Proceedings of the 17th World Congress
The International Federation of Automatic Control
Seoul, Korea, July 6-11, 2008

Adaptive extremum-seeking control applied to productivity optimization in yeast fed-batch cultures

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Abstract: In this study, we consider the problem of optimizing the productivity of fed-batch cultures of *S. cerevisiae*, which are characterized by strongly nonlinear kinetic models based on the bottleneck assumption of Sonnleitner and Käppeli [1986] and ethanol inhibition resulting from the fermentation of a possible excess of substrate feeding. In contrast with most published studies where the critical substrate level is assumed constant, we investigate the situation where this critical substrate level depends on the yeast respiratory capacity, and in turn on the oxygen and etahnol concentration in the culture medium. The challenge is thus to maintain the process at a high level of productivity by avoiding the accumulation of ethanol. To this end, an adaptive extremum seeking control scheme, coupled to an asymptotic observer, is developed based on Lyapunov stability arguments. *Copyright ©2008 IFAC*

Keywords: Extremum seeking, nonlinear adaptive control, asymptotic observer, fermentation process, biotechnology.

1. INTRODUCTION

Yeasts are one of the most important host microorganisms in manufacturing of biopharmaceuticals. Industrial vaccine production is usually achieved using fed-batch cultures of genetically modified yeast strains, which can express different kinds of recombinant proteins. From an operational point of view, it is necessary to determine an optimal feeding strategy (i.e. the time evolution of the input flow rate to the fed-batch culture) in order to maximize productivity. The main problem that can be encountered at this stage is due to fermentation of an excess of substrate (glucose), which can lead to the accumulation of ethanol in the culture medium, and in turn to the inhibition of the cell respiratory capacity.

To avoid this undesirable effect, a closed-loop optimizing strategy is required, which could take various forms (Chen et al. [1995], Akesson [1999], Renard [2006], Dewasme et al. [2007]). In particular, the use of extremum seeking strategies for bioprocess optimization has received an increasing attention in recent years (Ariyur and Krstic [2003], Guay and Zhang [2003], Guay et al. [2004], Marcos et al. [2004], Titica et al. [2004]).

In this study, we develop an adaptive extremum-seeking strategy based on Lyapunov stability arguments (in a similar way as in (Guay et al. [2004], Titica et al. [2004])). The main challenge is to consider the dependence of the critical substrate level on the respiratory capacity, itself influenced by the oxygenation level and the ethanol inhibition. A second difficulty rises from the strong nonlinearity of the kinetic laws, which is due to the switch between a respirative regime and a respiro-fermentative regime, depending again on the yeast respiratory capacity and, in turn, on the substrate concentration in the culture medium (i.e. the bottleneck assumption of Sonnleitner and Käppeli [1986]). In addition, the kinetic laws take account of the inhibitory effect of ethanol on this yeast respiratory capacity. Compared to previous studies, the underlying models therefore significantly depart from the classical Monod or Haldane laws and a new analysis is required. In particular, an adaptive extremum-seeking strategy including two adaptation laws is developed and a simplified more robust version of this strategy is also proposed. Moreover, the use of an asymptotic observer is considered so as to limit the number of required on-line measurements (Chen et al. [1995], Bastin and Dochain [1990]).

This paper is organized as follows. The next section introduces the macroscopic model of yeast fed-batch cultures used in this study and defines the optimal operating conditions. Section 3 presents the adaptive extremum seeking algorithm, the design of an asymptotic observer and a Lyapunov stability analysis. In Section 4, the performance of the algorithm is tested in simulation and discussed, whereas Section 5 draws some conclusions and perspectives.

2. MODELING YEAST FED-BATCH CULTURES

2.1 Nonlinear dynamic model

The yeast strain *S. cerevisiae* presents a metabolism that can be macroscopically described by the following three main reactions:

$$\text{Substrate oxidation :} \quad \mathrm{S} + k_5\,\mathrm{O} \xrightarrow{r_1} k_1\,\mathrm{X} + k_7\,\mathrm{P} \tag{1a}$$

$$\text{Substrate fermentation :} \quad \mathrm{S} \xrightarrow{r_2} k_2\,\mathrm{X} + k_4\,\mathrm{E} + k_8\,\mathrm{P} \tag{1b}$$

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

where X, S, E, O and P are, respectively, the concentration in the culture medium of biomass, substrate (typically glucose), ethanol, dissolved oxygen and carbon dioxide. k_i are the yield coefficients and r_1, r_2 and r_3 are the nonlinear reaction rates given by:

$$r_1 = \min\left(r_S, \frac{r_o}{k_5}\right) \tag{2}$$

$$r_2 = \max\left(0, r_S - \frac{r_o}{k_5}\right) \tag{3}$$

$$r_3 = \max\left(0, \min\left(r_E, \frac{r_o - k_5 r_S}{k_6}\right)\right) \tag{4}$$

where the kinetic terms associated with the substrate consumption r_S, the oxidative or respiratory capacity r_o and the ethanol oxidative rate r_E are given by:

$$r_S = \mu_S \frac{\mathrm{S}}{\mathrm{S} + K_S} \tag{5a}$$

$$r_o = \mu_O \frac{\mathrm{O}}{\mathrm{O} + K_O} \tag{5b}$$

$$r_E = \mu_E \frac{\mathrm{E}}{\mathrm{E} + K_E} \tag{5c}$$

These expressions take the classical form of Monod laws where μ_S, μ_O and μ_E are the maximal values of specific growth rates and K_S, K_O and K_E are the saturation constants of the corresponding element.

This kinetic model, which is often encountered in the literature, is based on Sonnleitner's bottleneck assumption (Sonnleitner and Käppeli [1986]) (Figure 1). During a culture, the yeast cells are likely to change their metabolism because of their limited respiratory capacity. When the substrate is in excess (concentration $S > S_{crit}$), the yeast cells produce ethanol through fermentation, and the culture is said in respiro-fermentative (RF) regime. On the other hand, when the substrate becomes limiting (concentration $S < S_{crit}$), the available substrate (typically glucose), and possibly ethanol (as a substitute carbon source), if present in the culture medium, are oxidized. The culture is then said in respirative (R) regime.

Component-wise mass balances give the following differential equations :

$$\frac{dX}{dt} = (k_1 r_1 + k_2 r_2 + k_3 r_3)X - DX \tag{6a}$$

$$\frac{dS}{dt} = -(r_1 + r_2)X + DS_{in} - DS \tag{6b}$$

$$\frac{dE}{dt} = (k_4 r_2 - r_3)X - DE \tag{6c}$$

$$\frac{dO}{dt} = -(k_5 r_1 + k_6 r_3)X - DO + OTR \tag{6d}$$

$$\frac{dP}{dt} = (k_7 r_1 + k_8 r_2 + k_9 r_3)X - DP - CTR \tag{6e}$$

$$\frac{dV}{dt} = F_{in} \tag{6f}$$

where S_{in} is the substrate concentration in the feed, F_{in} is the inlet feed rate, V is the culture medium volume and D is the dilution rate ($D = F_{in}/V$). OTR and CTR represent respectively the oxygen transfer rate from the gas phase to the liquid phase and the carbon transfer rate from the liquid phase to the gas phase. Classical models of OTR and CTR are given by:

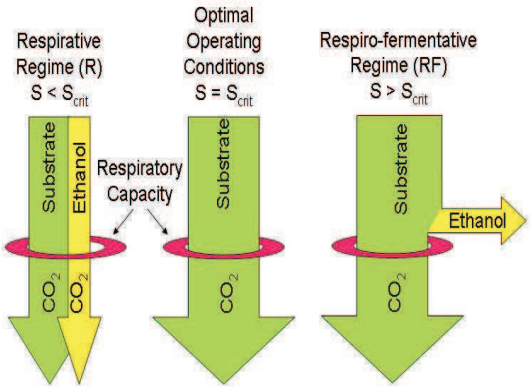

Fig. 1. Illustration of Sonnleitner's bottleneck assumption for yeast limited respiratory capacity.

$$OTR = k_L a(O_{sat} - O) \tag{7a}$$

$$CTR = k_L a(P - P_{sat}) \tag{7b}$$

where $k_L a$ is the volumetric transfer coefficient and, O_{sat} and P_{sat} are respectively the dissolved oxygen and carbon dioxide concentrations at saturation.

Ethanol has a detrimental effect on the cells growth because it directly inhibits the cells respiratory capacity (Pham [1999]). Taking this remark into account, a more detailed expression of r_o is given by:

$$r_o = \mu_O \frac{\mathrm{O}}{\mathrm{O} + \mathrm{K_O}} \frac{\mathrm{K_{i_E}}}{\mathrm{K_{i_E}} + \mathrm{E}} \tag{8}$$

where K_{i_E} is the inhibition constant of ethanol.

It is common in the literature to consider the critical substrate level S_{crit} constant. However, this level is actually not constant and depends on the respiratory capacity which is limited by a lack of oxygen and inhibited by an excess of ethanol. For illustration purposes, Figure 2 shows a simulation of the process where the substrate concentration in the culture medium is regulated around a constant theoretical value of $S_{set} = 0.0226 g/l$. It is apparent that ethanol is produced during the batch and that the biomass growth rate is lower than expected. A simple substrate regulation does not allow to avoid the production of ethanol, which in turn, reduces the respiratory capacity and the critical substrate level.

Considering we are in the optimal operating conditions ($S = S_{crit}$), the fermentation and ethanol oxidation rates are equal to zero and the substrate consumption rate r_S is equal to $\frac{r_o}{k_5}$. Consequently, after a trivial mathematical manipulation of (5a), a relation between the critical substrate concentration level and the cell respiratory capacity is obtained as:

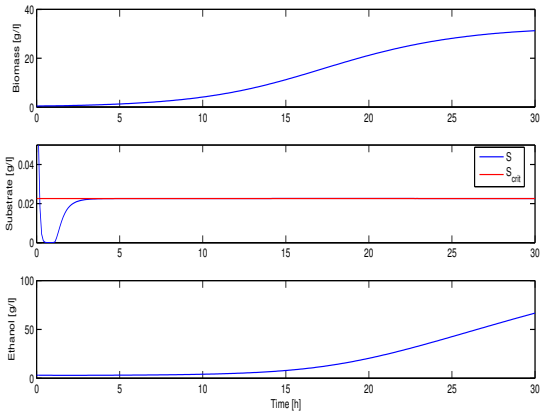

Fig. 2. Simulation of a fed-batch process controlled at a constant S_{crit} value.

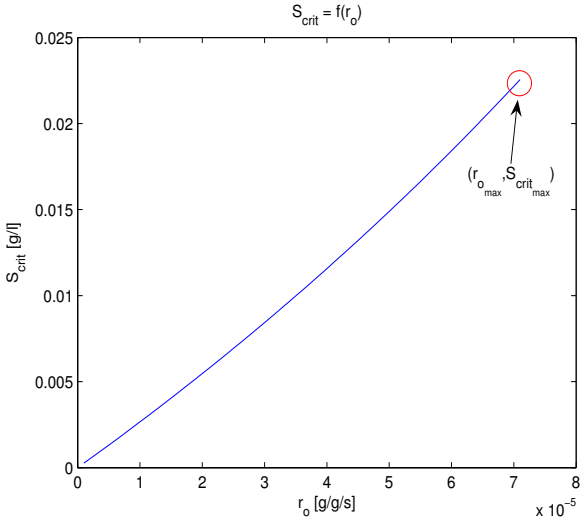

Fig. 3. S_{crit} as a function of r_o.

$$S_{crit} = \frac{K_s r_o}{k_5 \mu_s - r_o} \tag{9}$$

Figure 3 shows a plot of this relation where the point $[0,0]$ corresponds to a totally inhibited respiratory capacity, preventing any growth, and the point $[r_{o_{max}}, S_{crit_{max}}]$ corresponds to maximum productivity (i.e. absence of ethanol in the culture medium and a sufficient level of oxygenation). Obviously, the presence of ethanol in the culture medium can decrease the respiratory capacity and in turn the value of the critical substrate concentration $S = S_{crit}$. In order to maintain the system at the edge between the respirative and respiro-fermentative regimes, it is necessary to determine on-line the critical substrate concentration (S_{crit}) and to control the substrate concentration in the culture medium around this value.

3. AN ADAPTIVE EXTREMUM-SEEKING STRATEGY

3.1 Introduction and main principles

The adaptive extremum-seeking strategy that is chosen in this study is related to the techniques developed in Titica et al. [2004], Betancur et al. [2004], Guay and Zhang [2003], Guay et al. [2004] and Marcos et al. [2004], which take their roots

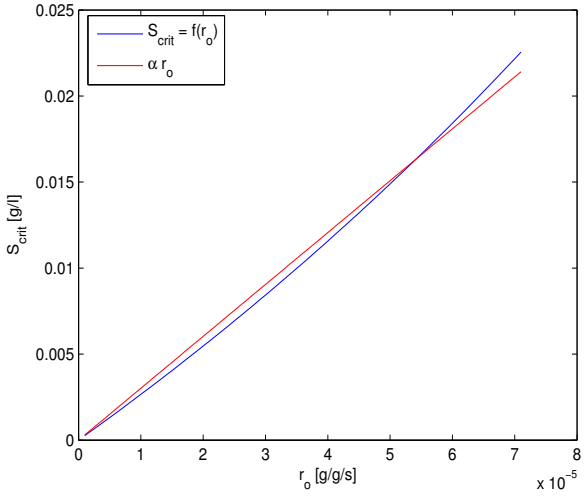

Fig. 4. S_{crit} as a function of r_o and linear approximation.

in the classical work of (Ariyur and Krstic [2003]). It consists in a permanent estimation of the system dynamics through the analysis of a "control error signal" (which in the present case is a function of the difference between S and the set point S_{crit}) following the injection of a periodical excitation signal d into the adaptive system. This allows the convergence of the parameter estimates to their true values and the stabilization of the error signal around zero (but not exactly zero as the excitation is permanent).

3.2 Controller design

We first define the main parameters to estimate. Then, we derive adaptation laws and a control law from the consideration of a candidate Lyapunov function ensuring system stability.

First, equation (6b) can be rewritten as follows:

$$\frac{dS}{dt} = -\theta X - D(S - S_{in}) \tag{10}$$

where $\theta = r_1 + r_2$ is considered as an unknown kinetic parameter.

As we wish to control the substrate concentration around its critical point, we need to assess its value at every moment. Unfortunately, equation (9) contains several unknown (or at least uncertain) parameters (μ_s, K_s, k_5, K_o, μ_o and K_{i_E}), which can be detrimental to the control quality. To avoid this possible lack of robustness, as the order of r_o is clearly smaller than $k_5\mu_S$, we propose to approximate expression (9) by the following one:

$$S_{crit} \approx \frac{K_S}{k_5 \mu_S} r_o = \alpha r_o \tag{11}$$

where α is a positive parameter which has to be adapted during the batch (as a modeling exercise, a residual mean error of 0.2% is obtained after a linear regression applied to (9) demonstrating the quality of a first-order approximation - see Figure 4).

Defining:

$$Z_s = k_p(S - S_{crit}) + k_i \int (S - S_{crit})dt - d \tag{12}$$

the control error variable, where d is the periodical "dither signal" and, k_p and k_i are positive tuning parameters,

$$\tilde{\theta} = \theta - \hat{\theta} \tag{13}$$

the estimation error on θ, and

$$\tilde{\alpha} = \alpha - \hat{\alpha} \tag{14}$$

the estimation error on α, we consider the following Lyapunov candidate function:

$$V = \frac{1}{2}Z_s^2 + \frac{1}{2\gamma}\tilde{\theta}^2 + \frac{1}{2\gamma_s}\tilde{\alpha}^2 \tag{15}$$

where γ and γ_s are strictly positive tuning parameters.

A stabilizing controller is obtained if one can prove the strict negativity of the Lyapunov function derivative. Differentiating V, we obtain:

$$\begin{aligned}\dot{V} = Z_s\,[k_p(-\theta X - D(S - S_{in}) - \alpha \dot{r_o}) + k_i(S \\ -\hat{\alpha} r_o) - \dot{d}\,] + \tilde{\theta}(-\frac{\dot{\hat{\theta}}}{\gamma}) + \tilde{\alpha}(-\frac{\dot{\hat{\alpha}}}{\gamma_s})\end{aligned} \tag{16}$$

Replacing (12), (13) and (14) in (16) and forcing $\dot{V}$ to be negative as in:

$$\dot{V} = -k_z Z_s^2 \tag{17}$$

where k_z is a strictly positive tuning parameter, we obtain:

$$\begin{aligned}-k_z Z_s = k_p(-\hat{\theta} X - D(S - S_{in}) - \hat{\alpha}\dot{r_o}) \\ + k_i(S - \hat{\alpha} r_o) - \dot{d}\end{aligned} \tag{18}$$

provided that:

$$\dot{\hat{\theta}} = -\gamma k_p Z_s X \tag{19a}$$

$$\dot{\hat{\alpha}} = -\gamma_s Z_s (k_p \dot{r_o} + k_i r_o) \tag{19b}$$

$$\hat{S}_{crit} = \hat{\alpha} r_o \tag{19c}$$

Finally, the control law is given by

$$D = \frac{\left[\frac{k_z Z_s - a + k_d d}{k_p} - \hat{\theta} X\right]}{S - S_{in}} \tag{20}$$

with a dither signal d chosen as:

$$\dot{d} = a + k_i(S - \hat{\alpha} r_o) - k_p \hat{\alpha} \dot{r_o} - k_d d \tag{21}$$

where a is a closed-loop excitation signal and k_d is a new strictly positive design parameter.

The proposed control law (20) requires several on-line measurements (X, S, O, E), which can nowadays be achieved using specific probes. However, these sensors are still quite expensive and their use is not widespread. In the following we consider the use of an asymptotic observer to provide estimation of biomass and ethanol from glucose, dissolved oxygen and carbon dioxide measurements (Chen et al. [1995]). The main advantage of the asymptotic observer is that it provides an estimation independent of the kinetic laws.

3.3 Asymptotic observer

The mass-balance equations (6) can be written in a compact form:

$$\xi = \mathrm{K}\phi - \mathrm{D}\xi + \mathrm{F} - \mathrm{Q} \tag{22}$$

where ξ is the state vector, K the yield coefficient matrix, ϕ the reaction rates vector, F the inlet flux vector when the external components are diluted in the culture medium and Q the outlet flux vector for gas components.

In the present case, the rank of K is equal to 3 so that 3 measurements are necessary in order to estimate all the states. We propose here to estimate the biomass and ethanol concentrations from measurements of glucose, dissolved oxygen and carbon dioxide:

$$\begin{aligned}\xi_1 &= [S\ O\ P]^T \\ \xi_2 &= [X\ E]^T\end{aligned} \tag{23}$$

This partition induces the corresponding partition of the yield matrix K, i.e., K_1 and K_2.

The definition of the auxiliary variables vector $z = A_1\xi_A + A_2\xi_B$ with $A_0 = A_1 = -K_2K_1^{-1}$ and $A_2 = I$, leads to the asymptotic observer structure:

$$\begin{bmatrix}\dot{z}_1 \\ \dot{z}_2\end{bmatrix} = -D\begin{bmatrix}z_1 \\ z_2\end{bmatrix} + A_0\begin{bmatrix}S_{in}D \\ OTR \\ -CTR\end{bmatrix} \tag{24a}$$

$$\begin{bmatrix}\hat{X} \\ \hat{E}\end{bmatrix} = \begin{bmatrix}z_1 \\ z_2\end{bmatrix} - A_0\begin{bmatrix}S \\ O \\ P\end{bmatrix} \tag{24b}$$

The convergence speed of this observer is linked to the dilution rate:

$$\frac{d\tilde{\xi}_2}{dt} = \frac{d}{dt}(\hat{\xi}_2 - \xi_2) = -D(\hat{\xi}_2 - \xi_2) \tag{25}$$

The dilution rate, given by (20), is persistently exciting and ensures the observer convergence.

In industrial practice, laboratory measurements are achieved at the beginning of each run so that the error on the initial state variables, $\tilde{\xi}_2$, is usually small and the dilution rate (which evolves exponentially so as to follow yeast growth) ensures a fast convergence of the asymptotic observer so that no stability problem of the closed-loop system (combining the asymptotic observer and the extremum-seeking controller) occurs.

Rigorously, however, the stability of the whole control system should be analytically demonstrated through the derivation of a new Lyapunov candidate function taking into account the introduction of the observer in the closed-loop.

$$V = \frac{1}{2}Z_s^2 + \frac{1}{2\gamma}\tilde{\theta} + \frac{1}{2\gamma_s}\tilde{\alpha} + \frac{1}{2}\tilde{z_1}^2 + \frac{1}{2}\tilde{z_2}^2 \tag{26}$$

The demonstration of stability is immediate considering that $\tilde{z} = z - \hat{z}$ and (24), i.e.

$$\dot{\tilde{z}} = -D\tilde{z} \tag{27}$$

Parameter	Value	Unit
γ_s	10	/
γ	10^{-7}	/
k_p	15	/
k_s	0.004	/
k_z	0.0015 X	/
k_d	0.01	/
ω_i	$\frac{2\pi\, i}{4000}$	rad/s

Table 1. Tuning parameter values.

We are thus ensured that the introduction of the observer does not affect the stability as it preserves the negativity of the Lyapunov function derivative.

4. SIMULATION RESULTS

In this section, we apply the controller designed in the previous section to a simulated case-study corresponding to classical small-scale (20 l bioreactor) culture conditions. The initial and operational conditions are:

$X_0 = 0.4g/l$, $S_0 = 0.5g/l$, $E_0 = 3g/l$, $O_0 = O_{sat} = 0.035g/l$, $P_0 = P_{sat} = 1.286g/l$, $V_0 = 6.8l$, $S_{in} = 350g/l$

For the kinetic and yield parameter values, the reader is referred to Sonnleitner and Käppeli [1986].

The selection of an appropriate dither signal is based on a persistence of excitation (PE) condition (Guay and Zhang [2003], Marcos et al. [2004], Adetola and Guay [2006]) which, once fulfilled, ensures the asymptotic convergence of the parameter estimates.

The excitation signal is here chosen as a simple sum of sinusoidal signals of the form:

$$a = \sum_{i=1}^{5} A_i sin(\omega_i\, t) \tag{28}$$

where A_i are normally distributed random numbers contained in $[-0.0005, 0.0005]$ and ω_i are the pulsations.

The initial substrate and ethanol concentrations are chosen at high values, so as to challenge (in a difficult situation) the controller convergence speed. Figures 5, 6 and 7 present the simulation results. The substrate concentration evolution (Figure 5) shows that the presence of ethanol at the beginning of the batch causes a decrease of the critical substrate concentration level. An adaptation of this critical substrate concentration is then needed so as to avoid an increased production of ethanol (due to the excess of substrate) and a serious inhibition of cell growth. At the end of the batch, ethanol is almost completely consumed so that the system is driven close to the optimum (see Figure 7). Figure 6 also shows the evolution of the feed rate F_{in}. θ converges to its true value, so as α through a judicious choice of the value of γ_s (see Table 1) as the convergence is generally very slow. The productivity is quite satisfactory as more than $150g/l$ of biomass are obtained within less than 40 hours, despite the high initial concentrations in substrate and ethanol.

The main drawback of this control strategy is the delicate choice of the tuning parameters, depending on the initial and operating conditions. This problem originates in the presence of the error control variable Z_s as a factor in (19b). If the substrate concentration quickly converges to its setpoint, i.e. the controller works efficiently and Z_s vanishes, the convergence of α is significantly affected. In turn, if the critical substrate level is overestimated, the control action can lead to the production of ethanol, and as a consequence, the inhibition of the respiratory capacity and a further decrease of the critical substrate level.

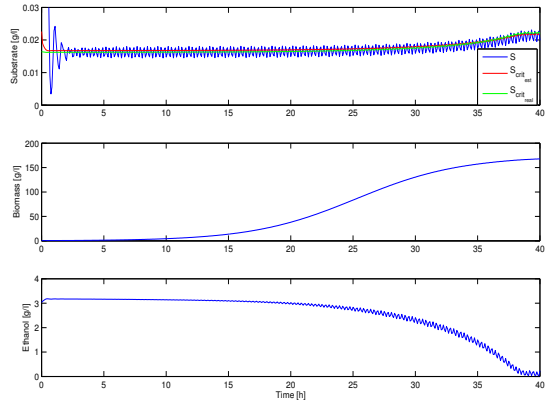

Fig. 5. Substrate (S, $\hat{S}_{crit}$ and S_{crit}), biomass (X) and ethanol (E) concentrations evolutions.

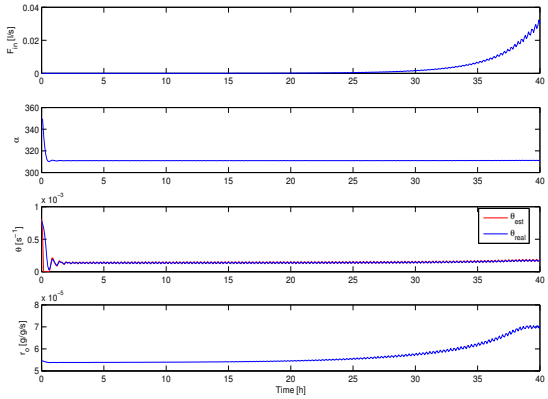

Fig. 6. Feed rate (F_{in}, α and θ parameters, and respiratory capacity (r_o) evolutions.

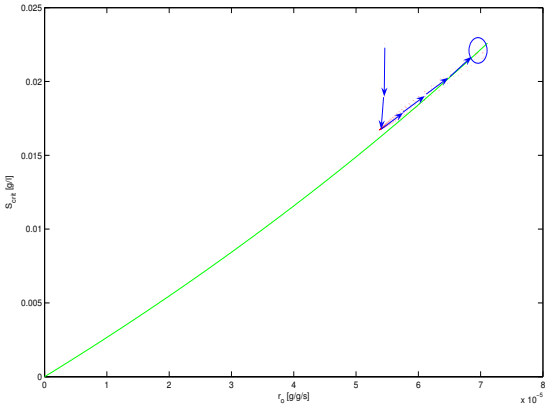

Fig. 7. Representation of the algorithm convergence through the evolution of S_{crit} as a function of r_o.

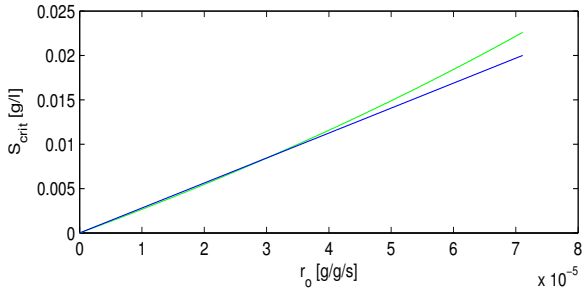

Fig. 8. S_{crit} as a function of r_o (in green) and reduced approximation (in blue).

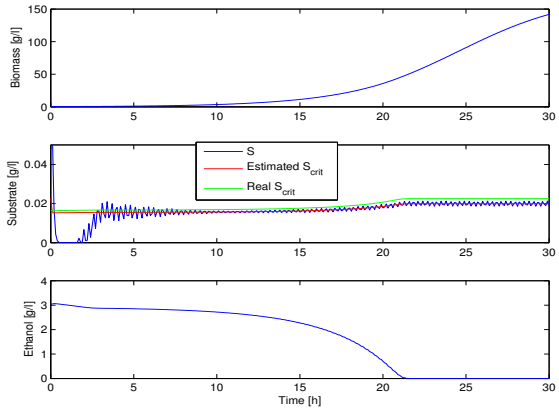

Fig. 9. Biomass (X), Substrate (S, $\bar{S}_{crit}$ and S_{crit}), and ethanol (E) concentrations evolutions.

With a bad choice of the tuning parameters, the biomass growth can therefore be seriously inhibited.

A simple way round this problem is to systematically underestimate the critical substrate level. This can be achieved by considering a lower linear approximation, i.e. a linear function below the real curve $S_{crit}(r_o)$ in the classical operating area (very low values of r_o are never reached in a controlled process). For instance, if we impose the point $(\mu_o, 0.02)$ to belong to this approximation (cfr Figure 8), the adaptation law of α is now the following one:

$$\bar{S}_{crit} = \bar{\alpha} r_o \tag{29}$$

Where $\bar{S}_{crit}$ and $\bar{\alpha}$ are the lower values of S_{crit} and α. α is thus equal to $\frac{\mu_o}{0.02}$.

Figure 9 shows simulation results using this modified strategy. The performance is now much more robust to the initial and operating conditions. The dither signal is simplified in $a = A\sin(\omega\, t)$ since θ is now the only parameter to be estimated ($A = 0.0005$ and $\omega = \frac{2\pi}{1000}$).

5. CONCLUSION

An adaptive extremum-seeking strategy is designed to control the substrate concentration in fed-batch cultures of *S. cerevisiae*. The challenge associated with this particular application is due to the dependence of the critical substrate level on the respiratory capacity ruling strong nonlinear kinetic laws describing the bottleneck assumption of (Sonnleitner and Käppeli [1986]). This yeast respiratory capacity is here considered as influenced by the oxygenation level and the ethanol inhibitory effect (Pham [1999]). Based on Lyapunov stability arguments, original adaptation laws are derived to estimate on-line unknown kinetic parameters. In addition, an asymptotic observer is designed so as to limit the need for expensive hardware sensors of the culture component concentrations. Finally, a simplified strategy is proposed, which provides a more robust estimation of the critical substrate level.

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 rests with its authors.

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