ADAPTIVE EXTREMUM SEEKING CONTROL OF ENZYME PRODUCTION IN FILAMENTOUS FUNGAL FERMENTATION

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Abstract: The aim of this paper is to investigate, via a case study, an adaptive extremum seeking control scheme for product formation in fed-batch bioreactors. The presented approach utilizes the structure information of the Haldane kinetic model, to derive an extremum seeking algorithm that drives the system to the desired set points with the objective to maximize the product formation rate. The adaptive extremum seeking algorithm consists of a control law and parameter learning laws designed by using Lyapunov's stability arguments. The adaptive extremum seeking control scheme is applied to the maximization of the enzyme production yield in filamentous fungal fermentation. Numerical simulations are provided in order to investigate the effectiveness of the proposed scheme.

Keywords: Extremum seeking, Lyapunov stability theory, fed-batch bioreactor, Haldane kinetics, filamentous fungal fermentation, enzyme production

1. INTRODUCTION

Filamentous fungi are extensively used in the fermentation industry for the production of a large number of products including primary metabolites, antibiotics, industrials enzymes and proteins (McIntyre et al., 2001).

Many industrial fermentations are carried out in fed-batch mode. The main challenge in industrial fermentations involving filamentous fungi remains how to design and operate the bioreactor for high-density cultivation. The purpose of the fermentation process is naturally to obtain as high enzyme yield as possible and also to minimize the energy used during the fermentation process (mainly related to stirring and aeration). The operation from moderate to high biomass concentrations is crucial to achieve high productivity, but it induces high viscosity and non-Newtonian behavior in the fermentation medium (Li et al., 2000). In these conditions, the oxygen supply to the cells becomes critical.

Consequently, the control issue in the operation of fed-batch filamentous fungal fermentation is to find a control strategy for substrate feeding that will maximize the enzyme production yield and avoid oxygen limitation. The classical approach to solve this problem is model-based optimal control, providing a theoretically realizable optimum. However, in practice, because of the modeling uncertainties, poor performance may be expected from such control strategies, and, although a priori attractive, optimal control has not been largely applied to industrial bioprocesses. An alternative is to use approaches that are aimed at handling the process uncertainties with an adaptive control scheme (Bastin and Dochain, 1990), (VanImpe, 1993).

The extremum seeking control is an interesting alternative to handle optimization problems, when the objective function is a function of unknown parameters or for selecting the desired states to keep a performance function at its extremum value (Zhang et al, 2003).

In this paper, we investigate an extremum seeking control scheme for the maximization of enzyme production rate in fed-batch bioreactors. The proposed scheme utilizes explicit structure information of the objective function that depends on system states and unknown parameters. In this approach, the optimum search is included in the adaptive control scheme. The Lyapunov's stability theory is used in the design of the extremum seeking controller structure and the development of the parameter learning laws. A similar approach has been considered for a simple microbial growth with Haldane kinetics (Titica et al., 2002).

The paper is organized as follows. Section 2 presents the dynamic model of filamentous fungal fermentation that has been used in this study. The model-based adaptive extremum seeking algorithm is developed in Section 3. Simulation results are discussed in Section 4.

2. FILAMENTOUS FUNGAL FERMENTATION MODEL

The model considered in this study is derived from a literature model, proposed by (Agger at al., 1998). This is a morphologically structured model, giving a description of growth and product formation of filamentous fungi, under no limiting oxygen conditions. In this study, the limitation by O_2 is considered via Monod expressions, introduced at the kinetic expressions level.

2.1. Modelling considerations

Growth kinetics. The morphologically structured model proposed by (Agger et al., 1989) is based on the division of the biomass in three compartments:

- *active region (X_a)*, responsible for the uptake of substrate and growth of the hyphal element. It is assumed also that only the active region is responsible for the enzyme production
- *extension region (X_e)*, where building of new cell wall and extension zone take place
- *hyphal region (X_h)* is the degenerated part of the hyphal elements, which are inactive.

Two metamorphosis reactions are considered in the model:

$$X_a \xrightarrow{q_1} X_e \quad (1)$$

$$X_a \xrightarrow{q_2} X_h \quad (2)$$

The corresponding kinetic expressions are represented in Eqs. 3 and 4:

$$q_1 = \frac{k_1 \cdot s}{a \cdot (s + K_{s1})} \cdot x_a \cdot \frac{o_2}{o_2 + K_{o_2}} \quad (3)$$

$$q_2 = k_2 \cdot x_a \quad (4)$$

The growth of the active region is given in Eq. 5:

$$q_3 = \frac{k_3 \cdot s}{s + K_{s3}} \cdot a \cdot x_e \cdot \frac{o_2}{o_2 + K_{o_2}} \quad (5)$$

The specific growth rate of total biomass is given in Eq. 6:

$$\mu = \frac{q_3}{x_e + x_a + x_h} \quad (6)$$

Specific rate of enzyme production. Enzyme production in filamentous fungi is a classical example of growth associated product formation. In this model, the specific rate of enzyme production is related to the active region. Also, enzyme production is subject to glucose (substrate) repression - described in this paper by an Haldane expression - and oxygen limitation - represented by a Monod equation - as given in Eq. 7:

$$r_{ps} = \frac{\mu_0 \cdot s}{K_s + s + \frac{s^2}{K_I}} \cdot \frac{o_2}{K_{o_2} + o_2} + k_c \quad (7)$$

The parameter k_c represents the constitutive level of enzyme production.

Specific rate of oxygen consumption and carbon dioxide formation are expressed in Eqs. 8 and 9:

$$r_{o2} = Y_{xo} \cdot \frac{q_3}{(X_e + X_a + X_h)} + Y_{PO} \cdot r_{ps} \cdot \frac{X_a}{(X_e + X_a + X_h)} + m_o \quad (8)$$

$$r_{co_2} = Y_{XC} \cdot \frac{q_3}{(X_e + X_a + X_h)} + m_c \quad (9)$$

2.2. Balance model equations

The model proposed by Agger et al. consists of a set of five balance equations for the three regions of the biomass, substrate and product concentrations, as represented in Eqs.10-14. For control and estimation purposes, supplementary mass balances are introduced in this study for describing the dissolved oxygen concentration in the bioreactor and the fraction of CO_2 in the gaseous phase that are both measurable on-line (Eqs. 15-16).

Morphological states

$$\frac{dX_e}{dt} = q_1 - D \cdot X_e \quad (10)$$

$$\frac{dX_a}{dt} = q_3 - q_1 - q_2 - D \cdot X_a \quad (11)$$

$$\frac{dX_h}{dt} = q_2 - D \cdot X_h \quad (12)$$

Glucose (s)

$$\frac{ds}{dt} = -\left[\left(\frac{1}{\alpha}\right) \cdot q_3 + r_{ps} \cdot \frac{1}{Y_{sp}} \cdot x_a + m_s \cdot (X_e + X_a + X_h)\right] + D \cdot (s_f - s) \quad (13)$$

Enzyme (p)

$$\frac{dp}{dt} = r_{ps} \cdot x_a - D \cdot p \quad (14)$$

Dissolved O_2 concentration (O_2)

$$\frac{dO_2}{dt} = -r_{o2} \cdot (X_e + X_a + X_h) + k_L a \cdot (O_2^* - O_2) - D \cdot O_2 \quad (15)$$

CO_2 concentration in the gaseous phase (CO_2)

$$\frac{dCO_2}{dt} = r_{CO_2} \cdot (X_e + X_a + X_h) \quad (16)$$

For more details about the morphological model and the significance and numerical values of the different parameters, the reader is referred to (Agger et al., 1989).

3. ADAPTIVE EXTREMUM SEEKING CONTROL ALGORITHM

3.1. Problem

The main control issue in the operation of fed-batch filamentous fungal fermentation is to find a control strategy for substrate feeding that maximizes the enzyme production. An approximation of the optimal solution for this problem can be obtained by maximizing the enzyme production rate during the fermentation duration. From this consideration and based on the model structure presented in the Section 2, we shall now concentrate on the design of an adaptive extremum-seeking control algorithm for the enzyme production rate.

3.2. Principle and assumptions

According to the kinetic model of the enzyme production (Eq.7), the maximum enzyme production rate with respect to the substrate concentration *s* is obtained if s can be stabilized at the set-point:

$$s^* = \sqrt{K_s \cdot K_i} \tag{17}$$

Since the exact values of Haldane model parameters K_s, μ_0 and K_I, are usually unknown (or at least poorly known), the adaptive extremum seeking algorithm is developed to search this unknown set-point such that the enzyme production rate is maximized.
So, the adaptive extremum seeking control scheme provides an adaptive control law of the dilution rate to control the substrate *s* at the desired set point *s**, coupled with parameter learning laws for Haldane model parameters estimation. This algorithm requires the on-line knowledge of substrate and biomass concentrations. Also, the growth kinetics are assumed to be known via CO_2 measurements, as well as the related yield coefficients. The algorithm design is done under the assumption that oxygen limitation does not occur.
The adaptive extremum seeking control scheme is designed using Lyapunov's stability theorem.

Parameter definition. Let define:

$$\theta_\mu = \frac{\mu_0}{K_s} \tag{18}$$

$$\theta_S = \frac{1}{K_s} \tag{19}$$

$$\theta_I = \frac{1}{K_s \cdot K_I} \tag{20}$$

$\theta = [\theta_\mu \ \ \theta_s \ \ \theta_I]^T$ represent the new set of kinetic parameters to be estimated on-line. The optimum for s (17) can be re-expressed as follows:

$$s^* = \frac{1}{\sqrt{\theta_I}} \tag{21}$$

By this transformation, the optimum value is function of only one unknown parameter that has to be estimated on-line.

3.3. Estimation and Controller Design

The controller design proceeds in different steps. First of all, the estimation equation for *s* is derived from the balance model equation, then the control law and the estimation of the unknown kinetic parameters are included in a Lyapunov based derivation framework.

Estimation equation for the substrate s
By considering Eq. 13 and the kinetic parameter definition, the predicted state *s* is generated by:

$$\frac{d\hat{S}}{dt} = -\frac{1}{\alpha} \cdot q_3 - k_2' \cdot \frac{\hat{\theta}\mu \cdot S}{1 + \hat{\theta}_S \cdot S + \hat{\theta}_I \cdot S^2} \cdot x_a + D \cdot (S_f - S) + k_S \cdot e_S \tag{21}$$

$k_2' = \frac{1}{Y_{sp}} \cdot \frac{o_2}{K_{o_2} + o_2}$ is supposed to be known and constant (this typically happens when o_2 is not limiting ($o_2 >> K_{o2}$) or when o_2 is controlled at some constant value). where $\hat{\theta}$ denote the estimate of the true parameter θ. $e_s = s - s^*$ represents the prediction error and k_s is a positive tuning parameter.

Design of the adaptive extremum seeking controller
Since the parameter θ_I is unknown, the desired set-point (21) can be re-expressed as follows:

$$s^* = \frac{1}{\sqrt{\hat{\theta}_I}} \tag{22}$$

The controller will be designed in order to drive the substrate concentration *s* to the estimated value of *s** (22). An excitation signal *d(t)* is design and injected into the adaptive system such that the estimated parameter $\hat{\theta}_I$ converges to its true value. The extremum seeking control objective can be achieved when the substrate concentration *s* is stabilized at the optimal operating set-point *s**.

Define the error control variable z_s as follows:

$$z_S = s - \frac{1}{\sqrt{\theta_I}} - d(t) \tag{23}$$

Consider the Lyapunov candidate function as follows:

$$V = \frac{z_S^2}{2} \cdot (1 + \theta_S \cdot s + \theta_I \cdot s^2) + \frac{1}{2} \cdot \left(\frac{\tilde{\theta}_\mu^2}{\gamma_\mu} + \frac{\tilde{\theta}_S^2}{\gamma_S} + \frac{\tilde{\theta}_I^2}{\gamma_I}\right) + \tag{24}$$
$$+ \frac{e_S^2}{2}(1 + \theta_S \cdot s + \theta_I \cdot s^2)$$

where $\tilde{\theta}_\mu = \theta_\mu - \hat{\theta}_\mu$, $\tilde{\theta}_s = \theta_s - \hat{\theta}_s$ and $\tilde{\theta}_I = \theta_I - \hat{\theta}_I$
γ_μ, γ_s and γ_I are positive tuning parameters.

The adaptive extremum seeking controller algorithm is derived from the derivative expression of Lyapunov candidate function *V*, in such a way $\dot{V}$ is negative.
After mathematical manipulations, we obtain the control law and the parameter learning laws as follows:

Control law:

$$D = \frac{1}{(s_f - s)} \cdot \left[\frac{1}{\alpha} \cdot q_3 + k_2^{'} \cdot \frac{\hat{\theta}\mu \cdot s}{1 + \hat{\theta}_S \cdot s + \hat{\theta}_I \cdot s^2} \cdot x_a \right] + \\ + \frac{1}{(s_f - s)} \cdot \left[-k_z \cdot z_s + a(t) - k_d \cdot d(t) \right] \quad (25)$$

Note that the resulting control law consists in two terms: the first one includes the specific rates of enzyme production and of the biomass growth, while the second is a correcting term proportional to the error output and to the dither signal, *d(t)* defined as:

$$\dot{d}(t) = a(t) - \frac{1}{2} \cdot \hat{\theta}_I^{-\frac{3}{2}} \cdot \frac{d\hat{\theta}_I}{dt} - k_d \cdot d(t) \quad (26)$$

where $a(t)$ acts as a dither signal on the closed-loop process and k_d is a strictly positive constant.

Parameter learning laws.

$$\frac{d\hat{\theta}_\mu}{dt} = -\gamma_\mu \cdot k_2^{'} \cdot s \cdot x_a \cdot (z_s + e_S) \quad (27)$$

$$\frac{d\hat{\theta}_S}{dt} = \gamma_S \cdot \frac{k_2^{'} \cdot s^2 \cdot x_a \cdot (z_s + e_S)}{1 + \hat{\theta}_s \cdot s + \hat{\theta}_I \cdot s^2} \quad (28)$$

$$\frac{d\hat{\theta}_I}{dt} = \gamma_I \cdot \frac{k_2^{'} \cdot s^3 \cdot x_a \cdot (z_s + e_S)}{1 + \hat{\theta}_s \cdot s + \hat{\theta}_I \cdot s^2} \quad (29)$$

3.4. Stability and convergence analysis

Substituting the Eqs. 25-29 into the $\dot{V}$ expression, we obtain:

$$\dot{V} = -(z_S{}^2 \cdot k_z + e_S{}^2 \cdot k_S) \cdot (1 + \theta_S \cdot S + \theta_I \cdot S^2) + \Gamma \quad (30)$$

$$\Gamma = \left[\frac{z_S{}^2}{2} + \frac{e_S{}^2}{2} \right] \cdot (\theta_S + 2 \cdot \theta_I \cdot S) \cdot \dot{S} \quad (31)$$

The states and parameters involved in the Eq. 30 being all positives, the first term of the right-hand side of Eq. 30 is always negative.

In order to obtain $\dot{V}$ negative:

$$\Gamma < 0 \Rightarrow \dot{S} < 0$$

e.g.

$$D < k_1 \cdot Q_{co2} + k_2^{'} \cdot \frac{\theta_\mu \cdot S}{1 + \theta_S \cdot S + \theta_I \cdot S^2} \cdot x_a \quad (32)$$

This means, the dilution rate D needs to be upper bounded, and then k_z and k_d parameters will be tuned according to the condition (32). The theoretical proof of the convergence of a similar adaptive extremum seeking algorithm can be found in (Titica et al., 2003).

4. SIMULATION RESULTS

The proposed adaptive extremum seeking controller has been tested in numerical simulation, performed using a realistic example of a fed-batch fermentation process, as given by (Agger et al, 1998).

The initial states used in simulation are:
$x_e(0) = 0.0005 g/kg$, $x_a(0) = 1.2 g/kg$
$x_h h(0) = 0.0005 g/kg$, $s(0) = 10 g/l$,
$p(0) = 0 FAU/l$, $o_2(0) = 100\%$
$s_f = 50 g/l$.

The Haldane kinetic model parameters used during the numerical simulation are identified from the Agger model as follows:
$\mu_0 = 227 FAU/(gactiveDW \cdot h)$
$K_s = 0.0211 g/l$
$K_I = 1.5 \cdot 10^{-3} g/l$

For the Haldane model, from Figure 1, the maximum on the specific rate of enzyme production occurs at *s*=0.0059* g/l. The control objective is to design a controller for the dilution rate, *D*, to regulate the substrate *s* at *s**. The controller requires knowledge of the substrate and biomass concentrations. The kinetic parameters determining the *s** are obtained using the estimation algorithm previously presented.

The initial values for the initial estimates of the kinetic parameters are:
$\theta_\mu = 1.6 \cdot 10^4$, $\theta_s = 23.69$, $\theta_I = 1.6 \cdot 10^4$

The design parameters for the extremum seeking controller are set to:

Table 1: Values of the tuning parameters, used during numerical simulations

Tuning parameter	Value
γ_μ	10
γ_s	0.5
γ_I	50
k_z	10
k_d	0.1
k_s	10

The dither signal *a(t)* is chosen as:
$a = 0.01 \cdot \exp(-0.01 \cdot t) \cdot (-\sin(0.1 \cdot t) - \sin(t))$

The design parameters in the adaptive controller and the adaptive laws are tuned in simulation, by trial-error. First, k_s design parameter is selected according

to the convergence rate and the quality of the estimate of *s*. Then, the design parameters $\gamma_\mu, \gamma_S, \gamma_i$ are tuned during the batch phase, according to the convergence of the kinetic parameter estimates to the true values. The design parameters of the control law, k_z and k_d are selected according to the control performances in regulation and set point tracking, during the fed-batch phase. The tuning of the parameters at a time, considering the whole operation duration, is difficult, because of existing interactions between design parameters.

The simulation results are illustrated in Figure 2. It is shown that the substrate concentration converges to the unknown optimum and the convergence of the kinetic parameters to their true values is achieved at the end of the batch phase. Figure 2 also illustrates the evolution of the dilution rate profile: *D* is maintained to zero, as long as the substrate concentration (*s*) in the reactor is higher than the optimum value, *s** (batch operation). The feeding of the reactor is started when *s* reaches *s**, determined by the kinetic parameter estimates, with a dilution rate progressively increasing. It is important to note that the control performance is strongly dependent on the convergence of θ_i parameter, determining the set point for the control law.

The maximum volume of the reactor gives the end of the process. The corresponding production (Figure 2) obtained at the end of the fed-batch is equal to the maximum expected during the definition of the control strategy. The feeding profile derived by the extremum seeking algorithm is the optimal feeding profile, if the constraints in terms of oxygen limitation are not reached.

The adaptive extremum seeking controller has been tested in presence of oxygen limitation. When limitation by oxygen occurs, the dilution rate is reduced proportionally to the specific production and growth rates, but because the optimum values s^* does not depend on the oxygen concentration, the optimum search doesn't take into account the oxygen limitation. As illustrated in Figure 3, the maximum value of the enzyme production specific rate depends on the oxygen concentration. When oxygen limitation occurs, the *s** given by the Haldane kinetic model is no more optimum. The real optimum s* diminishes such that the production rate follows the curve corresponding to the constraint value of the oxygen and in this way, oxygen limitation could be avoided.

5. CONCLUSIONS

In this paper, an adaptive extremum seeking control scheme for product formation in fed-batch bioreactors has been investigated. The proposed extremum seeking controller drives the substrate concentrations to unknown desired set-points that optimize the product formation rate. The proposed approach has been illustrated via a case study – the enzyme production in filamentous fungal fermentation. Numerical simulations have been performed in order to test the effectiveness of the proposed approach. The adaptive extremum seeking is shown to perform satisfactorily in the case where the product formation is not subject to oxygen limitation (or another substrate). When oxygen limitation occurs the proposed technique is not able to provide a feasible solution.

The main drawback of the present extremum seeking controller is its dependence on the kinetic expression structure. An alternative approach (Guay, Dochain and Perrier, 2003) considers limited knowledge of the growth kinetics that is approximated by using neural network approximation techniques. Such an approach is very appealing to solve our extremum seeking control problem under limiting oxygen conditions and will be considered in subsequent studies.

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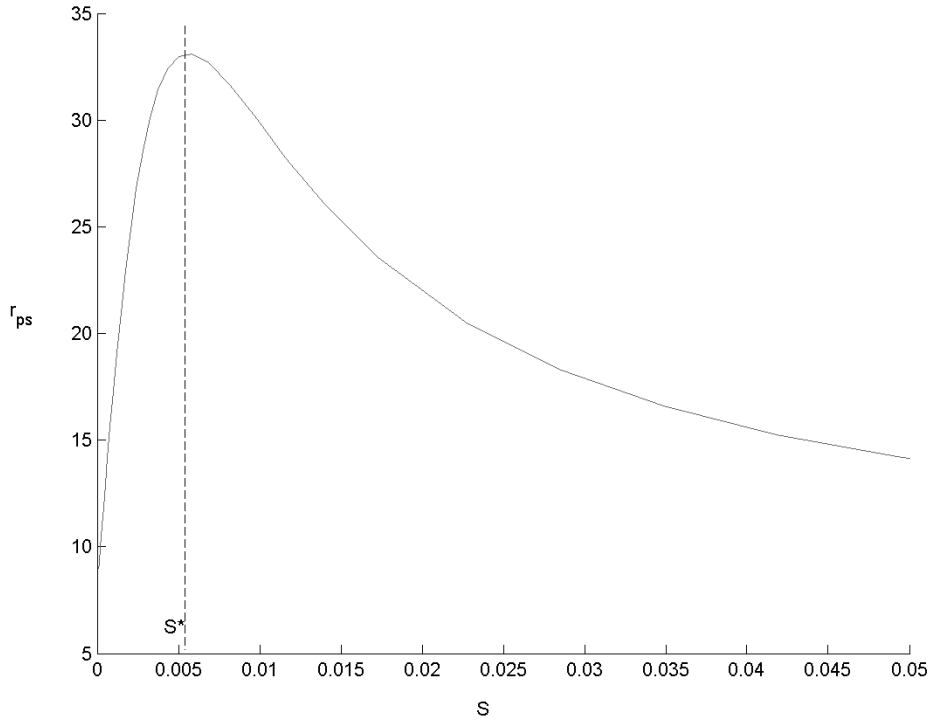

Figure 1: Rate of production formation
(The kinetic parameter of the Haldane model are: $\mu_0 = 227 FAU/(gactiveDW \cdot h)$, $K_s = 0.0211 g/l$, $K_I = 1.5 \cdot 10^{-3} g/l$)

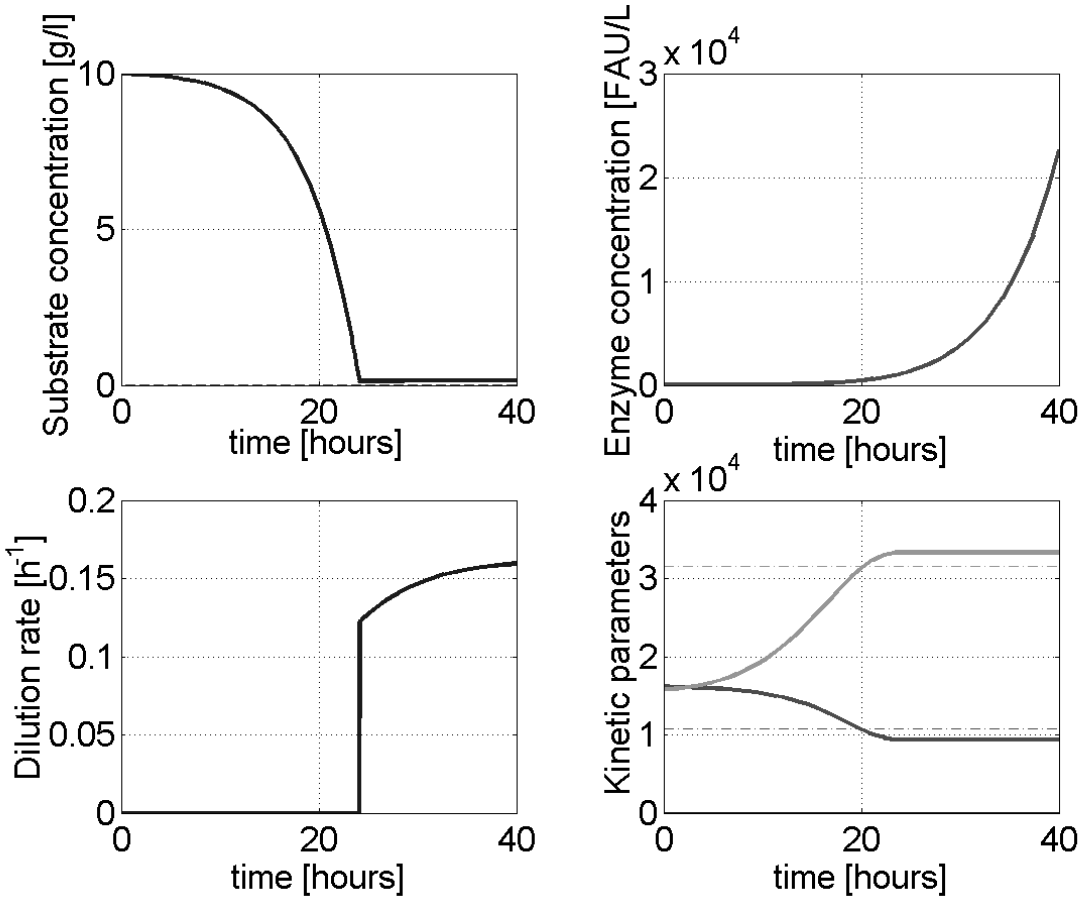

Figure 2: Simulation results: illustration of the convergence properties

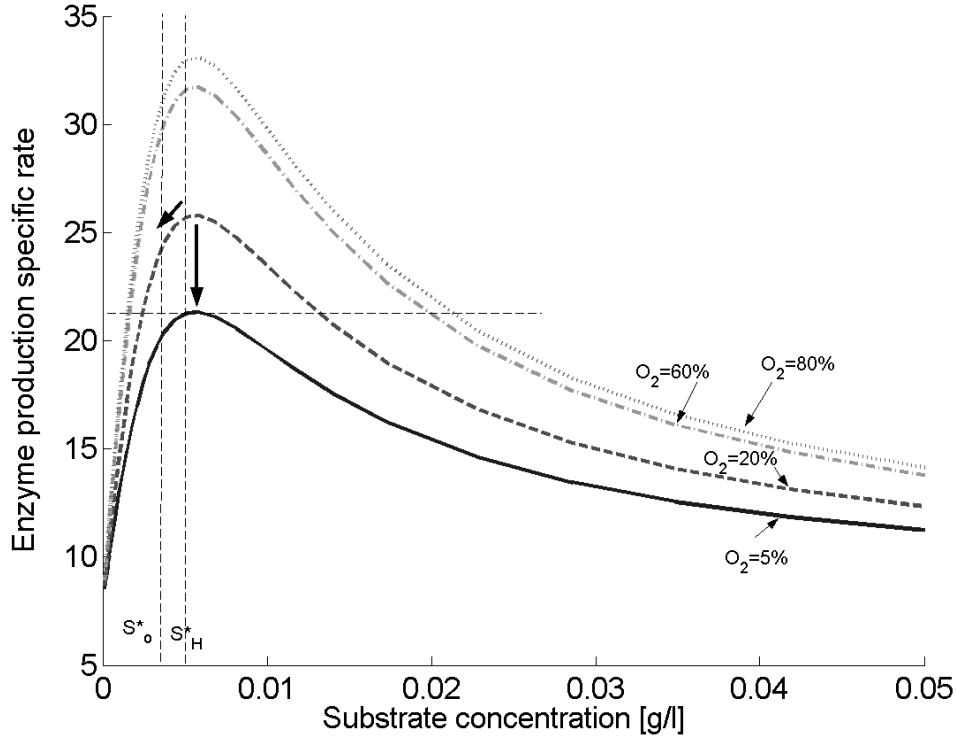

Figure 3 : Enzyme production specific rate evolution under oxygen limitation conditions. Evolution of the optimum value of s*