Adaptive extremum-seeking control of fed-batch cultures of micro-organisms exhibiting overflow metabolism

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Abstract: *Overflow metabolism* characterizes cells strains that are likely to produce inhibiting metabolites resulting from an excess of substrate feeding and a saturated respiratory capacity. The critical substrate level separating the two different metabolic pathways is generally not well defined. This paper proposes two non-model based extremum-seeking strategies preventing a too important accumulation of inhibiting metabolites in fed-batch cultures, by estimating the critical substrate level on the basis of 3 simple measurements related to the feeding, oxygen and carbon dioxide. A simple substrate controller based on Lyapunov stability arguments is then designed and tested in combination with the two extremum-seeking schemes.

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

1. INTRODUCTION

Industrial vaccine production is usually achieved using fed-batch cultures of genetically modified yeast or bacteria 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 encountered problem comes from the metabolic changes of such strains in presence of feeding overflow. This "overflow metabolism", also called "short-term Crabtree effect", is a metabolic phenomenon that is induced when the rate of glycolysis exceeds a critical value, leading to a generally inhibiting by-product formation from pyruvate (for not well understood reasons). It occurs for instance in *S. cerevisiae* cultures with aerobic ethanol formation, in *P. pastoris* with aerobic methanol formation, in *E. coli* cultures with aerobic acetate formation or in mammalian cell cultures with the aerobic lactate formation. To avoid this undesirable effect, a closed-loop optimizing strategy is required, which could take various forms (Pomerleau (1990), Chen et al. (1995), Akesson (1999), Renard (2006), Dewasme et al. (2007)).

In this study, a non-model based extremum-seeking strategy is chosen. Two original techniques are proposed and compared.

The first one is related to the work of Blackman in the 60's, revisited and improved in Ariyur and Krstic (2003) while the second one is based on a simple recursive least squares technique (RLS). Non-model based extremum-seeking has already been applied succesfully to dynamic optimization of continuous cultures in Wang et al. (1999).
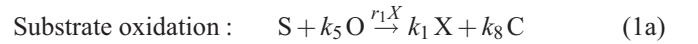
Alternatively, model-based extremum-seeking strategy as presented in the works of Guay et al. (2004), Titica et al. (2003a) and Titica et al. (2003b) could also be considered for the on-line determination of the critical glucose concentration. However, the convergence of this adaptation scheme is slow and lacks robustness (Dewasme and Vande Wouwer (2008)).
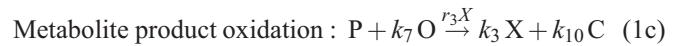
2. MODEL AND CONTROL OBJECTIVES

2.1 Modeling cultures of micro-organisms exhibiting overflow metabolism

In this study, we consider a generic model that would, in principle, allow the representation of the culture of different strains presenting an overflow metabolism (yeasts, bacteria, animal cells, etc). This model describes therefore the cell catabolism through the following three main reactions:

Substrate oxidation : $\quad \mathrm{S} + k_5 \mathrm{O} \xrightarrow{r_1 X} k_1 \mathrm{X} + k_8 \mathrm{C}$ \hfill (1a)

Overflow reaction (typically fermentation) :

$$\mathrm{S} + k_6 \mathrm{O} \xrightarrow{r_2 X} k_2 \mathrm{X} + k_4 \mathrm{P} + k_9 \mathrm{C} \quad (1b)$$

Metabolite product oxidation : $\mathrm{P} + k_7 \mathrm{O} \xrightarrow{r_3 X} k_3 \mathrm{X} + k_{10} \mathrm{C}$ (1c)

where X, S, P, O and C are, respectively, the concentration in the culture medium of biomass, substrate (typically glucose or glycerol), product (i.e. ethanol or methanol in yeast cultures, acetate in bacteria cultures or lactate in animal cells cultures), dissolved oxygen and carbon dioxide. k_i are the yield coefficients and r_1, r_2 and r_3 are the nonlinear specific growth rates given by:

$$r_1 = \min\left(r_S, r_{Scrit}\right) \quad (2)$$

$$r_2 = \max\left(0, r_S - r_{Scrit}\right) \quad (3)$$

$$r_3 = \max\left(0, \min\left(r_P, \frac{k_5(r_{Scrit} - r_S)}{k_7}\right)\right) \quad (4)$$

where the kinetic terms associated with the substrate consumption r_S, the critical substrate consumption r_{Scrit} (generally de-
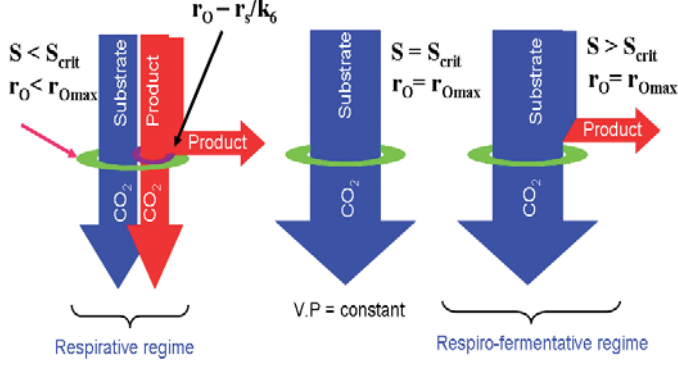
Fig. 1. Illustration of Sonnleitner's bottleneck assumption for cells limited respiratory capacity.

pendant on the cells oxidative or respiratory capacity r_O) and the product oxidative rate r_P are given by:

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

$$r_{Scrit} = \frac{r_O}{k_5} = \frac{\mu_O}{k_5} \frac{O}{O + K_O} \frac{Ki_P}{Ki_P + P} \tag{5b}$$

$$r_P = \mu_P \frac{P}{P + K_P} \tag{5c}$$

These expressions take the classical form of Monod laws where μ_S, μ_O and μ_P are the maximal values of specific growth rates, K_S, K_O and K_P are the saturation constants of the corresponding element, and Ki_P is the inhibition constant.

This kinetic model is based on Sonnleitner's bottleneck assumption (Sonnleitner and Käppeli (1986)) which was applied to a yeast strain *Saccharomyces cerevisiae* (Figure 1). During a culture, the cells are likely to change their metabolism because of their limited respiratory capacity. When the substrate is in excess (concentration $S > S_{crit}$), the cells produce a metabolite product P 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 the metabolite P (as a substitute carbon source), if present in the culture medium, are oxidized. The culture is then said in respiratory (R) regime. Component-wise mass balances give the following differential equations :

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

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

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

$$\frac{dO}{dt} = -(k_5 r_1 + k_6 r_2 + k_7 r_3)X - DO + OTR \tag{6d}$$

$$\frac{dC}{dt} = (k_8 r_1 + k_9 r_2 + k_{10} r_3)X - DC - CTR \tag{6e}$$

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

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:
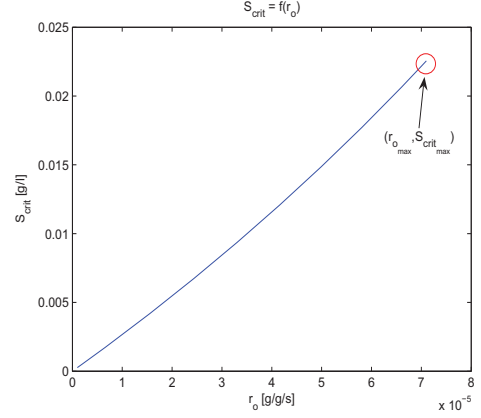

Fig. 2. S_{crit} as a function of r_o.

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

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

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.

2.2 Control objectives

First, we show that the respiratory capacity has an influence on the critical substrate concentration level. In the optimal operating conditions ($S = S_{crit}$), the fermentation and metabolite product oxidation rates are equal to zero and the substrate consumption rate r_S is equal to r_{Scrit} or $\frac{r_O}{k_5}$. Consequently, after a trivial mathematical manipulation of (5a), a relation between the critical substrate concentration level and the cell respiratory capacity is obtained as:

$$S_{crit} = \frac{K_S r_O}{k_5 \mu_S - r_O} \tag{8}$$

Figure 2 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 metabolite product in the culture medium and a sufficient level of oxygenation). Obviously, the presence of the product 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 respiration and respiro-fermentative regimes, it would be necessary to determine on-line the critical substrate concentration (S_{crit}) and to control the substrate concentration in the culture medium around this value (Dewasme and Vande Wouwer (2008)). Unfortunately, the substrate concentration measurement is a difficult task as typical concentration levels are below the resolution of currently available probes (or sensors).

An alternative solution is to reformulate the problem not as a maximazition of the respiratory capacity but as the maximization of the substrate consumption rate coupled to the minimization of the fermentation rate.

This can finally be formulated as follows:

$$max_{S_{crit}} Y = max_{S_{crit}} \varphi_1 - \varphi_2 \tag{9}$$

where:

- Y is the assumed measurable cost function;
- φ_1 and φ_2 correspond to the reaction rates $r_1 X$ and $r_2 X$, respectively.
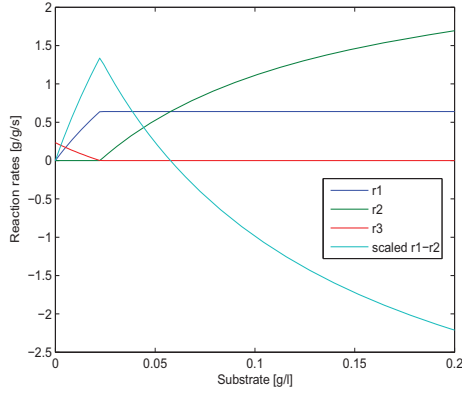
Fig. 3. Reaction rates and optimization criteria as a function of S.

In order to estimate the cost function Y online, we use a pseudo-steady state assumption. Indeed, assuming that the variations of substrate, oxygen and carbon dioxyde concentrations are equal to zero, we obtain from (6b), (6d) and (6e):

$$D(S_{in} - S) = (r_1 + r_2)X \qquad (10a)$$
$$-DO + OTR = (k_5 r_1 + k_6 r_2 + k_7 r_3)X \qquad (10b)$$
$$DC + CTR = (k_8 r_1 + k_9 r_2 + k_{10} r_3)X \qquad (10c)$$

Dilution terms can be considered as negligible in comparison with OTR, CTR and DS_{in}. Replacing the reaction rates $r_i X$ by φ_i $(i = 1, 2, 3)$, (10) can be written:

$$DS_{in} = \varphi_1 + \varphi_2 \qquad (11a)$$
$$OTR = k_5 \varphi_1 + k_6 \varphi_2 + k_7 \varphi_3 \qquad (11b)$$
$$CTR = k_8 \varphi_1 + k_9 \varphi_2 + k_{10} \varphi_3 \qquad (11c)$$

From this on, after some basic mathematical manipulations, it is possible to express a relation evolving proportionaly to $\varphi_1 - \varphi_2$, as a function of the yield coefficients, OTR, CTR and DS_{in}. We decide to call DS_{in} the "substrate intake rate" (SIR) and we finally obtain:

$$Y = \varphi_1 - \varphi_2 \propto y$$
$$y = 2\, k_{10}\, OTR - 2\, k_7\, CTR + (k_7 k_9 - k_5 k_{10} + k_8 k_7 - k_6 k_{10})SIR$$
$$(12)$$

This optimization criteria can thus be evaluated on the basis of 3 measurements (OTR, CTR and SIR) coupled to a sufficiently good identification of several yield coefficients. Figure 3 shows the evolution of the reaction rates and the criteria (scaled 20 times higher) as a function of the substrate concentration for a model of *S. cerevisiae* where the respiratory capacity is assumed to be constant (no oxygen limitation and no inhibition).

3. ADAPTIVE MODEL-FREE EXTREMUM-SEEKING STRATEGIES

Two adaptive extremum-seeking techniques are proposed in the following.

3.1 Extremum-seeking through a bank of filters

The objective of the extremum-seeking strategy is to determine on-line the parameter $\hat{\theta}$ (which in this case represents the critical glucose concentration). To this end, the system is excited by injection of a relatively slow sinusoidal dither signal $d = A\sin(\omega t)$, as shown in Figure 4.
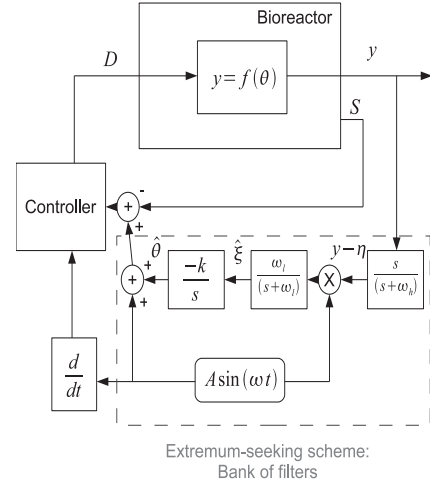

Extremum-seeking scheme:
Bank of filters

Fig. 4. Extremum-seeking scheme with a bank of filters.

The following equations describe the extremum-seeking method:
The corresponding equations to Figure 4 are:

$$y = f(\hat{\theta} + A\sin(\omega t)) \qquad (13a)$$
$$\dot{\hat{\theta}} = k\xi \qquad (13b)$$
$$\dot{\xi} = -\omega_l \xi + \omega_l (y - \eta) A\sin(\omega t) \qquad (13c)$$
$$\dot{\eta} = -\omega_h \eta + \omega_h y \qquad (13d)$$

where:

- $y = f(\hat{\theta} + A\sin(\omega t))$ is the measurable cost function;
- $\hat{\theta}$ is the estimation of the unknown parameter;
- k is the gain of the integrator;
- ξ can be seen as the gradient estimation ($\approx \frac{d\hat{\theta}}{dt}$);
- ω_l is the cut-off frequency of the low-pass filter;
- ω_h is the cut-off frequency of the high-pass filter;
- η is an intermediate variable explaining the absence of the low frequencies rejected from y in $y - \eta$ by the high-pass filter;

A first high-pass filter is used in order to reject the continuous component of y. The output is then multiplied by the dither signal in order to be "demodulated". As the dither signal and the output of the high-pass filter can only be in phase ($\hat{\theta} < \theta^*$) or out of phase ($\hat{\theta} > \theta^*$), there exists another continuous component inside the result of this demodulation. The second low-pass filter is used in order to isolate this new component containing the information of interest and sometimes residual mid-frequencies signals. This signal ξ is then filtered one last time by an integrator in order to attenuate the last "parasite" components and to recover the estimation of the unknown parameter from the integration of the continuous component. Following the theorem demonstrated in Krstic and Wang (1997), by choosing adequate values for all the parameters of the optimizing loop, the system should exponentially converge to an $O(\omega + A)$-neighborhood of the optimum.

3.2 Extremum-seeking through a RLS scheme

This second technique presents a scheme somewhat equivalent to the previous one where the bank of filters is actually replaced by a continuous recursive least squares (RLS) (Sastry and Bodson (1989)) scheme (see Figure 5) that computes the gradient ξ using a linear relationship, which is inspired from the shape of $r_1 - r_2$ as a function of the substrate concentration:
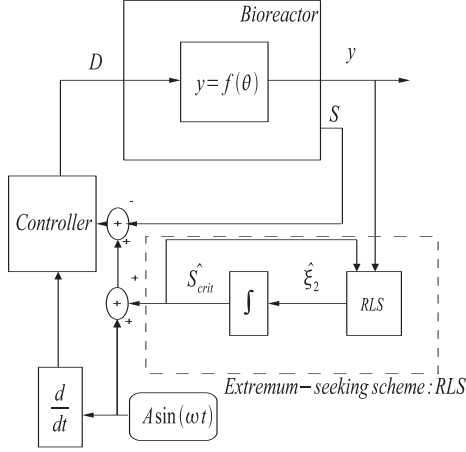
Fig. 5. Extremum-seeking scheme with RLS.

$$y = \hat{\xi}\Phi \tag{14}$$

where:

- $y = \varphi_1 - \varphi_2$
- $\hat{\xi} = [\hat{\xi}_1 \; \hat{\xi}_2]$
- $\Phi = [1 \; S_{crit}]$

In comparison with the previous extremum-seeking technique, (14) can be seen as the new relation replacing (12). The vector parameter $\hat{\xi}$ is then identified through the continuous RLS scheme that follows:

$$e = y - \hat{\xi}\Phi \tag{15a}$$

$$\dot{\hat{\xi}} = KR^{-1}\Phi^T e \tag{15b}$$

$$\dot{R} = K(\Phi^T\Phi - \lambda R) \tag{15c}$$

where:

- K is the strictly positive and constant adaptation gain;
- R is the inversed covariance matrix acting as a directional adaptation gain;
- λ is a forgetting factor used in order to avoid a "covariance wind-up problem" due to the absence of bounds in R growth (if $\lambda = 0$, $\dot{R} \geq 0$ (Sastry and Bodson (1989))).

$\hat{\xi}_2$ can be considered as the gradient estimation. This one is pushed to zero in average using an integral control of the form:

$$\dot{\hat{S}}_{crit} = k_i\hat{\xi}_2 \tag{16}$$

The conclusions about the convergence error are identical to the previous extremum-seeking technique.

3.3 Controller design

We derive adaptation and control laws from the consideration of a candidate Lyapunov function ensuring system stability. First, equation (6b) can be rewritten as follows:

$$\frac{dS}{dt} = -\nu X - D(S - S_{in}) \tag{17}$$

where $\nu = r_1 + r_2$ is considered as an unknown kinetic parameter. Defining:

$$Z_s = S_{crit} + d - S \tag{18}$$

the control error variable, where $d = A\sin(\omega t)$, is the periodical "dither signal".

$$\tilde{\nu} = \nu - \hat{\nu} \tag{19}$$

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

$$V = \frac{1}{2}Z_s^2 + \frac{1}{2\gamma}\tilde{\nu}^2 \tag{20}$$

where γ is a strictly positive tuning parameter.

A stabilizing controller is obtained if one can prove the strict negativity of the Lyapunov function derivative. Differentiating V and considering S_{crit} constant in order to decouple the control law from the extremum-seeking scheme (this can be done assuming that the controller converges significantly faster than the extremum-seeking scheme), we obtain:

$$\dot{V} = Z_s\left[\nu X + D(S - S_{in}) + \dot{d}\right] + \tilde{\nu}(-\frac{\dot{\hat{\nu}}}{\gamma}) \tag{21}$$

Replacing (18) and (19) in (21) and forcing $\dot{V}$ to be negative as in:

$$\dot{V} = -k_pZ_s^2 \tag{22}$$

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

$$-k_pZ_s = \hat{\nu}X + D(S - S_{in}) + \dot{d} \tag{23}$$

provided that:

$$\dot{\hat{\nu}} = -\gamma Z_s X \tag{24}$$

Finally, the control law is given by:

$$D = \frac{\left[k_pZ_s + \dot{d} + \hat{\nu}X\right]}{S_{in} - S} \tag{25}$$

This last expression explains the presence of the derivative $\dot{d}$ in the controller (Figure 4 and 5).

4. SIMULATION RESULTS

Coupling the controller designed in the subsection 3.3 with the extremum-seeking schemes, we apply the complete loop to a small-scale simulated yeasts culture (typically 20 l bioreactor). The initial and operating conditions are: $X_0 = 0.4g/l$, $S_0 = 0.5g/l$, $E_0 = 1g/l$, $O_0 = O_{sat} = 0.035g/l$, $C_0 = C_{sat} = 1.286g/l$, $V_0 = 5l$, $S_{in} = 350g/l$ where E_0 is the initial concentration of ethanol. For the kinetic and yield parameter values, the reader is referred to Sonnleitner and Käppeli (1986).

4.1 Application of the bank filters technique

The parameters for this extremum-seeking scheme are $A = 0.007$, $\omega = \frac{2\pi}{0.2}\ h^{-1}$, $\omega_h = 0.1\omega\ h^{-1}$, $\omega_l = 1.5\omega\ h^{-1}$, $k = 100$ and $k_p = 100$. The culture time is fixed to 24 h. Figures 6 and 7 show the results when no inhibition from ethanol accumulation is considered. This seems to be realistic as the ethanol concentration is below 4 g/l.

However, inhibition is an important phenomena that has to be taken into account. When included in our bioprocess model, the extremum-seeking results are as shown in Figure 8 and 9. It is apparent that the biomass level that can be achieved is significantly affected by the presence of ethanol, despite the set-point adaptation. Note that these results are very satisfactory in view of the situation where a constant substrate concentration is regulated. Indeed, a small error on S_{crit} would lead to a dramatical accumulation or reconsumption of ethanol and biomass growth would probably be affected beyond model prediction. As it is explained in Ariyur and Krstic (2003), the output error of the extremum-seeking algorithm achieves a local exponential convergence to an $O(A^2)$-neighborhood of the origin if it is
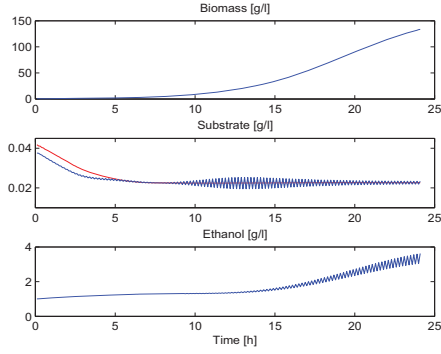
Fig. 6. Biomass (X), substrate (S in blue and $\hat{S}_{crit}$ in red), and ethanol (E) concentrations evolutions when no respiratory capacity inhibition is considered.
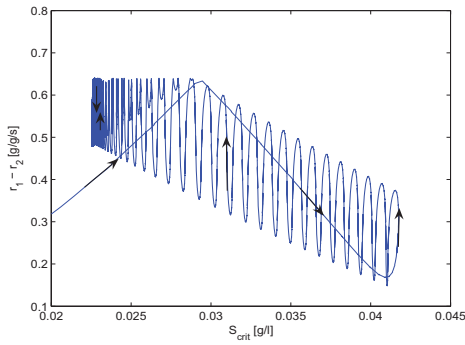

Fig. 7. Convergence of the optimization criteria $r_1 - r_2$ to the optimum when no respiratory capacity inhibition is considered.
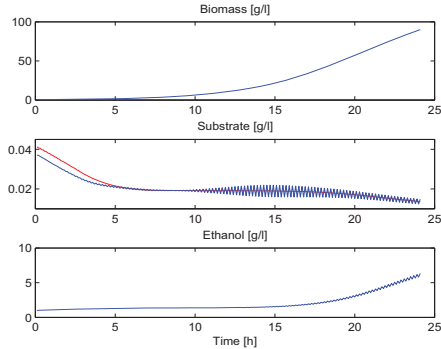

Fig. 8. Biomass (X), substrate (S in blue and $\hat{S}_{crit}$ in red), and ethanol (E) concentrations evolutions when inhibition is considered.

assumed that we are operating around a point of zero slope as it is typically the case for a convex function. As it can be observed in Figure 3, the criteria does not present a point of zero slope as the function has a discontinuous derivative at the optimum. Despite this difficulty, we see that the algorithm converges well and more particularly, the error is around an $O(A)$-neighborhood of the origin (Krstic and Wang (1997)). This last remark, which won't be elaborated in this paper, is clearly a source of bias in the set-point when the ethanol inhibition is considered (cfr Figure 9). As A needs to be chosen sufficiently large to create a significant variation on the system dynamics, a small error cannot be avoided and the ethanol is
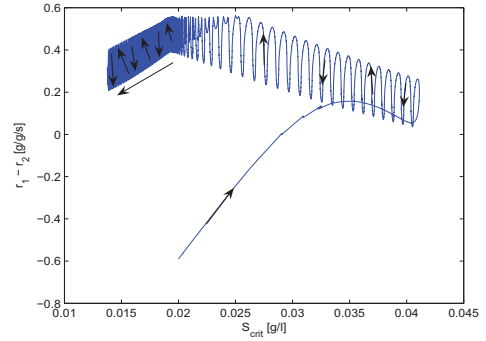

Fig. 9. Convergence of the optimization criteria $r_1 - r_2$ to the optimum when inhibition is considered.
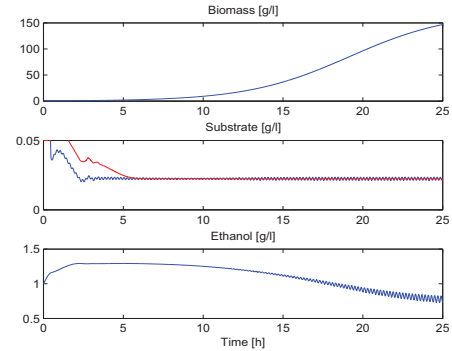

Fig. 10. Extremum-seeking with RLS: Biomass (X), substrate (S in blue and $\hat{S}_{crit}$ in red), and ethanol (E) concentrations evolutions when no respiratory capacity inhibition is considered.

accumulated while the algorithm goes on converging. As the ethanol concentration grows, the respiratory capacity slightly decreases and, following (8), S_{crit} does so.

4.2 Application of the RLS technique

The tuning parameters are defined as: $A = 0.001$, $K = 100$, $\lambda = 0.1$, $\omega = \frac{2\pi}{0.2}$, $k_i = 0.01$ and $k_p = 100$. The culture time is still fixed to $24\ h$. Figures 10 and 11 show the new results when no inhibition from ethanol accumulation is considered, and Figures 12 and 13 when the inhibition term is taken into account. The main observations are: (i) convergence is clearly faster. (ii) convergence is achieved around S_{crit} so that the ethanol concentration slowly decreases in the last hours.
When no inhibition is considered as in Figure 10, this set-point bias has no consequence on the extremum while, in Figure 12, when inhibition is taken into account, the set-point error is, by chance, playing a positive role so that ethanol is consumed.
In this application, the RLS algorithm is less computationally demanding, and easier to tune than the bank of filters strategy.

5. CONCLUSION

The high productivity of fed-batch cultures using genetically modified strains exhibiting overflow metabolism relies on a double condition: an optimal feeding strategy and the implied limitation of the inhibiting by-product formation. To this end, an adaptive controller using two different non-model based extremum-seeking strategies is designed for a general case of
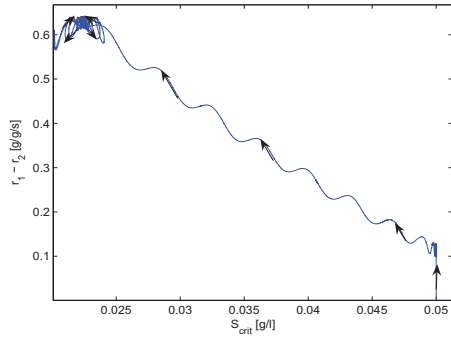
Fig. 11. Extremum-seeking with RLS: convergence of the optimization criteria $r_1 - r_2$ to the optimum when no respiratory capacity inhibition is considered.
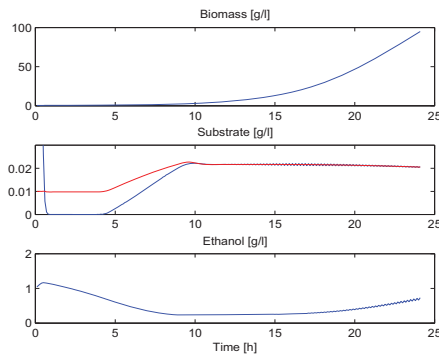

Fig. 12. Extremum-seeking with RLS: Biomass (X), substrate (S in blue and $\hat{S}_{crit}$ in red), and ethanol (E) concentrations evolutions when inhibition is considered.
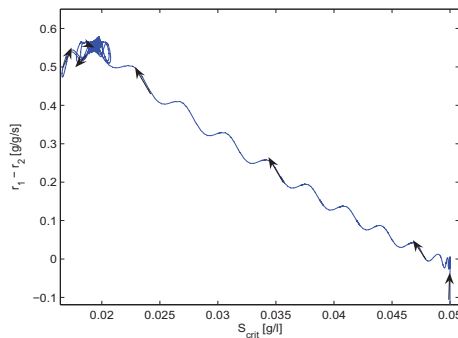

Fig. 13. Extremum-seeking with RLS: convergence of the optimization criteria $r_1 - r_2$ to the optimum when inhibition is considered.

overflow metabolized strain and is applied to the particular case of *S. cerevisiae*. The tracking of the critical substrate level (or, at least, its kinetic image), representing the optimum, is correctly performed by both extremum-seeking techniques, limiting the ethanol accumulation despite the considerations of an ethanol-inhibited respiratory capacity and discontinuous derivatives around the optimum.

6. ACKNOWLEDGMENT

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