ADAPTIVE ESTIMATION OF MICROBIAL GROWTH RATES

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ABSTRACT

In this paper, continuous-time estimation algorithms for the microbial growth-rate of a fermentation process are proposed. The microbial specific growth-rate is here considered as a time-varying unknown parameter and is estimated by using adaptive estimation schemes. Different input-output configurations are considered. Stability and convergence are analysed for each estimation algorithm.

Keywords: Adaptive systems, biology, non linear systems, time-varying systems.

1. INTRODUCTION

Continuous microbial growth in a completely stirred bioreactor is commonly described by the following state-space representation :

$$\frac{dX(t)}{dt} = [\mu(t) - D(t)]\, X(t) \qquad (1)$$

$$\frac{dS(t)}{dt} = - k_1 \mu(t) X(t) - k_2 X(t) + D(t)[S_{IN}(t) - S(t)] \qquad (2)$$

with: $X(t)$, the biomass concentration
$S(t)$, the limiting substrate concentration
$S_{IN}(t)$, the inlet substrate concentration
$D(t)$, the dilution rate (i.e. the flow rate)
$\mu(t)$, the specific growth-rate
k_1, k_2, the yield coefficients

In this representation, the specific growth-rate $\mu(t)$ is known to be a complex function of the biomass concentration $X(t)$, of the substrate concentration $S(t)$, of the pH, of the temperature, of inhibitors, etc. Many different analytical laws have been suggested for modeling $\mu(t)$. The most popular is certainly the "Monod law" :

$$\mu(t) = \frac{\mu^* S(t)}{K_m + S(t)}$$

where μ^* is the maximum growth rate and K_m the "Michaelis-Menten" parameter. But it is far from being the only one : during a recent investigation in the scientific literature, we registered more than thirty different expressions for $\mu(t)$.

Therefore, the choice of an appropriate analytical description of $\mu(t)$ is crucial in using state-space representations like (1)-(2) and is an object of controversy in the literature.

In this paper, we suggest to avoid this choice by considering $\mu(t)$ as a time-varying parameter estimated in real time.

To solve this estimation problem, extended Kalman filter ideas could clearly be applied (Nihtila et al., 1984; Stephanopoulos and Ka-Yiu-San, 1983) but stability and convergence analysis is difficult. The aim of this paper is to show that reliable real time estimates of $\mu(t)$ can be obtained by using very simple adaptive schemes. We describe the adaptive estimation of $\mu(t)$ for several input-output configurations of the system and, in each case, we prove the algorithm stability (in a BIBO sense) by using arguments from Anderson and Johnstone (1983).

The problem of parameter estimation of microbial growth processes has been previously considered by Aborhey and Williamson (1978) : they assume that $\mu(t)$ obeys to the Monod law and the estimation of the constant parameters μ^* and K_m is carried out via the use of state variable observers equations. Here we shall use a similar approach but, as already said, to estimate a time-varying parameter $\mu(t)$, independently of any analytical expression. Moreover, Aborhey and Williamson assume that both the biomass concentration $X(t)$ and the substrate concentration $S(t)$ are available for direct measurement. In the following, different estimation algorithms are presented, which depend on which variables are available for on-line measurement :
a) in section 3.1., we consider the case

when the biomass concentration X(t) is accessible for direct measurement ;

b) in section 3.2, an estimation scheme is described when both the biomass concentration X(t) and the substrate concentration S(t) are measured on line ;

c) in section 3.3, estimation results are presented when biomass concentration measurements are not available. Then reaction product measurements are necessary to implement estimation of $\mu(t)$. Two different reaction products (gaseous and liquid) are considered and described in section 2.

Practical implementations of the estimation algorithm have been carried out on two different fermentation processes and real life results are presented in section 4.

2. DESCRIPTION OF THE SYSTEM

As said in the introduction, we consider fermentation systems described by the state-space representation (1)-(2). In order to make this description fairly general, we shall specify output equations relating the synthesis product to the state of the system.

We shall distinguish between two cases.
1) When the reaction product is gaseous, we consider the following equation :

$$Q(t) = k_3 \mu(t) X(t) \qquad (3)$$

with $Q(t)$: gas flow rate.
k_3 : yield coefficient.

A typical example is the anaerobic fermentation process, where Q(t) is a methane gas flow rate (Andrews, 1969)

2) In the case of a liquid reaction product, we consider :

$$\frac{dP(t)}{dt} = k_3 \mu(t) X(t) + k_4 X(t) - D(t) P(t) \qquad (4)$$

$P(t)$: reaction product concentration,
k_2, k_3 : yield coefficients.

Equation (4) is derived from Luedeking and Piret (1959) and can be considered as fairly general. P(t) is, for instance, the ethanol concentration produced from a glucose fermentation process (Dourado and Calvet, 1983).

Throughout the paper we adopt the following (mild) assumptions (for all $t > t_o$):
1) $0 < D(t)$
2) $0 < S_{IN}(t) < S_{IN(max)}$
3) $0 < \mu(t) < \mu^*$
4) the experimental conditions are such that $S(t)$, $X(t) > 0$ and $Q(t) > 0$.

Under these assumptions, it can be shown (Dochain and Bastin, 1984) that X(t), S(t) and Q(t) are bounded.

The assumption $\mu(t) < \mu^*$ means that we assume the existence of an unknown upper bound on $\mu(t)$ (μ^* is the maximum growth-rate). This assumption is needed to ensure the boundedness of X, S and Q.

3. ESTIMATION OF THE SPECIFIC MICROBIAL GROWTH-RATE $\mu(t)$: ALGORITHMS.

3.1. The biomass concentration X(t) is available for direct measurement.

Let us denote by $\hat{X}$, the estimate of X and write the following observer equation :

$$\frac{d\hat{X}}{dt} = \hat{\mu} X - D X + C_1(t)(X - \hat{X}) \qquad C_1(t) > 0 \qquad (5)$$

The estimate $\hat{\mu}$ of the microbial growth rate is updated by using the following equation :

$$\frac{d\hat{\mu}}{dt} = C_2(t)(X - \hat{X}) \qquad C_2(t) > 0 \qquad (6)$$

It is worth noting that the estimation of μ is carried without the use of the reaction product values.

If we define the errors

$$e_X = X - \hat{X}$$

$$e_\mu = \mu - \hat{\mu}$$

the following "error system" can be written from (1), (5), (6) :

$$\frac{de_X}{dt} = - C_1(t) e_X + X e_\mu \qquad (7.a)$$

$$\frac{de_\mu}{dt} = - C_2(t) e_X + \frac{d\mu}{dt} \qquad (7.b)$$

The stability and convergence properties of the estimation algorithm (5) (6) are analysed under the following assumptions:

A.1. X(t) is strictly positive : $X(t) \geq \epsilon > 0$
A.2. $\frac{d\mu}{dt}$ is bounded : $\left|\frac{d\mu}{dt}\right| < M < \infty$

Then we have the following stability results

Theorem 3.1.1. : Under assumption A.1 and A.2, the error vector $\underline{e}^T = (e_X, e_\mu)$ is bounded as follows :

$$\|\underline{e}(t)\| < K_1 \|\underline{e}_o\| + K_2 M \qquad (8)$$

with $\underline{e}_o$: initial error vector
K_1, K_2 : constants

Proof : can be found in Bastin and Dochain, 1985.

Theorem 3.1.2.: Under assumptions A.1 and A.2, and if $C_1(t)$ and $C_2(t)$ are chosen as follows :

$$C_1(t) = \bar{C}_1 X(t), \quad \bar{C}_1 > 0 \qquad (9.a)$$

$$C_2(t) = \bar{C}_2 X(t), \quad \bar{C}_2 > 0 \qquad (9.b)$$

with $\bar{C}_2 = \frac{\alpha}{4}\bar{C}_1^2 \quad 0 < \alpha < 1$ (9.c)

Then, $\lim_{t\to\infty} \|\underline{e}(t)\| = \delta < \dfrac{M[1+\alpha\bar{C}_1]}{\epsilon\,\alpha_2\,\bar{C}_1^2}$

where δ, α_1, α_2 are positive constants.

Proof : can be found in Bastin and Dochain, 1985.

Comments: a) in theorems 3.1.1 and 3.1.2, we have to assume that $d\mu/dt$ is bounded in order to ensure boundedness of the errors e_x and e_μ. This assumption is a rather mild one, since almost all the microbial growth models suggested in the literature fulfill this requirement.
b) In theorem 3.1.2, δ can be made arbitrary small by choosing $\bar{C}_1$ sufficiently large.

3.2. The biomass concentration X(t) and the substrate concentration S(t) are available for direct measurements.

In the preceeding paragraph, we have presented an estimation scheme of the microbial growth rate μ when the biomass concentration X(t) is measured on-line. Yet, it is generally considered that growth-rate $\mu(t)$ depends on the substrate concentration S(t). Therefore, if both X(t) and S(t) are available for direct measurement, it would be interesting to implement an estimation algorithm of the microbial growth-rate by using both measurements.

In order to do so, we suggest to use the following "minimal" dependence relation between the microbial growth-rate and the substrate concentration :

$$\mu(t) = \rho(t)\,S(t) \tag{10}$$

and to estimate the parameter $\rho(t)$ instead of $\mu(t)$.

This procedure was first introduced in Dochain and Bastin (1984) in order to solve convergence problems of a methane gas production control algorithm. The relation (10) between μ and S is plainly justified by the fact that most of the growth-rate analytical models are compatible with it.

For simplicity, we consider that, in equation (2), $k_2 = 0$.

But extension to the case when $k_2 \neq 0$ is very easy.

If we define $\varphi(t) = k_1\rho(t)$ and if we introduce the expression (10) in equation (1) (2), estimation of ρ and φ can be carried out by using the following equations :

$$\begin{aligned}
\frac{d\hat{X}}{dt} &= \hat{\rho}SX - DX + C_1(t)(X-\hat{X}) \\
\frac{d\hat{\rho}}{dt} &= C_2(t)(X-\hat{X}) \\
\frac{d\hat{S}}{dt} &= -\hat{\rho}SX + D(S_{in}-S) + C_3(t)(S-\hat{S})
\end{aligned} \tag{11}$$

$$\frac{d\hat{\rho}}{dt} = -C_4(t)(S-\hat{S})$$

Under the following assumptions :

A.3. X(t) and S(t) are strictly positive: $X(t)S(t) \geq \epsilon > 0$

A.4. $\frac{d\rho}{dt}$ is bounded : $\left|\frac{d\rho}{dt}\right| < M$

We have stability and convergence properties as in Par. 3.1.

Theorem 3.2.1 : The error vector $\underline{e}^T = [e_x,\ e_\rho,\ e_s,\ e_\varphi]$ is bounded as follows :

$$\|\underline{e}\| < K_1\,\|\underline{e}_o\| + K_2 M$$

with $\underline{e}_o$: initial error vector

K_1, K_2 : constants

Proof : can be found in Bastin and Dochain, 1985.

Theorem 3.2.2 : If we choose $C_i(t)$, i = 1 to 4, such that

$$C_i(t) = X(t)S(t)\bar{C}_i$$

$$\bar{C}_2 = \frac{\alpha}{4}\bar{C}_1 \quad 0 < \alpha < 1$$

$$\bar{C}_4 = \frac{\beta}{4}\bar{C}_3 \quad 0 < \beta < 1$$

Then, $\lim_{t\to\infty} \|\underline{e}(t)\| = \delta$

$$< \frac{M}{\epsilon}\left[\frac{1+\alpha_1\bar{C}_1}{\alpha_2\bar{C}_1^2} + \frac{1+\alpha_3\bar{C}_3}{\alpha_4\bar{C}_2^2}\right]$$

Proof : similar to theorem 3.1.2

3.3. The biomass concentration X(t) is not available for direct measurement.

If biomass concentration measurements are not available, values of the reaction product are necessary to implement microbial growth-rate estimation. In this paragraph, two different cases are considered, depending on the type of reaction product, i.e. the gaseous reaction product case and the liquid reaction product case.

3.3.1. Gaseous reaction product : $Q(t) = k_3\mu(t)X(t)$

Let us calculate the derivative of the reaction product from equation (3) :

$$\frac{dQ}{dt} = \mu Q - DQ + k_3 X\frac{d\mu}{dt} \tag{12}$$

Therefore, if we assume that Q is available for measurement, the microbial growth rate $\mu(t)$ can be estimated via the following observer equation of the reaction product flow rate Q :

$$\begin{aligned}
\frac{d\hat{Q}}{dt} &= \hat{\mu}Q - DQ + C_1(t)(Q-\hat{Q}) \\
\frac{d\hat{\mu}}{dt} &= C_2(t)(Q-\hat{Q})
\end{aligned} \tag{13}$$

where $\hat{Q}$ and $\hat{\mu}$ are estimates of Q and μ, and $C_1(t)$ and $C_2(t)$ are positive.

Let us define the error terms :

$e_Q = Q - \hat{Q}$

$e_\mu = \mu - \hat{\mu}$

The error system is deduced from equations (12), (13) :

$$\frac{d}{dt}\begin{bmatrix} e_Q \\ e_\mu \end{bmatrix} = \begin{bmatrix} -C_1(t) & Q \\ -C_2(t) & 0 \end{bmatrix}\begin{bmatrix} e_Q \\ e_\mu \end{bmatrix} + \begin{bmatrix} k_3 X \\ 1 \end{bmatrix}\frac{d\mu}{dt} \qquad (14)$$

Let us introduce the following assumption in order to emphasize stability properties of the estimation algorithm (13) :

A.5. Q(t) is strictly positive :
$Q(t) \geq \epsilon > 0$.

Theorem 3.3.1 : Under assumptions A.2 and A.5 the error vector $\underline{e}^T = [e_Q, \ e_\mu]$ is bounded as follows :

$$\|\underline{e}\| < K_1 \|\underline{e}_o\| + K_2 M$$

with $\underline{e}_o$: initial error vector

K_1, K_2 : constants

Proof : similar to theorem 3.1.1.

Remark 3.3.1

Similarly as in paragraph 3.2, an estimation algorithm of $\rho(t)$ can be implemented by using measurements of Q and S. And the same stability property can be deduced.

3.3.2. Liquid reaction product : $P = k_3\mu X + k_4 X - DP$

The estimation procedure is somewhat different from what has been done above. The estimate of the microbial growth-rate μ is deduced from two independent estimation algorithms of the biomass concentration X and of the "biomass activity" μX.

We assume that :

a) The substrate concentration S and the product concentration P are available for direct measurement.
b) The value of yield parameters k_1, k_2, k_3, k_4 are known. (They may have determined from a batch experiment, for instance).

a. Estimation of X

We define

$$Z = \frac{1}{2} k_1 X + \frac{1}{2}\frac{k_1}{k_3} P + S \qquad (15)$$

We calculate the derivative of Z, by using equations (1) (2) (4)

$$\frac{dZ}{dt} = DS_{in} - DZ + (\frac{1}{2}\frac{k_1 k_4}{k_3} - k_2)X \qquad (16)$$

By noting that $X = \frac{2}{k_1}(Z - \frac{1}{2}\frac{k_1}{k_3} P - S)$, equation (16) becomes :

$$\frac{dZ}{dt} = DS_{in} - (D - \left[\frac{k_4}{k_3} - 2\frac{k_2}{k_1}\right])Z + (\frac{k_4}{k_3} - 2\frac{k_2}{k_1})(S - \frac{1}{2}\frac{k_1}{k_3} P) \qquad (17)$$

Therefore, we can write the following estimation equation for Z :

$$\frac{d\hat{Z}}{dt} = DS_{in} - (D - \left[\frac{k_4}{k_3} - 2\frac{k_2}{k_1}\right])\hat{Z} + (\frac{k_4}{k_3} - 2\frac{k_2}{k_1})(S - \frac{1}{2}\frac{k_1}{k_3} P) \qquad (18)$$

And the estimate of X follows :

$$\hat{X} = \frac{2}{k_1}[\hat{Z} - \frac{1}{2}\frac{k_1}{k_3} P - S] \qquad (19)$$

Lemma 3.3.1. : If D(t) is such that :

$$D(t) > \frac{k_4}{k_3} - 2\frac{k_2}{k_1} \quad \text{for all } t$$

Then $\hat{X}$ converges exponentially to X.

Proof : can be found in Bastin and Dochain, 1985.

Remark 3.3.2: it is worth noting that the speed of convergence of the estimation algorithm (18) (19) only depends on the value of D(t), i.e. there is no parameter which could be calibrated as in previous sections. Therefore, the speed of convergence strongly depends on the experimental conditions.

b. Estimation of $k_1\mu X + k_2 X$

Let us define :

$$\varphi = k_1\mu X + k_2 X \qquad (20)$$

The estimate of φ is computed via an observer equation of the substrate concentration S :

$$\frac{d\hat{S}}{dt} = -\hat{\varphi} + C_1(S - \hat{S}) + D(S_{in} - S)$$
$$\frac{d\hat{\varphi}}{dt} = -C_2(S - \hat{S}) \qquad (21)$$

Let us define the error terms e_s and e_φ :

$$e_s = S - \hat{S}$$
$$e_\varphi = \varphi - \hat{\varphi} \qquad (22)$$

We introduce the following assumption :

A.6 $\frac{d\varphi}{dt}$ is bounded : $|\frac{d\varphi}{dt}| < M$

Lemma 3.3.2 : Under assumption A.6 and if C_1 and C_2 are chosen as

$$C_2 = \frac{\alpha}{4} C_1^2 \ , \ 0 < \alpha < 1$$

then, $\lim_{t\to\infty} \|\underline{e}(t)\| = \delta < \frac{M(1+\alpha_1 C_1)}{\epsilon\, \alpha_2\, C_1^2}$

with $\underline{e}^T = [e_s, e_\varphi]$

Proof : can be found in Bastin and Dochain, 1985.

Remark 3.3.3 : Since X(t) is bounded, the boundedness of $d\varphi/dt$ derives from the boundedness of $d\mu/dt$.

c. Estimation of μ

The estimate of μ can be calculated from a. and b. :

$$\hat{\mu} = (\hat{\varphi} - k_2\hat{X})/(k_1\hat{X}) \qquad (23)$$

provided that $\hat{X}$ is different from zero.

The convergence properties of the estimation of the microbial specific growth-rate μ result from those of the estimation algorithms of X and φ.

Remark 3.3.4 : when $k_4 = 0$, equation (4) becomes :

$$dP/dt = k_3\mu X - DP$$

Consider now the case when not only the product concentration P but also its derivative dP/dt are available for measurement.

Then, if we define :

$$Q = dP/dt + DP$$

equation (4) is equivalent to equation (3), and the estimation algorithm (13) can be applied to the liquid reaction product case.

4. ESTIMATION OF THE SPECIFIC MICROBIAL GROWTH RATE $\mu(t)$: PRACTICAL RESULTS.

In this section, real-life estimation results are shown, which illustrate two different estimation algorithms presented above.

4.1. Estimation of $\mu(t)$ from biomass concentration measurements.

The process is a H_2-producing fermentation process by Rhodopseudomonas capsulatas micro-organisms. The biomass concentration is measured online via optical sensors, and data are available every hour (C. Vialas, 1984 ; C. Vialas and A. Cheruy, 1985).

A discretized version(Euler discretization scheme) of the algorithm (5) (6) has been implemented on a sequence of 35 hours experiment :

$$\begin{cases} \hat{X}_{t+1} = \hat{X}_t + \hat{\mu}_t T X_t - D_t T X_t + \bar{C}_1 T X_t (X_t - \hat{X}_t) \\ \hat{\mu}_{t+1} = \hat{\mu}_t + \bar{C}_2 T (X_t - \hat{X}_t) \end{cases}$$

where T is the sampling period (T = 1 hour) and t is the time index.

The process has been started with the following operating conditions :

$$D = 0.055\ h^{-1} \qquad S_{in} = 5\ mM$$

Fig. 1 shows the results of the estimation of $\mu(t)$. The parameters $\bar{C}_1$ and $\bar{C}_2$ were set to the following values :

$$\bar{C}_1 = 0.98 \qquad \bar{C}_2 = 0.24$$

The initial value of $\hat{X}_t$ was set to 0.5, while two different initial conditions for the specific growth rate estimate has been tested ($\hat{\mu}_o = 0.055$, $\hat{\mu}_o = 0.11$). Notice that the influence of the initial conditions has disappeared after 15 estimation steps.

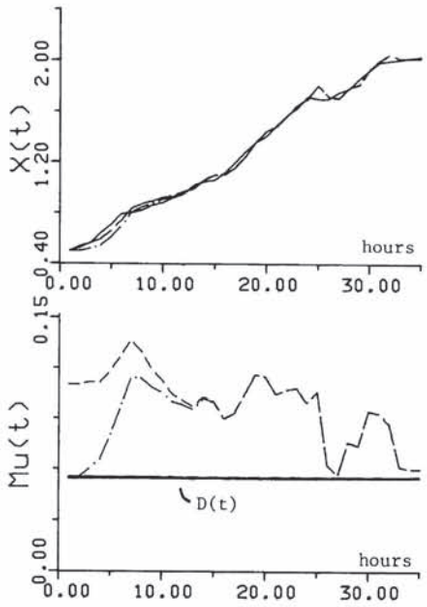

fig. 1. On-line estimation of $\mu(t)$ of a H_2-producing fermentation process. Dotted lines represent the estimates.

4.2. Estimation of $\mu(t)$ from gaseous reaction product measurements.

Here, the estimation algorithm (13) has been implemented on an anaerobic digestion process, where methane gas is produced by methanogenic bacteria (Dochain D. and G. Bastin, 1984).

Methane gas production rate is measured on-line and measurements are available every hour.

Estimation has been carried out over a period of 14 days . The operating conditions were the following ones : the dilution rate D was set to 0.1 day^{-1}, while an inlet substrate concentration step (from 10 gDCO/l.day to 20 gDCO/l.day) was applied to the process on the third day.

The discretized equations of the estimation algorithm (13) can be written as follows :

$$\begin{cases} \hat{Q}_{t+1} = \hat{Q}_t + \hat{\mu}_t T Q_t - D_t T Q_t + \bar{C}_1 T Q_t (Q_t - \hat{Q}_t) \\ \hat{\mu}_{t+1} = \hat{\mu}_t + \bar{C}_2 T Q_t (Q_t - \hat{Q}_t) \end{cases}$$

Fig. 2 shows the evolution of both estimates $\hat{Q}$ and $\hat{\mu}$. The sampling period T = 1 hour. And the following parameter and initial variables values were used :

$\bar{C}_1 = 10 \quad \bar{C}_2 = 10 \quad \hat{\mu}_o = 0.1 \quad \hat{Q}_o = 0.8$

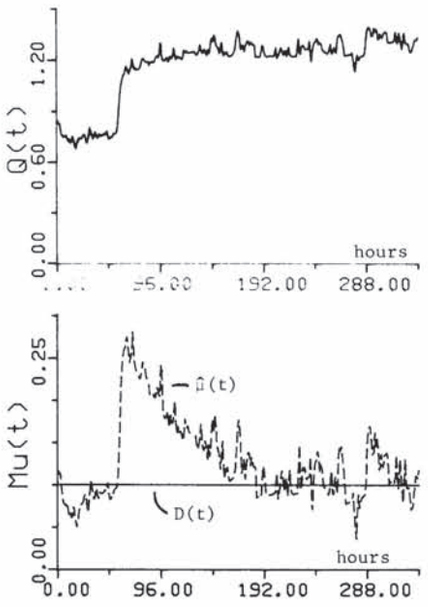

fig. 2. On-line estimation of $\mu(t)$ of an anaerobic digestion process.

5. CONCLUSIONS.

This paper has dealt with the problem of designing estimation schemes for the specific growth-rate of fermentation processes, when it is considered as a time varying unknown parameter.

Continuous-time estimation algorithms of the specific growth-rate $\mu(t)$ have been proposed, depending on which variables are available from measurement. If the biomass concentration X(t) is available for direct measurement, estimation schemes have been implemented, which are independent of the type of reaction product. If the biomass concentration X(t) cannot be measured on-line, different estimation algorithms have been proposed, depending on the input-output configuration.

Stability and convergence properties of the estimation algorithms have been analyzed. It has been shown that, for each estimation scheme the error vector is bounded, and that the bound is proportional to the bound of $d\mu(t)/dt$. Moreover, for three of the algorithms, we have shown that the estimation error is asymptotically smaller to a bound that can be made arbitrarily small by a proper choice of the design parameters.

It is also worth noting that the proposed algorithms can be coupled, if such an information is desired, with on-line adaptive observers of the state variable (like X or S) or with adaptive controllers (Dochain and Bastin, 1984 and 1985).

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