ROBUST CONTROL WITH YOULA PARAMETRIZATION OF YEAST FED-BATCH CULTURES

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Abstract: Optimal productivity of *S. cerevisiae* cultures can be achieved through the regulation of the ethanol concentration at a low value. In this study, a robust control strategy is developed, which requires very little knowledge about the process, i.e. only one yield coefficient and the on-line measurement of the ethanol concentration. A Youla parametrization is selected in order to reject asymptotically the exponential growth disturbance and to robustify the control scheme against unstructured uncertainties and measurement noise. The performance of the control scheme is illustrated with real on-line experimental data.

Keywords: Robust control; Adaptive control; Fed-batch fermentation; Biotechnology;

1. INTRODUCTION

Saccharomyces cerevisiae are among the most popular industrial microorganisms for their robustness and ability to utilize cheap materials for growth and production. They have been used since the very early days of microbial fermentation history for brewing wine and beer. Recently, with achievement of modern gene technology, *S. cerevisiae* can be used as host organisms for production of recombinant proteins (production of insulin, vaccines, ...).

Due to the economic importance of these products, there is an obvious motivation to maximize the biomass productivity of the process. One method commonly used to ensure optimal operating conditions consists in regulating the ethanol concentration at a low value. Several methods have been proposed to this end (see, e.g. Chen *et al.*, 1995; Pomerleau, 1990). However, they often require an extensive knowledge of the reaction scheme stoichiometry and several on-line measurements (dissolved oxygen, oxygen uptake rate, dissolved carbon dioxide and carbon dioxide production rate, ...).

This study develops a strategy for robust control of the ethanol concentration assuming the knowledge of only one stoichiometric coefficient and only one on-line measurement: the ethanol concentration. Following the line of thought of Valentinotti *et al.* (2003), two simple linear models are derived from the global non linear model of Sonnleitner and Käppeli (1986). The first model depicts the relationship between the substrate feed and the ethanol production, while the second model describes the exponential cell growth. Therefore as far as the regulation of the ethanol concentration is concerned, the exponential cell growth can be considered as a disturbance to be rejected.

Then, an original control strategy which uses a RST controller with a Youla parametrization is developed. The Youla parameter is chosen in order to reach two objectives:

- An asymptotic rejection of the exponential growth disturbance (Valentinotti *et al.*, 2003). As the

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growth rate can evolve during the culture, an adaptive version of the robust control algorithm is also considered.

- An improvement of the robustness against unstructured uncertainties and a reduction of measurement noise on the control signal (Rodriguez, 2003).

A particular structure of the Youla parameter is chosen in order to take account of these two objectives with two stable transfer functions. The design of the first one is based on the internal model principle whereas the second one results from a convex optimisation problem expressing the frequency and temporal constraints of the second objective.

2. MODELING OF YEAST FED-BATCH CULTURES

2.1 *Nonlinear dynamic model*

The metabolism of yeast depends strongly on the culture conditions. During the aerobic growth, glucose and ethanol can be used as carbon sources according to the following reaction scheme:

$$\text{Glucose oxidation :} \quad \mathrm{G} + k_5\,\mathrm{O} \xrightarrow{r_1} k_1\,\mathrm{X} + k_7\,\mathrm{P} \quad (1a)$$

$$\text{Glucose fermentation :} \quad \mathrm{G} \xrightarrow{r_2} k_2\,\mathrm{X} + k_4\,\mathrm{E} + k_8\,\mathrm{P} \quad (1b)$$

$$\text{Ethanol oxidation :} \quad \mathrm{E} + k_6\,\mathrm{O} \xrightarrow{r_3} k_3\,\mathrm{X} + k_9\,\mathrm{P} \quad (1c)$$

where X, G, E, O, P are, respectively, the concentration in the culture medium of biomass, glucose, ethanol, dissolved oxygen and dissolved carbon dioxide, and k_i are the pseudo-stoichiometric coefficients.

The reaction rates associated with these reactions are:

$$r_1 = \min\left(r_G, \frac{r_{Omax}}{k_5}\right) \quad (2)$$

$$r_2 = \max\left(0, r_G - \frac{r_{Omax}}{k_5}\right) \quad (3)$$

$$r_3 = \max\left(0, \min\left(r_E, \frac{r_{Omax} - k_5 r_G}{k_6}\right)\right) \quad (4)$$

The kinetic terms associated with the glucose consumption r_G, the respiratory capacity r_{Omax} and the potential ethanol oxidative rate r_E are:

$$r_G = \mu_G \frac{\mathrm{G}}{\mathrm{G}+K_G},\ r_{Omax} = \mu_O \frac{\mathrm{O}}{\mathrm{O}+K_O},\ r_E = \mu_E \frac{\mathrm{E}}{\mathrm{E}+K_E}$$

where μ_G, μ_O and μ_E are the maximal values of specific growth rates, K_G, K_O and K_E are the saturation constants of the corresponding substrate.

This kinetic model is based on the bottleneck hypothesis developed by Sonnleitner and Käppeli (1986). It assumes a limited oxidation capacity of yeast, leading to the formation of ethanol under conditions of oxygen limitation and/or high glucose concentration. The glucose concentration at which the oxidative capacity saturates is defined as G_{crit}, for which $r_G = r_{Omax}/k_5$. According to the glucose concentration value, two different operating regimes can be distinguished. At low glucose concentrations ($G \leq G_{crit}$), the system is said in respirative regime. The glucose consumption rate is smaller than the maximal respiratory capacity ($r_G \leq r_{Omax}/k_5$) and the rate of the oxidative glucose metabolism is determined by the glucose consumption rate (2). Whereas, at high glucose concentrations ($G \geq G_{crit}$), the system is said in respiro-fermentative regime. The glucose consumption rate is larger than the maximal respiratory capacity ($r_G \geq r_{Omax}/k_5$) and the respiratory capacity of the cells determines the rate of the oxidative glucose metabolism (2). If the glucose flux is higher than the maximal respiratory capacity, the excess of glucose will be metabolized by the fermentative metabolism (3). If the glucose flux does not take up the whole respiratory capacity of the cell, ethanol may be oxidized in parallel with glucose and the rate of the oxidative ethanol metabolism depends on the excess of respiratory capacity and the available ethanol (4). Under oxygen starvation conditions, the fermentative metabolic pathway always predominates.

Based on the reaction scheme (1), the following macroscopic mass balances can be derived:

$$\frac{d(VX)}{dt} = (k_1 r_1 + k_2 r_2 + k_3 r_3) VX \quad (5a)$$

$$\frac{d(VG)}{dt} = -(r_1 + r_2) VX + F_{in} G_{in} \quad (5b)$$

$$\frac{d(VE)}{dt} = (k_4 r_2 - r_3) VX \quad (5c)$$

$$\frac{dV}{dt} = F_{in} \quad (5d)$$

where F_{in} is the inlet feed rate, V is the culture medium volume and G_{in} is the glucose concentration in the feed.

2.2 *Optimal operating conditions*

For economic reasons, there are strong motivations to maximize the amount of biomass produced with minimum operating time. Therefore the optimization problem consists in establishing the feeding strategy that maximizes the biomass productivity. It is shown (see, e.g. Valentinotti *et al.*, 2004) that the optimal solution corresponds to a feeding profile $F_{in}(t)$ which fills exactly the bottleneck. Thus the optimal operating point is situated at the boundary between the respiro-fermentative and respirative operating regimes. In this case the glucose concentration is maintained at a constant value G_{crit} such that the glucose consumption rate r_G is equal to the maximal glucose oxidative rate r_{Omax}/k_5. Only the glucose oxidation reaction takes place and there is no ethanol production or consumption. The total amount of ethanol VE is kept constant.

Therefore, in order to always operate the system around the optimal operating point, the control strategy must be able to maintain the product VE constant.

It requires an accurate measurement of both volume and ethanol concentration. As it is difficult to take into account all liquid additions (evaporation, sampling, base addition, ...), E-control is more usual than VE-control. Although the E-control is a suboptimal strategy, it comes quite close to the optimal one as the reference value E_{ref} for the ethanol concentration approaches zero.

2.3 *Simplified linear models*

As the optimal operating point is situated at the boundary between the respiro-fermentative and respirative operating regimes, a simple tailor-made model is derived for each regime when G tends to G_{crit}. The modeling procedure is directly inspired from the work of Valentinotti *et al.* (2003). For both models, it is assumed that the total amount of glucose VG is at quasi-steady state:

$$\frac{d(VG)}{dt} = -(r_1 + r_2)VX + F_{in}G_{in} = 0 \qquad (6)$$

This assumption is justified since a small variation in the substrate feed rate F_{in} will result in an almost instantaneous change in the amount of substrate VG in the bioreactor.

Respiro-fermentative model ($r_2 \neq 0$; $r_3 = 0$)
This model is valid when E is regulated at E_{ref} near 0. In this case, G is slightly larger than G_{crit} and only a small production of ethanol is needed to counteract the dilution effect. This way, reaction (1a) is saturated ($r_1 = \mu_O/k_5$ if oxygen is not limiting) and the cell growth rate μ can be assimilated to a constant parameter $\bar{\mu}$:

$$\mu = k_1 r_1 + k_2 r_2 + k_3 r_3 \approx k_1 r_1 = k_1 \frac{\mu_O}{k_5} \triangleq \bar{\mu} \qquad (7)$$

Together with (7), Equation (5a) gives:

$$VX = V_0 X_0 \exp(\bar{\mu}(t - t_0)) \qquad (8)$$

With $r_3 = 0$, (5c) and (6) lead to the ethanol production dynamics:

$$\frac{d(VE)}{dt} = k_4 G_{in}\,(F_{in} - d) \qquad (9)$$

where $d = \frac{\mu_O}{k_5 G_{in}} VX$ is considered as an input disturbance corresponding to the substrate flux needed for biomass growth.

During a fed-batch culture, the volume increases with time from V_0 to V_f. Thus, considering a constant average volume $\bar{V} = (V_0 + V_f)/2$, the ethanol dynamics in (9) can be rewritten as:

$$\frac{dE}{dt} = \frac{k_4 G_{in}}{V}\,(F_{in} - d) \qquad (10)$$

With Equation (8), the disturbance d corresponding to the exponential substrate oxidation is given by:

$$d(t) = K_d \exp(\bar{\mu}(t - t_0)) \qquad (11)$$

where $K_d = \frac{\mu_O V_0 X_0}{k_5 G_{in}}$.

Respirative model ($r_3 \neq 0$; $r_2 = 0$)
This model is valid when the excess oxidative capacity is small. Therefore, if the ethanol concentration is sufficient (i.e. $r_E \geq (r_{Omax} - k_5 r_G)/k_6$), the glucose flux r_G is near r_{Omax}/k_5 and (7) still holds. In this case, the ethanol oxidative rate remains small and can be written as follows:

$$r_3 = \frac{\mu_O - k_5 r_1}{k_6} \qquad (12)$$

Considering (5c) and (6) with $r_2 = 0$ and the average volume $\bar{V}$, the ethanol dynamics is given by:

$$\frac{dE}{dt} = \frac{k_5}{k_6}\frac{G_{in}}{V}\,(F_{in} - d) \qquad (13)$$

where the disturbance d is the same as in the respiro-fermentative model.

Finally, for both operating regimes, disturbance and ethanol dynamics can be expressed by the same discrete transfer functions:

$$\begin{aligned} E(k) &= \frac{K_E\, q^{-1}}{1 - q^{-1}}\,(F_{in}(k) - d(k)) \\ &= \frac{B(q^{-1})}{A(q^{-1})}\,(F_{in}(k) - d(k)) \end{aligned} \qquad (14)$$

$$d(k) = \frac{K_d}{1 - \gamma q^{-1}}\,\delta(k) = \frac{C(q^{-1})}{D(q^{-1})}\,\delta(k) \qquad (15)$$

with $\gamma = \exp(\bar{\mu} T_s)$ and $\delta(k)$ the unit pulse.

This simplified model can be associated to the following modeling uncertainties:

- K_E variations according to the operating regime (respiro-fermentative regime : $K_E = T_s k_4 G_{in}/\bar{V}$; respirative regime : $K_E = T_s \frac{k_5}{k_6} G_{in}/\bar{V}$, where T_s is the sampling time),
- neglected ('high frequency') glucose dynamics,
- γ variations. In fact, γ is the only kinetic parameter of the simplified model, which is *a priori* unknown.

3. CONTROL STRATEGY

The controller used in this work is a RST controller with Youla parametrization (see, e.g. Maciejowski, 1989). The corresponding block diagram is shown in Figure 1. The Youla parametrization of the initial controller $\mathcal{R}_0 \mathcal{S}_0 \mathcal{T}_0$ leads to the following stabilizing polynomials:

$$\bar{\mathcal{T}} = \mathcal{T}_0 - A_0 Q_2, \;\; \bar{\mathcal{R}} = \mathcal{R}_0 - B Q_1, \;\; \bar{\mathcal{S}} = \mathcal{S}_0 + A Q_1 \qquad (16)$$

where Q_1 and Q_2 are stable transfer functions.

The closed loop transfer function can be written as follows:

$$y = \frac{B(\mathcal{T}_0 - A_0 Q_2)}{A\mathcal{R}_0 + B\mathcal{S}_0}\, w + \frac{B(\mathcal{R}_0 - B Q_1)}{A\mathcal{R}_0 + B\mathcal{S}_0}\, d \qquad (17)$$

Two remarks can be done: Q_2 modifies only the tracking behaviour, and, if the model is exact, the

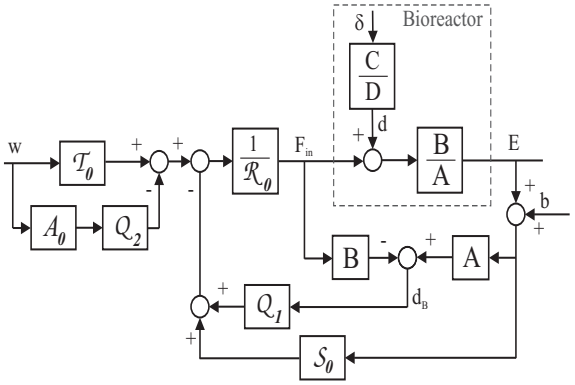

Fig. 1. Closed-loop diagram of fed-batch yeast culture process controlled by a RST controller with Youla parametrization ($u = F_{in}$, $y = E$ and $w = E_{ref}$).

characteristic equation A_0A_c is not modified by the parametrization:

$$A_0A_c = A\bar{\mathcal{R}} + B\bar{\mathcal{S}} = A\mathcal{R}_0 + B\mathcal{S}_0 \tag{18}$$

Q_2 is set to zero since tracking is ensured by the initial controller design. Disturbance rejection can be achieved by tuning Q_1, for which a particular structure is chosen:

$$Q_1 = Q_{11} + DQ_{12} \tag{19}$$

where Q_{11} and Q_{12} are stable transfer functions: Q_{11} is designed to ensure an asymptotic rejection of disturbance d, while Q_{12} is designed to maximize the robustness against modeling uncertainties.

3.1 Q_{11} *design*

The Q_{11} design follows the work of Valentinotti *et al.* (2003) about the adaptive rejection of unstable disturbances. Considering Equations (15) and (17), the disturbance effect on the output is defined as:

$$e_d = \frac{B(\mathcal{R}_0 - BQ_1)}{A_0A_c}\frac{C}{D}\delta \tag{20}$$

According to the internal model principle (Francis and Wonham, 1976), the unstable poles of e_d must be present in the $\mathcal{R}$ denominator in order to ensure the disturbance rejection. Since the characteristic equation is stable by construction, the disturbance effect has only one unstable pole, the pole γ of the D polynomial. Therefore, e_d converges asymptotically to zero if the polynomial D is a factor of the $\bar{\mathcal{R}}$ polynomial. With the particular structure (19) of Q_1 where Q_{12} is already convoluted with D, Q_{11} can be designed independently of Q_{12} by solving the following Diophantine equation:

$$\mathcal{R}_0 - BQ_{11} = MD \tag{21}$$

where M is an arbitrary polynomial in q^{-1}.

3.2 Q_{12} *design*

Q_{12} is designed to satisfy two kinds of specifications: frequency and temporal specifications (Rodriguez, 2003).

3.2.1. *Frequency specifications* With regard to the simplified model, the uncertainties on the gain K_E and the neglected glucose dynamics can be represented by a multiplicative direct uncertainty. Assuming the system and controller structured as in Figure 1, the P representation for this kind of uncertainty is:

$$P = -\frac{B(\mathcal{S}_0 + AQ_{11})}{A_0A_c} - \frac{BAD}{A_0A_c}Q_{12} \tag{22}$$

Considering the small gain theorem (Maciejowski, 1989), the robustification against unstructured uncertainties is achieved by minimizing an H_∞ norm:

$$\min_{Q_{12}\in RH_\infty} \left\| P(q^{-1})W(q^{-1}) \right\|_\infty \tag{23}$$

where W is a weighting transfer function and RH_∞ is the space of all proper and stable transfer functions. The Youla parametrization allows linear dependency between P and the Youla parameter Q_{12} as shown in Equation (22). So, the specifications defined by Equation (23) are convex in Q_{12} (see e.g. Boyd and Barrat, 1991).

3.2.2. *Temporal specifications* A temporal specification on the control signal response to a measurement noise is considered. Let H_{ub} be the transfer function between the noise b and the control signal u:

$$H_{ub} = -\frac{A(\mathcal{S}_0 + AQ_{11})}{A_0A_c} - \frac{A^2D}{A_0A_c}Q_{12} \tag{24}$$

and denote by $s(t)$ the response of H_{ub} to a specific input $b(t)$ like a white noise sequence. Temporal specifications consist of a template inside which $s(t)$ must remain constrained. This template can be specified by the minimal and maximal amplitudes $\underline{s}$ and $\bar{s}$. So, the set of all Q_{12} parameters that satisfy this constraint is:

$$C_{env} = \{Q_{12} \,|\, \Phi_{env}(Q_{12}) \le 0\} \tag{25}$$

with $\Phi_{env}(Q_{12}) = \max\left(\max_{t\ge 0}(s(t) - \bar{s}(t), \underline{s}(t) - s(t))\right)$

The transfer function (24) being linearly parametrized by the Youla parameter Q_{12}, the temporal specifications are also convex in Q_{12} (see e.g. Boyd and Barrat, 1991).

In conclusion, the design of Q_{12} consists of a H_∞ minimisation problem (23) under constraints imposed by the temporal specifications (25):

$$\min_{\substack{Q_{12}\in RH_\infty \\ \Phi_{env}(Q_{12})\le 0}} \left\| P(q^{-1})W(q^{-1}) \right\|_\infty \tag{26}$$

This is a convex optimisation problem leading to a Q_{12} parameter varying in an infinite dimensional

space. To the authors' knowledge, there is no solution to this optimization problem, and a sub-optimal solution can be obtained by considering a finite dimentional sub-space generated by an orthonormal base of stable transfer functions. This way, the H_∞ norm minimisation and the temporal constraints can be approximated by a minimisation under linear inequality constraints (Rodriguez, 2003).

3.3 Q_{11} *adaptation*

Equation (21) depends on the unstable pole γ of the D polynomial. However, this pole is *a priori* unknown since the critical growth rate $\bar{\mu}$ can vary from a yeast strain to another or also during the culture. Therefore, Q_{11} has to be adapted on-line in order to minimize the disturbance effect. With (21) and (19), the disturbance effect (20) can be rewritten as:

$$e_d = \left(\frac{M - BQ_{12}}{M}\right)\left(\frac{\mathcal{R}_0 - BQ_{11}}{A_0 A_c}\right) d_B \qquad (27)$$

where $d_B(k) = B(q^{-1})d(k) = A(q^{-1})y(k) - B(q^{-1})u(k)$ is a filtered expression of disturbance d. Both terms into brackets are parametrized independently by Q_{12} and Q_{11}. If M is stable, it is enough to minimize on-line the last term and it can be written as a linear regression problem:

$$\min_{Q_{11}} \|\varepsilon_1 - \varepsilon_2 Q_{11}\|^2 \qquad (28)$$

where the signals ε_1 and ε_2 are defined as:

$$\varepsilon_1 = \frac{\mathcal{R}_0}{A_0 A_c} d_B \quad \text{and} \quad \varepsilon_2 = \frac{B}{A_0 A_c} d_B$$

An on-line adaptation of Q_{11} can be done using standard algorithms (Ljung, 1999).

4. EXPERIMENTAL RESULTS

4.1 *Experimental setup*

A new strain of genetically modified *S. cerevisiae* is considered in this study. A 20-l stirred tank bioreactor (BioLafitte, France) is used for the cell culture. Temperature, dissolved oxygen, pH and air flow rate are controlled by the bioreactor control box. The fed-batch process is started with 5.8 l of fresh medium without glucose and the inoculation gives an initial biomass concentration of 0.7 g/l. The inoculum also introduces a small quantity of ethanol. The feed medium contains 350 g/l of glucose and its composition (ammonium sources, vitamins, trace elements, ...) has not already been optimized for the considered yeast strain.

The ethanol concentration is measured with an ethanol probe (Frings, Bonn, Germany) immersed in the culture medium. A LABVIEW-based bioprocess management and control environment, BioOPT, is used to supervise the process (Valentinotti *et al.*, 2003).

4.2 *Controller design*

When E is regulated to the setpoint $E_{ref} = 0.7$ g/l, the system operates in respiro-fermentative regime. Thus, the only nonoperational parameter required to compute the controller is the stoichiometric coefficient k_4. The value proposed by Sonnleitner and Käppeli (1986) is chosen, $k_4 = 0.48$ [g of E/g of G]. With a sampling period $T_s = 0.1$ h, a feed glucose concentration $G_{in} = 350$ g/l and an average volume $\bar{V} = 9$ l, the gain of the production/consumption process (14) is $K_E = 1.87$. The initial controller is designed by pole placement with $A_0 A_c = 1 - 0.95\, q^{-1}$. The resulting proportional controller is given by $\mathcal{R}_0 = 1$, $\mathcal{S}_0 = 0.027$ and $\mathcal{T}_0 = A_0 A_c(1)/B(1) = 0.027$. The minimal degree solution of the Diophantine equation (21) corresponds to $M = 1$ and Q_{11} is a scalar equal to γ/K_E. As $\gamma = \exp(\bar{\mu} T_s)$, an initial value of Q_{11} can be computed from an initial estimation of the critical growth rate $\bar{\mu}$.

K_E varies according to the operating regime and the neglected glucose dynamics can be modeled by a direct multiplicative uncertainty (22). The frequency domain of glucose dynamics being situated in high frequencies, those frequencies are more heavily weighted thanks to the following weighting function: $W(q^{-1}) = (1 - 0.5\, q^{-1})/0.5$. Temporal specifications $\Phi_{env}(Q_{12})$ correspond to a template for measurement noise/control transfer (24). With a pseudo-random noise of zero mean and 0.05 variance, these specifications set a limit for the measurement noise effect on the control signal, restricting u variations due to noise within a ± 0.15 range. The optimization problem (26) is solved by a quadratic minimization algorithm under inequality constraints and leads to:

$$Q_{12}\left(q^{-1}\right) = \frac{-0.3281 + 0.1460\, q^{-1}}{1 - 1.1225\, q^{-1} + 0.2408\, q^{-2}}$$

Figure 2 shows the Black diagram for three different controllers. It is well known that ensuring a modulus lower than 6 dB for σ_d and lower than 3 dB for σ_c provides a good stability robustness. It is therefore apparent that the introduction of the unstable pole γ in $\bar{\mathcal{R}}$ deteriorates the robustness at high frequencies. On the other hand, the full robustified controller has good robustness for all frequencies, as well as better gain and phase margins.

4.3 *Results and discussion*

An experimental test is performed with the controller and the results are shown in Figure 3. After a short latency phase during which a small feed rate is used, the controller is started. Figure 3 shows that the control algorithm is able to bring E to the setpoint $E_{ref} = 0.7$ g/l and to subsequently regulate E around this setpoint throughout the first 18 h. During this period, the cell growth is exponential and, after a short transient, the

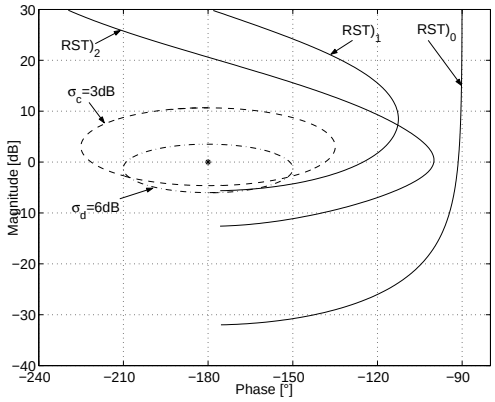

Fig. 2. Black diagram for initial controller RST)$_0$ ($Q_1 = 0$), controller RST)$_1$ ($Q_1 = Q_{11}$), controller RST)$_2$ ($Q_1 = Q_{11} + DQ_{12}$). σ_c and σ_d are the complementary and direct sensitivity functions.

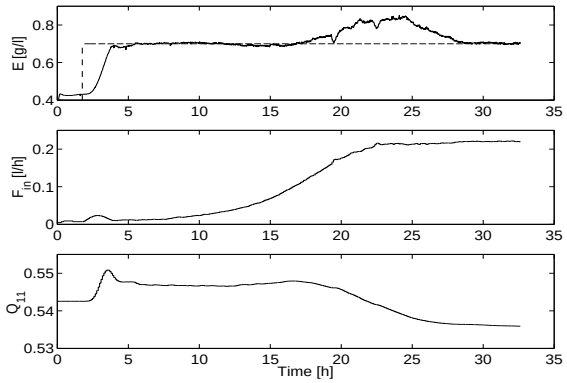

Fig. 3. Results for a yeast fed-batch culture controlled with the robust and adaptive control algorithm.

adaptation algorithm estimates very satisfactorily the Q_{11} parameter.

After 18h, a limitation phenomenon occurs because the feed medium is not opimized for the new yeast strain considered in this study. Therefore, the ethanol concentration increases slightly while the adaptation algorithm tries to decrease Q_{11}, which is the image of the decreasing growth rate $\overline{\mu}$. The adaptation dynamics being slower than the controller dynamics, a small drift is observed when $\overline{\mu}$ is overestimated.

Around 22h, the cell growth becomes nearly linear and the feed rate F_{in} becomes nearly constant. When the estimated $\overline{\mu}$ becomes satisfactory, around 25h, the controller is able to maintain E near E_{ref} again. Thus, this experiment shows that the controller ensures the tracking of the optimal trajectory despite strong limitation phenomena.

5. CONCLUSION

The optimal operating point ($G = G_{crit}$) corresponds to the boundary between the respiro-fermentative and respirative regime. Around this operating point, yeast fed-batch cultures can be modeled by a simple linear model describing the main macroscopic processes: exponential glucose uptake for cell growth and small ethanol production/consumption according to the operating regime. The first process is considered as a disturbance to be rejected, and the second one models the plant to be controlled. This modeling methodology allows several uncertainties to be associated with the simplified model, i.e. the gain variation of the ethanol production/consumption process and the glucose dynamics which has been neglected.

A RST controller with Youla parametrization is used to ensure the asymptotic rejection of unstable disturbances, a good robustness against uncertainties and a noise attenuation on the control signal. Moreover, the control algorithm includes a disturbance model adaptation since the growth rate is *a priori* unknown and can evolve during the culture. This control strategy is tested experimentally with a fed-batch culture of a new genetically modified yeast strain. The results are quite promising, and show that the controller is able to deal with metabolic changes such as a substrate limitation phenomenon.

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