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Brief Paper

On-line Estimation of Microbial Specific Growth Rates*

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Key Words—Fermentation processes; time varying systems; parameter estimation.

Abstract—Continuous time algorithms for the on-line estimation of microbial specific growth rates of fermentation processes are proposed. An important feature of the proposed algorithm is that they do not require any kind of analytical description of the specific growth rate which is simply considered as an unknown bounded time varying parameter. Four different input–output configurations are considered. In each case, the stability and convergence properties of the algorithms are described and their feasibility is illustrated by real life experiments.

1. *Introduction*

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

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

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

with $X(t)$ the biomass concentration
$S(t)$ the limiting substrate concentration
$S_{in}(t)$ the inlet substrate concentration
$D(t)$ the dilution rate
$\mu(t)$ the specific growth rate
k_1 the yield coefficient.

The growth of microorganisms in bioreactors is often accompanied by the formation of synthesis products, either soluble in the culture or given off in gaseous form.

When the formation of products is "growth-associated" (Bailey and Ollis, 1977), the production rate per unit of volume is written as

$$Q(t) = k_2\mu(t)X(t), \tag{3}$$

where k_2 is a yield coefficient. A typical example is the anaerobic fermentation process where $Q(t)$ is a methane gas flow rate (e.g. Andrews, 1969).

In the case of a liquid reaction product, the mass balance in the bioreactor leads to the dynamical equation:

$$\frac{dP(t)}{dt} = Q(t) - D(t)P(t) \tag{4}$$

or

$$\frac{dP(t)}{dt} = k_2\mu(t)X(t) - D(t)P(t) \tag{5}$$

with $P(t)$ the reaction product concentration. A typical example is alcohol fermentation (e.g. Luedeking, 1967).

It is clear from (1)–(5) that the specific growth rate $\mu(t)$ is a key parameter for the description of both biomass growth and products formation. This parameter $\mu(t)$ is known to be a complex function of many physico-chemical and biological factors like the biomass concentration X, the substrate concentration S, the product concentration P, the pH, the temperature, and various other inhibitors.

Many different analytical laws have been suggested for modelling $\mu(t)$. The most popular is certainly the "Monod law":

$$\mu(t) = \frac{\mu^* S(t)}{K_m + S(t)}, \tag{6}$$

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, the authors registered more than 50 different expressions of $\mu(t)$ to account for all the factors influencing the microbial growth. Therefore the choice of an appropriate analytical description of $\mu(t)$ is critical in using state-space representations like (1)–(5) in specific applications; it is an object of continuing controversy in the literature.

To avoid this choice, $\mu(t)$ can be considered as a time varying parameter estimated in real time. This paper is devoted to the design of adaptive algorithms for the tracking of the specific growth rate $\mu(t)$ from input–output data.

Obviously, extended Kalman filtering could be used to solve this estimation problem (Stephanopoulos and Ka-Yiu-San, 1984) but this approach leads to complex non-linear algorithms whose stability and convergence properties are difficult to evaluate.

The contribution of this paper is to show that simple algorithms for the tracking of $\mu(t)$ can be proved to be stable, to analyse their asymptotic convergence properties and to illustrate their feasibility by real life experiments.

The problem of on-line estimation of specific growth rate parameters has been previously considered by Aborhey and Williamson (1978): they assume that $\mu(t)$ obeys the Monod law (6) and they propose a stable algorithm for the on-line estimation of the constant parameters μ^* and K_m from *noise free* measurements of *both* biomass concentration $X(t)$ and substrate concentration $S(t)$. Here algorithms for the estimation of a completely unknown time varying parameter $\mu(t)$, are described.

Furthermore, it is assumed that only *noisy* measurements of *one* state variable are available. Different estimation algorithms are presented depending on which variable is measured (Section 3: measurements of X, Section 4: measurements of S, Section 5: measurements of P, Section 6: measurements of Q). The real life experiments are described in Section 7, while the basic

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assumptions for the derivation and the stability analysis of the algorithms are stated in Section 2.

2. *Basic assumptions*

The analysis of the algorithms presented in the next sections will be based on the following *mild* and *realistic* assumptions:

(A1) The specific growth rate $\mu(t)$ is positive and bounded (the maximum growth rate μ^* is unknown):

$$0 \leqslant \mu(t) \leqslant \mu^*. \tag{7}$$

(A2) The inputs $D(t)$ (dilution rate) and $S_{in}(t)$ (influent substrate concentration) are positive and bounded:

$$D(t) \leqslant D_{max}, \quad S_{in}(t) \leqslant S_{max}. \tag{8}$$

(A3) There is no growth without substrate:

$$\mu(t) = 0 \text{ when } S(t) = 0. \tag{9}$$

(A4) The time derivative of $\mu(t)$ is bounded:

$$\left|\frac{d\mu(t)}{dt}\right| \leqslant M_1. \tag{10}$$

Under these assumptions, and provided $k_1X(0) + S(0) \leqslant S_{max}$, it can be shown (Dochain and Bastin, 1984) that $X(t)$, $S(t)$ and $Q(t)$ are bounded as follows for all t:

$$0 \leqslant S(t) \leqslant S_{max} \tag{11}$$

$$0 \leqslant X(t) \leqslant \frac{S_{max}}{k_1} \triangleq X_{max} \tag{12}$$

$$0 \leqslant Q(t) \leqslant \frac{k_1}{k_2}\mu^* S_{max} \triangleq Q_{max}. \tag{13}$$

In addition to these basic assumptions, throughout the paper, the inputs $D(t)$ and $S_{in}(t)$ are assumed known (either by measurement or by a choice of the user) and the yield coefficients k_1 and k_2 are unknown (and therefore cannot be used in the algorithms).

3. *On-line estimation of* μ(t) *from noisy measurements* X(t)

Statement of the algorithm. Assume that a noisy measurement $X_m(t)$ of the biomass concentration $X(t)$ is available on line:

$$X_m(t) = X(t) + \varepsilon(t) \tag{14}$$

with $\varepsilon(t)$ the measurement noise. Then the following algorithm can be used to estimate $\mu(t)$:

$$\frac{d\hat{X}(t)}{dt} = [\hat{\mu}(t) - D(t) + c_1\{X_m(t) - \hat{X}(t)\}]X_m(t) \tag{15a}$$

$$\frac{d\hat{\mu}(t)}{dt} = c_2 X_m(t)[X_m(t) - \hat{X}(t)] \tag{15b}$$

with $c_1 > 0$, $c_2 > 0$ design parameters.

Stability analysis. Define the estimation errors as

$$\tilde{X}(t) = X(t) - \hat{X}(t) \tag{16a}$$

$$\tilde{\mu}(t) = \mu(t) - \hat{\mu}(t). \tag{16b}$$

Assuming that:

(B1) the measurement noise is bounded:

$$|\varepsilon(t)| \leqslant M_2; \tag{17}$$

(B2) the biomass concentration measurement $X_m(t)$ is strictly positive:

$$X_m(t) \geqslant \eta > 0; \tag{18}$$

the following stability and convergence properties can be established (Dochain, 1986).

Theorem 1. Under assumptions (A1)–(A4), (B1) and (B2), the algorithm (16a, b) is globally stable (i.e. the errors $\tilde{X}(t)$ and $\tilde{\mu}(t)$ are bounded for all t).

This theorem shows that the estimation error $\tilde{\mu}(t)$ is bounded, provided the design parameters c_1 and c_2 are taken positive. But it does not provide any useful information on the accuracy of the estimation of the specific growth rate $\mu(t)$. Actually, for a slightly restricted choice of c_1 and c_2, an upper bound of the asymptotic accuracy of $\tilde{\mu}(t)$ can be calculated explicitly.

Assuming that:

(B3) the design parameters c_1 and c_2 are chosen such that A has real distinct eigenvalues

gives the following convergence result.

Theorem 2. Under assumptions (A1)–(A4) and (B1)–(B3), the error $\tilde{\mu}(t)$ is asymptotically bounded as follows.

$$\limsup_{t\to\infty} |\tilde{\mu}(t)| = \frac{1}{\eta}\left\{B_1M_2 + \frac{c_1}{c_2}(M_1 + c_2B_2M_2)\right\} \tag{19}$$

with $B_2 \triangleq X_{max} + M_2$ and $B_1 \triangleq \max[D_{max}, (\mu^* + c_1B_2)]$.

Simulation results. In the previous paragraph, the global stability and the asymptotic accuracy of the estimation error $\tilde{\mu}(t)$ was analysed. To complete this analysis, the following simulation illustrates the transient behaviour of the algorithm. Consider a "true" fermentation process given by equations (1), (2) with a Monod specific growth rate (6), and with the following parameter and initial values:

$$\mu^* = 0.4 \quad K_m = 0.4 \quad k_1 = 25$$
$$S(0) = 0.893 \quad X(0) = 2.054$$
$$\hat{\mu}(0) = 0.06 \quad \hat{X}(0) = X(0).$$

Figure 1 shows an experiment with a constant dilution rate $D = 0.05$, a square wave input $S_{in}(t)$, an additional output white noise ($\sigma = 0.2$) and design parameters $c_1 = 1$, $c_2 = 0.24$.

4. *On-line estimation of* μ(t) *from noisy measurements of* S(t)

Statement of the algorithm. Assume that a noisy measurement $S_m(t)$ of the substrate concentration $S(t)$ is available on line:

$$S_m(t) = S(t) + \varepsilon(t). \tag{20}$$

The basic idea for the derivation of the estimation algorithm is that, in microbial growth, the rate of biomass production is proportional to the rate of substrate consumption. Therefore, in a first stage, the on-line measurements of $S(t)$ can be exploited to provide "pseudo" measurements of $k_1X(t)$, without any knowledge of $\mu(t)$ being necessary (Williamson, 1977). Then, in a second stage, this pseudo measurement is used to estimate $\mu(t)$ by an algorithm analogous to that of Section 3.

Let the auxiliary state variable $Z(t)$ be defined by:

$$\frac{dZ(t)}{dt} = D(t)[S_{in}(t) - Z(t)] \tag{21}$$

with $0 \leqslant Z(t) < \infty$, arbitrary. Clearly, for given $Z(0)$, $Z(t)$ can be computed in real time, in parallel to the process operation, since $D(t)$ and $S_{in}(t)$ are known by assumption (Section 2). Then, an on-line pseudo measurement of $Y(t) \triangleq k_1X(t)$ is given by

$$Y_m(t) = Z(t) - S_m(t). \tag{22}$$

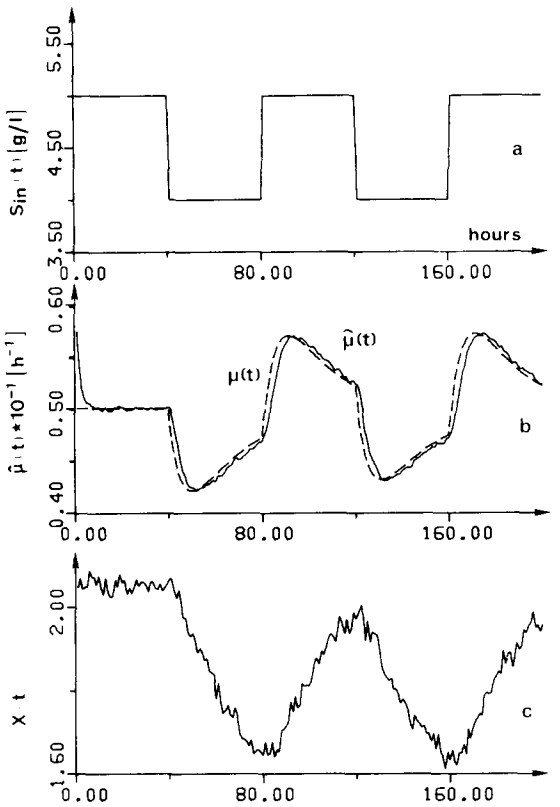

FIG. 1. On-line estimation of $\mu(t)$ from $X(t)$: simulation result.

Finally, the adaptive estimation of $\mu(t)$ is performed by an algorithm similar to (15a, b):

$$\frac{d\hat{Y}(t)}{dt} = \{\hat{\mu}(t) - D(t) + c_1[Y_m(t) - \hat{Y}(t)]\}Y_m(t) \quad (23a)$$

$$\frac{d\hat{\mu}(t)}{dt} = c_2 Y_m(t)[Y_m(t) - \hat{Y}(t)]. \quad (23b)$$

The motivation for the introduction of $Z(t)$ and $Y_m(t)$ is given by the following analysis.

Stability and convergence properties. From (1), (2):

$$\frac{d}{dt}[Y(t) + S(t)] = D(t)[S_{in}(t) - (Y(t) + S(t))]. \quad (24)$$

Then, comparing with (21), $Z(t)$ can obviously be considered as an on-line estimate of $Y(t) + S(t)$, with an estimation error

$$\omega(t) = Z(t) - [Y(t) + S(t)] \quad (25)$$

governed by the stable dynamical equation

$$\frac{d\omega(t)}{dt} = -D(t)\omega(t), \quad \omega(0) = Z(0) - Y(0) - S(0). \quad (26)$$

The expression (22) for $Y_m(t)$ follows readily and can also be written

$$Y_m(t) = Y(t) + \omega(t) - \varepsilon(t) = Y(t) + \bar{\varepsilon}(t). \quad (27)$$

If the measurement noise $\varepsilon(t)$ is bounded, the pseudo measurement noise $\bar{\varepsilon}(t)$ is also bounded:

$$|\bar{\varepsilon}(t)| \leqslant M_2 + |\omega(0)|. \quad (28)$$

The stability and convergence results of Section 3 can then be applied without restriction. Furthermore, if the dilution rate is strictly positive ($D(t) \geqslant \delta > 0$, for all t), it is evident from (26) that $\omega(t)$ converges exponentially to 0 (with rate δ at least). In such a case, the effect of the arbitrary choice of $Z(0)$ vanishes exponentially and the pseudo measurement $Y_m(t)$ becomes corrupted only by the measurement nosie $\varepsilon(t)$.

5. *On-line estimation of* $\mu(t)$ *from noisy measurement of* $P(t)$

Assume that a noisy measurement $P_m(t)$ of the liquid product concentration $P(t)$ is available on line:

$$P_m(t) = P(t) + \varepsilon(t). \quad (29)$$

The derivation of the algorithm is based on the fact that the product formation rate is, by definition, proportional to the biomass growth rate (5). Hence, the derivation closely follows that of the previous section and only essential explanations are included.

An auxiliary state variable $Z(t)$ is defined by:

$$\frac{dZ(t)}{dt} = -D(t)Z(t) \quad 0 < Z(0) < \infty, \text{ arbitrary.} \quad (30)$$

Here, it is obvious that $Z(t)$ can be considered as an estimate of $P(t)$-$Y(t)$, with $Y(t) \triangleq k_2 X(t)$. Then, the pseudo measurement of $Y(t)$ is defined as:

$$Y_m(t) = P_m(t) - Z(t) \quad (31)$$

and the algorithm (23a, b) is used for the estimation of $\mu(t)$. If $D(t)$ is strictly positive ($D(t) \geqslant \delta > 0$), then $\lim Z(t) = 0$ when $t \to \infty$ and the pseudo measurement $Y_m(t)$ tends to the actual measurement $P_m(t)$.

6. *On-line estimation of* $\mu(t)$ *from noisy measurements of* $Q(t)$

Assume that a noisy measurement $Q_m(t)$ of the production rate $Q(t)$ is available on line:

$$Q_m(t) = Q(t) + \varepsilon(t). \quad (32)$$

The derivative of (3) can be written

$$\frac{dQ(t)}{dt} = \alpha(t)Q(t) - D(t)Q(t) \quad (33)$$

with

$$\alpha(t) = \mu(t) + \frac{1}{\mu(t)}\frac{d\mu}{dt}. \quad (34)$$

Equation (33) is clearly analogous to the biomass growth equation (1). Therefore an algorithm similar to (15a, b) can be used to estimate $\alpha(t)$:

$$\frac{d\hat{Q}(t)}{dt} = \{\hat{\alpha}(t) - D(t) + c_1[Q_m(t) - \hat{Q}(t)]\}Q_m(t) \quad (35a)$$

$$\frac{d\hat{\alpha}(t)}{dt} = c_2 Q_m(t)[Q_m(t) - \hat{Q}(t)], \quad (35b)$$

while an on-line estimate of $\mu(t)$ readily derives from (34):

$$\frac{d\hat{\mu}}{dt} = -\hat{\mu}(t)[\hat{\mu}(t) - \hat{\alpha}(t)]. \quad (36)$$

The stability and convergence properties of algorithm (35a, b) follow from Theorems 1 and 2 (provided $\mu(t) > 0$ for all t). On the other hand, it is evident that (36) is globally stable provided $\hat{\mu}(0) > 0$.

7. *Real-life applications*

In this section, three applications on data from real life bioreactors are presented. In these applications, the estimation algorithms have been implemented numerically by simply using Euler discretization.

Estimation of μ(t) *from biomass measurements* X(t). The process is a continuous fermentation of lactoserum by *Rhodopseunomonas capsulata* microorganisms, producing hydrogen (H_2). the biomass concentration was measured on line via optical sensors with a sampling period of 1 h. the data were kindly provided by C. Vialas (1984) from the LAG.

The experiment under interest (Fig. 2) is a start-up of the reactor, with constant inputs

$$D = 0.055\,\mathrm{h}^{-1} \quad S_{in} = 5\,\mathrm{mM}$$

and the design parameters are set to

$$c_1 = 1.0 \quad c_2 = 0.24.$$

The initial value of X is 0.5 while two different initial conditions of $\hat{\mu}$ are tried (0.055 and 0.11). Figure 2b shows that the initial conditions effect vanish after 15 h.

As a matter of validation, Fig. 2c shows an on-line estimation of the substrate concentration, based on the on-line estimate $\hat{\mu}(t)$ and given by the following expression (which derives readily from (2)):

$$\frac{d\hat{S}(t)}{dt} = -k_1\hat{\mu}(t)X(t) - D(t)[S_{in}(t) - \hat{S}(t)] \qquad (37)$$

with a value $k_1 = 2.403$ obtained from an off-line identification study (Vialas, 1984).

One can observe the very good agreement between this on-line estimate $\hat{S}(t)$ and a few measurements obtained by off-line chemical analysis which are also indicated in the figure.

On the choice of the design parameters c_1 *and* c_2. In these real-life applications, this choice is made empirically after a set of simulations of a process model which is presumed to behave approximately as the "true" system.

This strategy is well illustrated by the foregoing application.

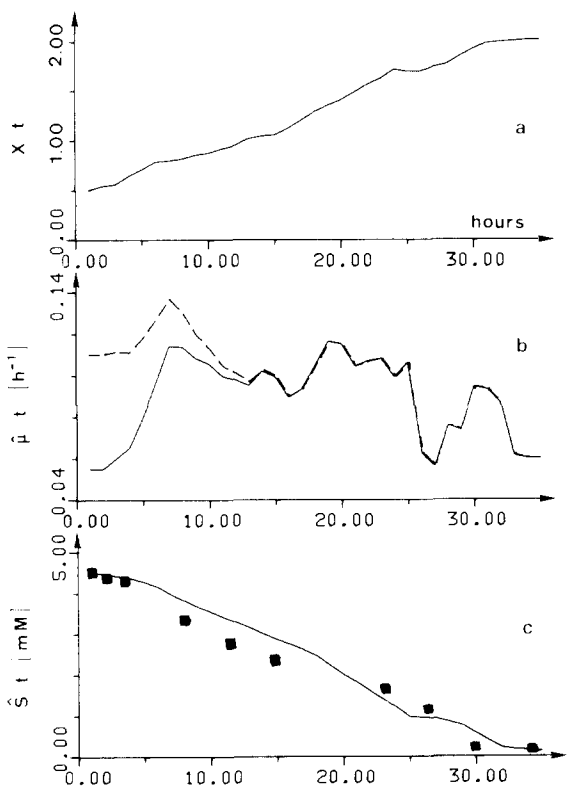

FIG. 2. On-line estimation of $\mu(t)$ and $S(t)$ from $X(t)$: real life result.

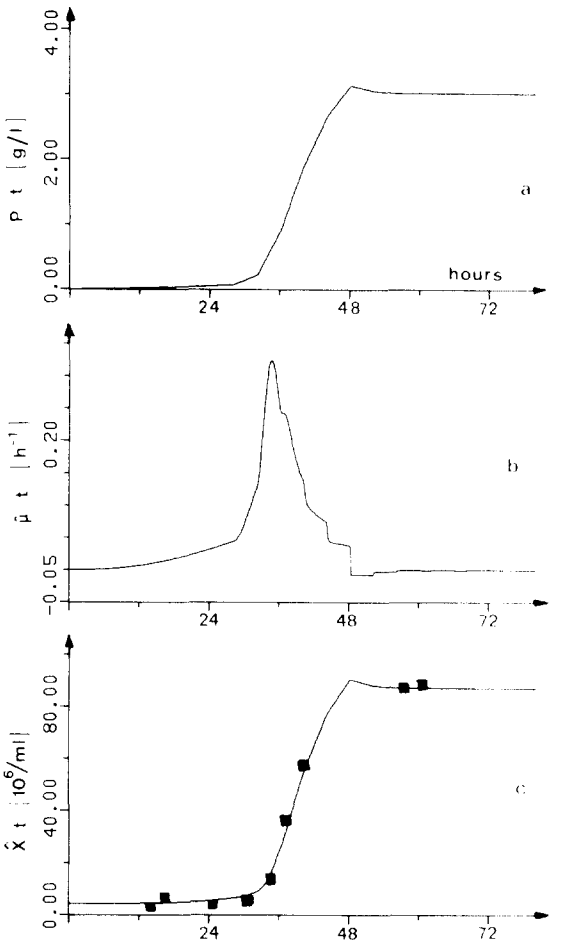

FIG. 3. On-line estimation of $\mu(t)$ from $P(t)$: real life result.

Indeed, the same dilution rate, the same inlet substrate concentration, the same sampling period and the same design parameters c_1 and c_2 are used in the simulation (Fig. 1) and in the real-life experiment (Fig. 2), clearly leading to satisfactory results in both cases. The choice of c_1 and c_2 can also be validated from off-line additional measurements (as in Fig. 2c): this will be illustrated further in the next application.

If the bounds M_1 and M_2 are known to the user from prior knowledge on the process, the asymptotic bound on $\tilde{\mu}$ (19) could be a useful tool for an optimal choice of the design parameters: a detailed discussion can be found in Dochain (1986).

Estimation of μ(t) *from liquid product concentration measurements.* The process is a batch anaerobic non-sterile fermentation of orange juice by yeasts, producing ethanol. The ethanol concentration (i.e. $P(t)$) is measured with a sampling period of 10 min. The data were provided by A. Pauss (1986) from the Unit of Bioengineering (University of Louvain).

The experiment is conducted under the conditions:

$$Z(0) = 0 \qquad \hat{\mu}(0) = 0 \qquad \hat{Y}(0) = 0$$
$$c_1 = 3.80 \qquad c_2 = 3.80.$$

In this application, the design parameters c_1 and c_2 have been calibrated from the nine off-line measurements of the yeast concentration (plate counting). They are chosen such that the estimate of X,

$$\frac{d\hat{X}}{dt} = \hat{\mu}\hat{X},$$

fits the off-line data as well as possible (Fig. 3c).

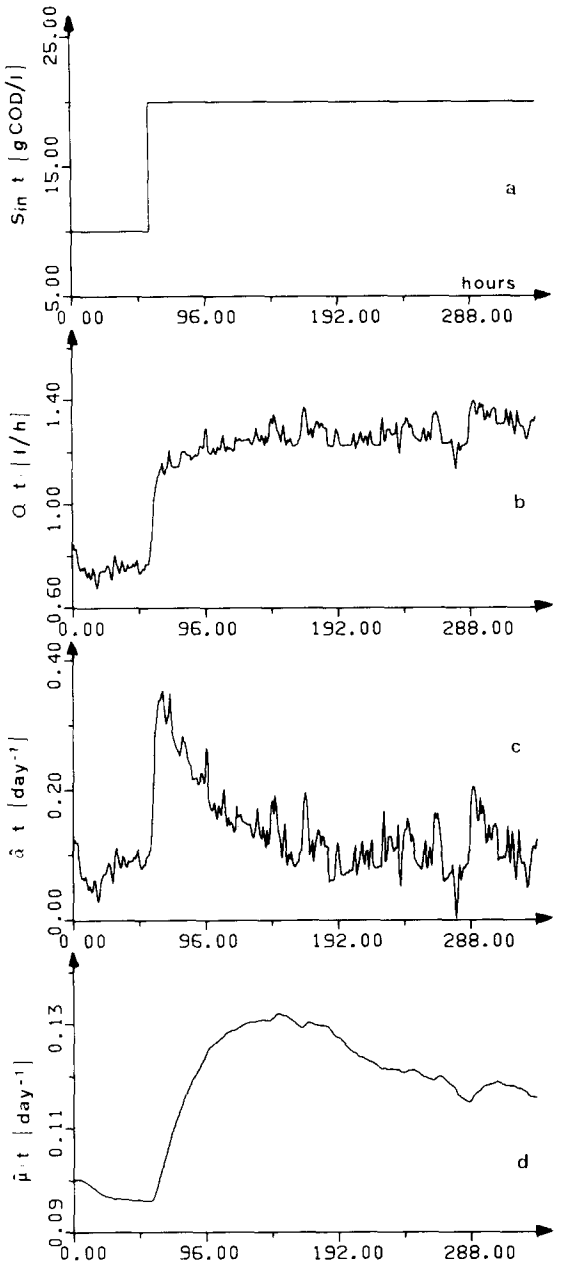

FIG. 4. On-line estimation of $\mu(t)$ from $Q(t)$: real life result.

The result, given in Fig. 3, clearly shows a realistic behaviour of the growth rate estimate $\hat{\mu}$.

Estimation of $\mu(t)$ *from production rate measurements* $Q(t)$. The process is an anaerobic digestion pilot plant, with methane gas production (Bastin *et al.*, 1983). The methane gas flow rate (which is here the production rate $Q(t)$) is measured on line through a gas meter, with a sampling period of 1 h.

The estimation experiment was carried out over a period of 14 days. The operating conditions were a constant dilution rate $D = 0.1\ \text{day}^{-1}$ and a step of inlet substance concentration (from 10 to $20\ \text{g COD l}^{-1}\ \text{day}^{-1}$). The following design parameters and initial conditions were used.

$$c_1 = 15,\quad c_2 = 20,\quad \hat{x}(0) = \hat{\mu}(0) = 0.1\ \text{day}^{-1},\quad \hat{Q}(0) = 0.8\ \text{day}^{-1}.$$

Figure 4 shows the evolution of the estimates $\alpha(t)$, $\mu(t)$ and $Q(t)$ during the experiment.

8. *Conclusions*

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

Continuous time adaptive algorithms for the estimation of $\mu(t)$ have been proposed, depending on which variables are available from measurements. The stability and convergence properties of the algorithms have been analysed. An analytical expression of the relationship between the design parameters and the asymptotic bound on the estimation error has been calculated.

It is also worth noting that the proposed algorithms can be coupled, if desired, with adaptive observers of the other state variables (Dochain and Bastin, 1985) or with adaptive regulators (Dochain and Bastin, 1984, 1985; Bastin and Dochain, 1985).

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