - DC - k_La(C_{sat} - C) \\ \frac{dV}{dt} &= F_{in} \end{aligned} \tag{11}$$

where S_{in} is the inlet substrate concentration; F_{in} is the inlet feed rate; V is the bioreactor volume; D the dilution rate, expressed as $D = F_{in}/V$; k_La is the volumetric transfer coefficient; O_{sat} and C_{sat} are the dissolved oxygen and carbon dioxide saturation concentrations, respectively. For convenience, the state vector, the control input and the measurements are often represented by

$$\mathbf{x} = [\ x_1 \ \ x_2 \ \ x_3 \ \ x_4 \ \ x_5 \ \ x_6\ ]' = [\ X \ \ S \ \ A \ \ O \ \ C \ \ V\ ]' \ ,$$

$$u = D \quad \text{and} \quad y = C_y \mathbf{x} \ ,$$

respectively, with C_y being a given constant matrix to be defined later.

3. CONTROL STRATEGY

In order to maximize the biomass production, a normal procedure is to drive the substrate concentration to its critical level S_{crit}. However, it is delicate to keep S close to S_{crit} since the sensitivity of substrate probes are too low for small concentrations. This practical disadvantage can be circumvented by a suboptimal strategy involving the regulation of the by-product concentration at a low level (Valentinotti et al., 2003). In contrast with the strategy of Coutinho and Vande Wouwer (2010) which considers the estimation of μ_1 and μ_3, in this paper, an observer-based partial feedback linearizing control law is applied to regulate x_3 around its set point reference value x_3^* (typically, very close to zero) as illustrated in Figure 2.

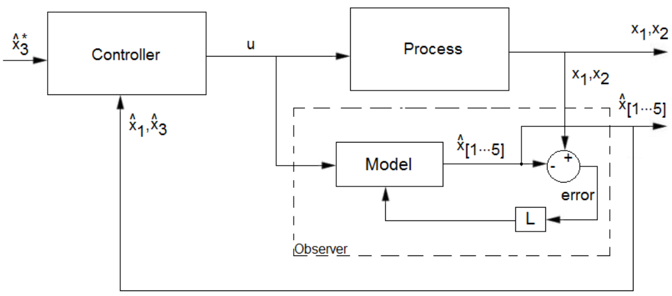

Fig. 2. Proposed control strategy.

In the following, the partial feedback linearization and the observer design are detailed.

3.1 Robust Control Law

The following assumptions for the control design are formulated: (i) the theoretical value of S_{crit} is very small (typically smaller than 0.1 g/L) and (ii) there is no accumulation of glucose in the bioreactor. Thus, the small amount of glucose is instantly consumed by the microorganisms, and in turn, $\frac{dx_2}{dt} \approx 0$ and $x_2 \approx 0$. Thus, the following relation can be derived from the second equation of (11)

$$\mu_2 x_1 \approx -\frac{k_1\mu_1 x_1 + S_{in}u}{k_2} \tag{12}$$

Taking the by-product dynamics into account and applying equation (12) yields the following reduced-order model:

$$\dot{x}_3 = \left(-\frac{k_3k_1\mu_1}{k_2} - k_4\mu_3\right)x_1 + \left(\frac{k_3S_{in}}{k_2} - x_3\right)u \tag{13}$$

To simplify the feedback linearizing design, it is assumed that $\mu_3 \approx 0$, due to the extremely low production of by-product when the optimal conditions are reached. Moreover, the specific growth rate μ_1 is modeled by the following time-varying parameter:

$$\mu_1 = \mu_1(t) = \bar{\mu}_1 + \sigma(t)\hat{\mu}_1 \ , \ \sigma(t) \in [-1,1] \tag{14}$$

where $\bar{\mu}_1$ is the mean value of $\mu_1(t)$ and the term $\sigma(t)\hat{\mu}_1$ is the deviation from $\bar{\mu}_1$. Assuming that the maximum ($\mu_{1_{\max}}$) and minimum ($\mu_{1_{\min}}$) values are known, $\bar{\mu}_1$ and $\hat{\mu}_1$ can be computed using the following equations:

$$\bar{\mu}_1 = \frac{\mu_{1_{\max}} + \mu_{1_{\min}}}{2}, \ \hat{\mu}_1 = \frac{\mu_{1_{\max}} - \mu_{1_{\min}}}{2}. \tag{15}$$

In view of the above simplifications and the reduced-order model in (13), the following control law is proposed:

$$u_{PL} = \frac{1}{\frac{k_3S_{in}}{k_2} - x_3}\left(\frac{k_3k_1\bar{\mu}_1x_1}{k_2} + \lambda(x_3^* - x_3)\right) \tag{16}$$

where $\lambda(x_3^* - x_3)$ is a free linear dynamic to be designed later in this paper. This control law approximately drives x_3 towards x_3^*, which is set as a small value. Note that S_{in} is always positive and $k_3S_{in}/k_2 - x_3$ is typically always positive (since the controller only acts when the process is in fed-batch mode and x_3 has a small positive value) which guarantees that (16) is non singular.

A robust control setting is considered for designing the parameter λ, where the time-varying parameter $\sigma = \sigma(t)$ is used to bound the non-canceled nonlinearity of the growth rate μ_1 in the closed-loop system of (13) with (16). Precisely, the closed-loop dynamics can be cast as follows:

$$\dot{x}_3 = -\lambda x_3 + \lambda x_3^* - k_4\mu_3x_1 + \frac{k_3k_1}{k_2}(\bar{\mu}_1 - \mu_1)x_1$$

Applying the assumption of $\mu_3 \approx 0$, the above dynamics can be further simplified to the following:

$$\dot{x}_3 = \lambda(x_3^* - x_3) + \frac{k_3k_1}{k_2}(\bar{\mu}_1 - \mu_1)x_1 \tag{17}$$

By defining $\xi = x_3^* - x_3$, the following input-output mapping can be written to represent the tracking error by-product dynamics:

$$\mathcal{M} \ : \ \begin{cases} \dot{\xi} &= -\lambda\xi - \hat{\mu}_1\sigma(t)w \\ z &= \xi \end{cases} \tag{18}$$

where $w := \frac{k_3k_1}{k_2}x_1$ is the input disturbance to the system $\mathcal{M}$, $\sigma(t) \in \Delta := [-1,1]$ and z represents the performance variable (i.e., the tracking error).

In this study, the parameter λ is computed by means of the LMI framework. More precisely, a convex optimization problem in terms of parameter-dependent LMI constraints is derived in order to minimize an upper bound on the $\mathcal{H}_\infty$ norm of the *quasi-LPV* model, ensuring robustness against model uncertainties and exogenous disturbances.

Considering a finite time interval $[0,T]$,the $\mathcal{L}_2$-gain of $\mathcal{M}$ is defined by assuming zero initial conditions:

$$\|\mathcal{M}_{wz}\|_{\infty,[0,T]} := \sup_{\sigma\in\Delta, 0\neq w} \frac{\|z\|_{2,[0,T]}}{\|w\|_{2,[0,T]}} \tag{19}$$

where T is the batch period and w is assumed to be a signal with finite energy in T.

An upper-bound α on $\|\mathcal{M}_{wz}\|_{\infty,[0,T]}$ can be minimized by means of the following optimization problem:

$$\min_{\lambda,\alpha} \ \alpha \ : \ \|\mathcal{M}_{wz}\|_{\infty,[0,T]} \leq \alpha \ , \ \sigma \in \Delta \tag{20}$$

subject to the stability of $\mathcal{M}$, where λ and α are the decision variables.

Based on this idea, the following candidate control-Lyapunov function is considered:

$$V(\xi) = \xi' Q \xi \tag{21}$$

where Q is a positive scalar to be determined.

The condition $\|z\|_{2,[0,T]} \leq \alpha\|w\|_{2,[0,T]}$ and the closed-loop stability are ensured by means of the following Lyapunov-like stability condition (Boyd et al., 1994):

$$\min_{\sigma\in\Delta} \ \alpha \ : \ V(\xi) > 0 \ , \ \dot{V}(\xi) + \frac{1}{\alpha}z'z - \alpha w'w < 0 \tag{22}$$

The time derivative of $V(\xi)$ can be cast as follows:

$$\begin{aligned} \dot{V}(\xi) &= 2\xi' Q\dot{\xi} = 2\xi' Q(-\lambda\xi - \hat{\mu}_1\sigma w) \\ &= \begin{bmatrix} \xi \\ w \end{bmatrix}' \begin{bmatrix} -2Q\lambda & -\hat{\mu}_1 Q\sigma \\ -\hat{\mu}_1\sigma Q & 0 \end{bmatrix} \begin{bmatrix} \xi \\ w \end{bmatrix} \end{aligned} \tag{23}$$

Applying (23) in (22) yields the following inequality:

$$\begin{bmatrix} \xi \\ w \end{bmatrix}' \begin{bmatrix} -2m & -\hat{\mu}_1 Q\sigma \\ -\hat{\mu}_1\sigma Q & -\alpha \end{bmatrix} \begin{bmatrix} \xi \\ w \end{bmatrix} - \frac{1}{\alpha}z'z < 0 \tag{24}$$

with $m = \lambda Q$ being the controller parameter.

Using the Schur's complement in the later expression leads to the following convex optimization problem:

$$\min_{Q,m,\alpha} \alpha : \begin{cases} \alpha > 0 \ , \ Q > 0 \ , \ m > 0 \\ \begin{bmatrix} -2m & -\hat{\mu}_1\sigma_1 Q & 1 \\ -\hat{\mu}_1\sigma_1 Q & -\alpha & 0 \\ 1 & 0 & -\alpha \end{bmatrix} < 0 \\ \begin{bmatrix} -2m & -\hat{\mu}_1\sigma_2 Q & 1 \\ -\hat{\mu}_1\sigma_2 Q & -\alpha & 0 \\ 1 & 0 & -\alpha \end{bmatrix} < 0 \end{cases} \tag{25}$$

for $\sigma_1 = -1$ and $\sigma_2 = 1$.

The LMI constraints on the right hand side of (25) are sufficient conditions to ensure the local asymptotic stability of system $\mathcal{M}$. If there exists a solution to the above optimization problem, then the parameter λ is obtained by means of $\lambda = mQ^{-1}$.

3.2 Extended Adaptive Luenberger Observer Design

In the preceding section, the controller is based on the measurements of all needed outputs. Nonetheless, this is not the case for *E. Coli* cultures, as acetate on-line probes are barely available (Dewasme et al., 2013). To overcome this problem, an extended Luenberger observer estimating x_3 is proposed. Note that, the model observability with respect to substrate and biomass measurements has been proved by Dewasme et al. (2013). Since the bioreactor dynamics depend on the operation regimes (respirative and respiro-fermentative), the proposed extended Luenberger observer has adaptive gains, precisely: one for the respirative regime (L_R) and another for the respiro-fermentative (L_{RF}) regime. When the process reaches low concentrations of substrate, the observer starts to switch between L_R and L_{RF} depending on the regime (larger substrate concentration than S_{crit} or smaller, respectively).

To implement an observer that adapts between the two regimes it is assumed that the process dynamic can be expressed as $f_j(\mathbf{x}, u)$ with $\mathbf{x} = [x_1;\ x_2;\ x_3;\ x_4;\ x_5;\ x_6]$ and $j = [R \quad RF]$ (i.e.: the dynamic $f_R(\mathbf{x}, u)$ represents the process dynamics when the system is in the respirative regime). Consequently, the Luenberger observer is designed taking the nonlinear switching system into account:

$$\begin{aligned} \dot{\mathbf{x}} &= f_j(\mathbf{x}, u) + B_w w \\ y &= C_y \mathbf{x} + D_w w \end{aligned} \tag{26}$$

where $\mathbf{x} \in \mathcal{X} \subset \Re^n$ is the state vector, $u \in \mathcal{U} \subset \Re^{n_u}$ is the input vector, $w \in \mathcal{W} \subset \Re^{n_w}$ is the vector of disturbance signals, $y \in \mathcal{Y} \subset \Re^{n_y}$ is the output vector and $B_w \in \Re^{n \times n_w}$, $C_y \in \Re^{n_y \times n}$ and $D_w \in \Re^{n_y \times n_w}$ are constant matrices.

Based on equation (26), a Luenberger observer (Figure 2) is proposed:

$$\begin{aligned} \dot{\hat{\mathbf{x}}} &= f_j(\hat{\mathbf{x}}, u) + L_j(y - \hat{y}) \\ \hat{y} &= h(\hat{\mathbf{x}}) \end{aligned} \tag{27}$$

with $\hat{\mathbf{x}}$ the state vector estimate, L_j the observer gain.

Defining the observation error as $e := \mathbf{x} - \hat{\mathbf{x}}$, the error dynamics is approximated as follows:

$$\dot{e} = (F_j(\hat{\mathbf{x}}, u) - L_j C)\, e + (B_\omega - L_j D_\omega)\omega \tag{28}$$

where the state dependent elements of $F_j(\mathbf{x}, u)$ are considered as bounded time-varying parameters $\theta_j(t)$ in order to determine the mode dependent observer gains L_j via a convex optimization problem computed offline. Hence, the error dynamics are redefined as follows:

$$\dot{e} = [\, F_j(\theta_{\mathbf{j}}, u) - L_j C_y \quad B_w - L_j D_w \,] \begin{bmatrix} e \\ w \end{bmatrix} \tag{29}$$

where $\theta_{\mathbf{j}} \in \Theta_j \subset \mathcal{R}^q$ and Θ_j is a known polytope.

Next, the Lyapunov theory is used to ensure the stability of the system. For this purpose, the following quadratic function is considered:

$$V(t) = V(e) = e' P e \ , \ P = P' > 0 \ . \tag{30}$$

The error dynamics will be locally asymptotically stable if $\dot{V}(e) < 0$. In view of (30), the time derivative of $V(e)$ is:

$$\dot{V}(e) = \dot{e} P e' + e' P \dot{e} \tag{31}$$

Taking (29) and (31) into account and defining $\upsilon = \begin{bmatrix} e' & w' \end{bmatrix}'$ the time derivative of $V(e)$ can be expressed as:

$$\dot{V} = \upsilon' \begin{bmatrix} PF_j + F_j' P - PL_j C_y - C_y' L_j' P & * \\ B_w' P - D_w' L_j' P & 0 \end{bmatrix} \upsilon \tag{32}$$

where the asterisks (*) denoting block matrices are inferred by symmetry. Note that as both gains are computed with the same Lyapunov function, the switching stability of both are guaranteed (refer to Liberzon (2003)).

In addition to stability, an upper bound constraint on the $\mathcal{H}_\infty$ norm of the state estimation error over the state measurement error is designed. Note that, as mentioned in Section 3, the substrate sensor has low sensitivity for low concentrations, resulting in a noisy measurement. To this end, the same methodology proposed for the controller design, equation (19), is used for the observer design, but this time considering ω as the measurement disturbances. The minimization problem can be rewritten as:

$$\dot{V}(\mathbf{x}) + e' C_z' \gamma_j^{-1} C_z e - \gamma_j \mathbf{x}'\mathbf{x} - \gamma_j w' w < 0 \tag{33}$$

Replacing the time derivative of the Lyapunov function (31) in the last condition and applying the Schur complement, the following optimization problem arises:

$$\min_{P,R_j,\gamma_j} \gamma_j : \begin{cases} P > 0 \\ \begin{bmatrix} \Pi_j & * & * & * \\ B_w' P - D_w' R_j' & 0 & -\gamma_j I_{nw} & 0 \\ C_z & 0 & 0 & -\gamma_j I_{nz} \end{bmatrix} < 0 \end{cases} \tag{34}$$

with $\Pi_j = F_j(\theta_{\mathbf{j}})' P - C_y' R_j' + P F_j(\theta_{\mathbf{j}}) - R_j C_y$, $R_j = L_j^T P$.

The variables for the optimization problem under LMI constraints are γ_j, P and R_j from which the observer gains are obtained by solving the LMIs at the vertices of θ_j applying $L_j = P^{-1} R_j$.

With this method, the observer may have a bias on its estimated states. This can be resolved by restricting the matrix R_j in a way that $\|L_j\| \leq \sqrt{\psi} I$. Thus, the restriction equation (35) is solved at the same time as (34).

$$\min_{P,R_j,\gamma_j} \gamma_j : \begin{cases} (P - \psi I) \geq 0 \\ \begin{bmatrix} \psi I & R_j' \\ R_j & I \end{bmatrix} \geq 0 \end{cases} \tag{35}$$

In order to obtain $F_j(\theta_{\mathbf{j}}, u)$, the nonlinearities are grouped in two polytopes to obtain L_R and L_{RF}, one for respirative regime $\theta_{\mathbf{R}} = [\,\theta_2\ \theta_3\ \theta_6\ \theta_8\ \theta_9\,]$ and another for respiro-fermentative regime $\theta_{\mathbf{RF}} = [\,\theta_1\ \theta_4\ \theta_5\ \theta_7\ \theta_{10}\,]$

$$\theta_1 = \frac{q_S}{k_1}; \quad \theta_2 = \frac{q_{S,crit}}{k_2}; \quad \theta_3 = \frac{(\theta_1 - \theta_2)}{k_3};$$
$$\theta_4 = \frac{(q_O - q_S k_{OS}/k_{OA})}{k_4}; \quad \theta_5 = (\theta_1)'_{x_2} x_1; \quad \theta_6 = (\theta_2)'_{x_3} x_1;$$
$$\theta_7 = (\theta_4)'_{x_3} x_1; \quad \theta_8 = (\theta_2)'_{x_2} x_1; \quad \theta_9 = (\theta_1)'_{x_3} x_1;$$
$$\theta_{10} = (\theta_3)'_{x_2} x_1;$$

where $(\theta_{ind})'_{x_{ind}}$ denotes the partial derivative of θ_{ind} by x_{ind}.

4. SIMULATION RESULTS

The process simulation uses the parameters presented in Table 1 and the process stops when the maximum tank volume of five liters is reached. The critical concentration of substrate is very small and is set to 0.03 g/L for this application. The implemented control law is redefined as follows:

$$u_{PL} = \frac{1}{\frac{k_3 S_{in}}{k_2} - \hat{x}_3} \left(\frac{k_3 k_1 \bar{\mu}_1 \hat{x}_1}{k_2} + \lambda (x_3^* - \hat{x}_3) \right) \qquad (36)$$

The controller gain (equation (25)) and the observer gains (equation (34) and (35)) are solved using the parser Yalmip (Löfberg, 2004) and solver SDPT-3 in Matlab (Toh et al., 1998).

The proposed control law is designed and the robust feedback gain is computed by equation (25). The robust gain is $\lambda = 3.0631 \times 10^3$ and the by-product set-point (x_3^*) is set to 0.1 g/L. Note that the optimization routine computes the largest gain possible, i.e. a robust gain, that guaranties the stability objectives within the selected boundaries.

Next, the extended adaptive Luenberger observer gains L_R and L_{RF} are designed assuming x_1 (biomass) and x_2 (substrate) measurements, while taking the following two polytopes of admissible values for the states into account to design the observer:

$$\mathcal{X}_R = \{0 \le S \le 2.5; 0.2 \le A \le 1.2; 0 \le X \le 35; 0 \le D \le 0.045\},$$
$$\mathcal{X}_{RF} = \{0 \le S \le 0.1; 0.1 \le A \le 1.2; 0 \le X \le 35; 0 \le D \le 0.045\}.$$

Taking into account that the designed observer has two groups of five nonlinearities, this implies that the stability condition system has 2^{10} vertices. The observer gains resulting from (34) and (35) with $\psi = 0.01$, $B_w = 1.0e^6 * I_{n\times n}$, $D_w = \left[1.0e^6\ 0\ 0\ 0\ 0; 0\ 1.0e^3\ 0\ 0\ 0\right]$ and $C_y = [\,1\ 0\ 0\ 0\ 0; 0\ 1\ 0\ 0\ 0\,]$ are obtained from $L_R = P^{-1} R_R^T$ and $L_{RF} = P^{-1} R_{RF}^T$:

$$L_R = \begin{bmatrix} 0.0173 & -0.0105 \\ -0.0244 & 0.0153 \\ -0.0647 & 0.0391 \\ 0.0001 & -6.0482e^{-5} \\ -0.0002 & 0.0001 \end{bmatrix}, L_{RF} = \begin{bmatrix} 0.0120 & -0.0206 \\ -0.0168 & 0.0632 \\ -0.0124 & 0.0603 \\ -5.5303e^{-5} & 0.0003 \\ 4.4974e^{-5} & -0.0003 \end{bmatrix}$$

For a more realistic simulation, disturbance with a normal distribution and deviation of 15 % and 30 % of each signal are added to the biomass and substrate measurement respectively. The difference of percentages emulate less reliability of substrate sensors.

X_0	5.0	g/L	S_0	2.0	g/L
A_0	0.5	g/L	V_0	3.17	L
F_{in}	0.001	L/h	S_{in}	250	g/L
$\mu_{S,max}$	1.832	g/g h	k_1	3.164	g/g
K_S	0.1428	g/L	k_2	25.22	g/g
k_{OS}	2.020	g/L	k_3	10.90	g/g
$\mu_{O,max}$	0.7218	g/g h	k_4	6.382	g/g
K_{iO}	6.952	g/L	k_5	1.074	g/g
$\mu_{A,max}$	0.0967	g/g h	k_6	11.89	g/g
K_A	0.5236	g/L	k_7	6.089	g/g
k_{OA}	1.996	g/L	k_8	1.283	g/g
K_{iA}	5.85	g/L	k_9	19.09	g/g
			k_{10}	6.57	g/g

Table 1. Inputs and model parameters (Hafidi et al., 2008; Rocha, 2003).

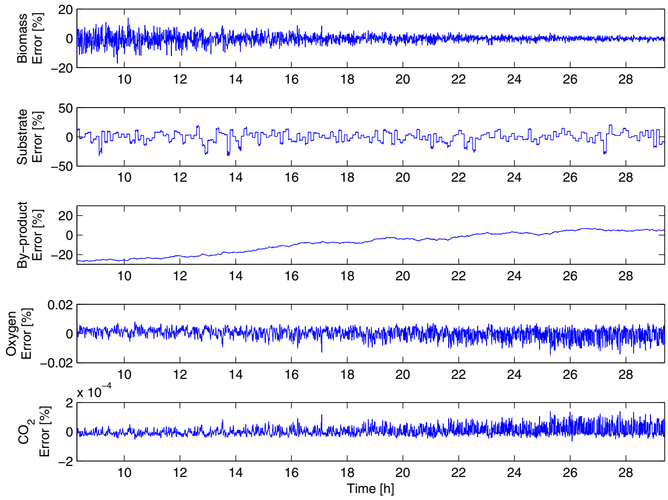

Fig. 3. Relative errors for the robust observer.

Taking advantage of the observer tolerance w.r.t noise, instead of using the biomass measurement x_1 for the controller input, the estimated biomass ($\hat{x}_1$) is used. Figure 3 shows the relative errors of the observer and Figure 4 shows the process response, the aim of which is to maximize biomass productivity. The process starts with a batch until the acetate concentration reaches the set-point. The controlled input flow rate then starts to act to maintain the acetate at its setpoint. Due to the low production of acetate and the nonlinearities, the process switches between the respiro-fermentative and respirative gains (see Figure 5). The robust observer guarantees for each mode the fast convergence of the state estimator for each different mode. This fast convergence allows to combine observer and controller benefits.

5. CONCLUSION

This paper presents a control strategy to maximize production of biomass in a fed-batch bioreactor using a robust controller coupled to an observer. According to Lyapunov's arguments, it is possible to rewrite the problem in a LMI framework that ensures the convergence and robustness of the controller. As this requires the by-product concentration measurement, an extended adaptive Luenberger observer is designed using the biomass and substrate concentration measurements. The achieved biomass productivity is similar to earlier studies (Santos et al., 2012; Dewasme et al., 2011) but is reached with more robustness with

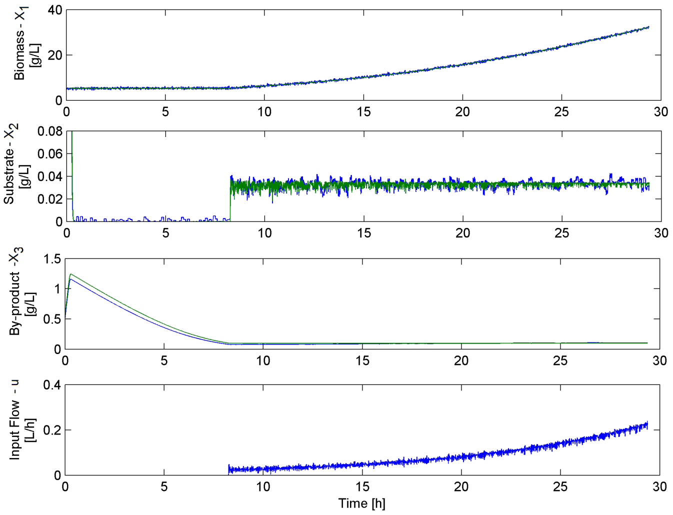

Fig. 4. Simulation Results. Estimated states in green and real values in blue.

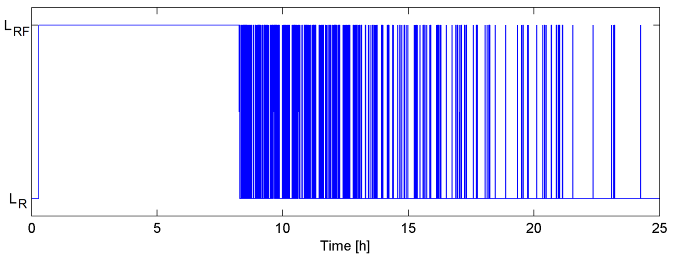

Fig. 5. Observer switching between respirative gain and respiro-fermentative gain.

respect to uncertainties and perturbations, and with the use of only two measurement probes, decreasing the cost of the established strategy.

ACKNOWLEDGEMENTS

This paper presents research results of the Belgian Network DYSCO (Dynamical Systems, Control, and Optimization), funded by the Interuniversity Attraction Poles Programme, initiated by the Belgian State, Science Policy Office. The scientific responsibility lies with its authors. The authors also acknowledge the support of FNRS and CNPq in the framework of a bilateral research project.

REFERENCES

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

Axelsson, J.P. (1988). On the role of adaptive controllers in fed-batch yeast production. In *IFAC Adaptive Control of Chemical Processes*, 103–108.

Axelsson, J.P. (1989). *Modeling and Control of Fermentation Processes*. Ph.D. thesis, Lund Institute of Technology – Sweden.

Bastin, G. and Dochain, D. (1990). *On-line estimation and adaptive control of bioreactors*. Elsevier.

Boyd, S., Ghaoui, L.E., Feron, E., and Balakrishnan, V. (eds.) (1994). *Linear matrix inequalities in system and control theory*. SIAM - Society for Industrial and Applied Mathematics.

Chen, L., Bastin, G., and Breusegem, V. (1995). A case study of adaptive nonlinear regulation of fed-batch biological reactors. *Automatica*, 31, 55–65.

Coutinho, D. and Vande Wouwer, A. (2010). A robust H_∞ quasi-LPV approach for designing nonlinear observers. In M. Diehl, F. Glineur, E. Jarlebring, and W. Michiels (eds.), *Recent Advances in Optimization and its Applications in Engineering*, 21–30. Springer Berlin Heidelberg.

Crabtree, H. (1929). Observations on the carbohydrate metabolism of tumors. *Biochemical Journal*, 23, 536–545.

Deken, R. (1966). The crabtree effect: a regulatory system in yeast. *Journal of General Microbiology*, 44, 149 – 156.

Dewasme, L., Coutinho, D., and Vande. Wouwer, A. (2011). Adaptive and robust linearization control strategies for fed-batch cultures of microorganisms exhibiting overflow metabolism. In *Informatics in Control, Automation and Robotics*. Springer Berlin Heidelberg.

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 culture. *Journal of Process Control*, 23, 148–157.

Hafidi, G., Tebbani, D., Dumur, D., and Vande Wouwer, A. (2008). Robustness analysis of nonlinear predictive control of fed-batch E. coli cultures. In *16th Mediterranean Conference on Control and Automation*, 25–27.

Liberzon, D. (2003). *Switching in Systems and Control*. Birkhauser, Boston.

Löfberg, J. (2004). Yalmip : a toolbox for modeling and optimization in MATLAB. In *Proceedings of the CACSD Conference*, 284 – 289.

Rocha, I. (2003). *Model-based strategies for computer-aided operation of recombinant E. coli fermentation*. Ph.D. thesis, Universidade do Minho – Portugal.

Santos, L., Dewasme, L., Coutinho, D., and Vande Wouwer, A. (2012). Nonlinear model predictive control of fed-batch cultures of micro-organisms exhibiting overflow metabolism: Assessment and robustness. *Computers and Chemical Engineering*, 39, 143–151.

Sonnleitner, B. and Käppeli, O. (1986). Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: formulation and verification of a hypothesis. *Biotechnology and Bioengeneering*, 28(6), 927–937.

Toh, K.C., Todd, M., Tütüncü, R., and Tutuncu, R.H. (1998). Sdpt3 - a matlab software package for semidefinite programming. *Optimization Methods and Software*, 11, 545–581.

Valentinotti, S., Srinivasan, B., Holmberg, U., Bonvin, D., Cannizzaro, C., Rhiel, M., and von Stockar, U. (2003). Optimal operation of fed-batch fermentations via adaptive control of overflow metabolite. *Control engineering practice*, 11, 665–674.

