Model Parameter Tracking in Microbial Growth Processes

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Introduction

The general dynamical model of a microbial growth process is described by a set of mass balances, where the rates of change of state variables are expressed as functions of operating conditions and model parameters. These are coupled with one or more kinetic models for specific growth rates. A knowledge of the physically meaningful parameters of a culture is essential for model building and verification. The application of modern control theory to biotechnology requires accurate estimation schemes, because relatively small deviations in parameters may indicate process disturbances or faults. Identification of growth models is known to be a difficult task due to the empirical nature of the models. The estimation of a unique set of physically meaningful parameters may not be always feasible (Nihtilä and Virkkunen, 1977; Holmberg, 1982; Stephanopoulos and San, 1984). Parameters in bioprocesses seldom remain constant; variability is induced by the sensitivity and selectivity exhibited by microorganisms to their environment. It has been shown that the maximum specific growth rate and half saturation constant in the Monod law exhibit strong temperature dependency (Topiwala and Sinclair, 1971).

Many different techniques are in use for on-line parameter estimation of continuous-culture processes; adaptive algorithms of the recursive least-squares (RLS) method are often used to track time-varying parameters (Aborhey and Williamson, 1978; Holmberg and Ranta, 1982). Golden and Ydstie (1989) reported Monod parameter estimation by RLS with forgetting factors. The RLS algorithms suggested in the literature are adaptations of schemes developed for stationary systems, and they fare poorly when used for monitoring parameters that drift with time, besides being sensitive to noise (Holmberg and Ranta, 1982).

In general, the functionality of a specific growth rate with respect to state variables and operating conditions is inadequately known. The available analytical expressions are limited in scope because they are often system-specific and extensions to wider operating regions may not be feasible (Stephanopoulos and San, 1984). Several researchers have suggested model-independent estimation of specific growth rate as a time-varying parameter. The extended Kalman filter (EKF) is widely applied to this effect (Stephanopoulos and San, 1984; Shimizu et al., 1989). The EKF is derived from the application of a Kalman filter to the linearized model of a nonlinear system. In microbial growth systems, which are characterized by strong nonlinearities, these algorithms often produce biased estimates (Zhou and Cluett, 1996). It is also extremely difficult to evaluate the stability and convergence properties of EKF algorithms over wide ranges of operation. Bastin and Dochain (1986) developed an adaptive observer to estimate the unmeasurable states and specific growth rate on-line. The approach, though simple in structure, is known to be difficult to tune (Oliveira et al., 1996). Lubenova (1996) suggested an adaptive algorithm for specific growth rate from oxygen uptake rate (OUR) data and their derivative.

The modulating-functions method belongs to a class of estimation methods for continuous-time systems, which apply an integral transform over a finite period of time to convert the original differential equation into a set of algebraic equations. It is particularly appealing for parameter estimation in microbial growth processes. The original nonlinear model is used without recourse to linearization, noisy data are directly processed, and it is not necessary to estimate initial conditions. Although several versions have been developed in the last two decades, applications of these methods to chemical and biochemical processes are almost nonexistent (Preisig and Rippin, 1993). To demonstrate the efficacy of the method, we present the results of on-line identification of a microbial growth model with the recursive modulating functions method developed earlier (Co and Ungarala, 1997). The computationally efficient algorithm is included here in a summarized form. The saprotrophy model for protozoan feeding on bacteria (Sambanis et al., 1987) is used for this simulation study. The set of four coupled differential equations contains three specific growth rates, two yield coefficients, and two death/maintenance coefficients. The seven parameters are estimated as time-varying parameters independent of any

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growth kinetic expressions. The internal parameters of models of specific growth rates, like the Monod law, are estimated in a subsequent step from the estimates of the specific growth rates obtained previously.

Protozoan–Bacterial Interactions

In mixed cultures the organisms influence one another, resulting in complex interactions like competition, predation, and parasitism. The predator–prey interaction is a common phenomenon in nature, where one species (prey) grows independently on the substrate and is simultaneously consumed by the other (predator). Consumption of bacteria by bacterivorous protozoa is a common example and an important process both ecologically and industrially (Fredrickson, 1983). Mixed microbial populations are commonly employed in industrial and biological waste treatment plants. In aquatic systems, protozoan predators feed on bacterial prey, establishing one of the first trophic levels in aquatic food webs. The saprotrophy model of protozoan–bacterial interactions accounts for the release of substances by protozoa that can support bacterial growth (Sambanis et al., 1987). These substances, produced by the death and lysis of protozoan cells, are termed autochthonous substrates to differentiate them from the nutrients fed to the culture, which are called allochthonous substrates. It has been shown that accounting for the growth of bacteria on autochthonous substrates aids as a stabilizing factor for models of protozoa–bacteria coexistence.

The saprotrophy-model equations for a continuous culture in a single-stage chemostat with sterile nutrient feed are written as follows:

$$\frac{dp}{dt} = -Dp + (Xn - \nu_d)p \tag{1a}$$

$$\frac{db}{dt} = -Db + (m + m')b - np \tag{1b}$$

$$\frac{ds}{dt} = -Ds - \frac{m}{Y}b + a\nu_d p \tag{1c}$$

$$\frac{ds'}{dt} = D(s'_f - s') - \frac{m'}{Y}b, \tag{1d}$$

where p is protozoan biovolume; b is bacterial density; s and s' are concentrations of autochthonous and allochthonous substrates, respectively; m and m' are specific growth rates of bacteria on autochthonous and allochthonous substrates, respectively; n is the specific growth rate of protozoa on bacteria; X is the yield coefficient for protozoa, and the yield coefficient, Y, is assumed to be the same for both substrates; ν_d is the rate of loss of protozoan biovolume due to death and lysis; a is a constant for the production of autochthonous substrate; D is the dilution rate; and s'_f is the concentration of allochthonous substrate in the nutrient feed.

The model is combined with algebraic relations, like the Monod law, for the three specific growth rates. Depending on the operating conditions, the model predicts coexistence of protozoa and bacteria in steady states or sustained oscillations of population densities. The saprotrophy model serves as an excellent example to illustrate the modulating-functions method. It is a more complicated system than a single-organism fermentation model, it is highly nonlinear, contains several time-varying growth rates, and offers persistent excitation of signals when oscillations are present. However, the oscillations tend to depend strongly on initial conditions and, for certain experimentally reasonable conditions, the saprotrophy model fails to predict the coexistence of the species, both of which are contrary to experimental observations. Along with the experimentally verified saprotrophy mechanism (Canale et al., 1973; Habte and Alexander, 1978; Sambanis et al., 1987), other processes may be simultaneously occurring. So far, saprotrophy is the most widely accepted model for the persistence of bacterial growth in the presence of protozoan predation.

Recursive Modulating-Functions Method

The modulating-functions method can be used to estimate the parameters of a general nonlinear differential equation, expressed in the following affine structure:

$$\sum_{j=1}^{p} \frac{d^{k_j}}{dt^{k_j}} \zeta_j(t,y,u) = \sum_{j=1}^{q} \alpha_j \frac{d^{k_j}}{dt^{k_j}} \psi_j(t,y,u), \tag{2}$$

where α_j are the parameters to be estimated; and ζ_j and ψ_j are nonlinear functions of input and output in time. Co and Zhong (1997) have recently extended the method to differential equations containing parameters in transcendental forms by using Lie symmetry transformations to obtain affinity in parameters.

The affine equation is modulated by taking the inner product with a modulating function, $\phi(t)$, over a batch of time-series data. The following adjoint relation shifts the differentiation operation from the time-series data, $f(t)$, on to the modulating function, ϕ,

$$\int_0^T \phi \frac{d^j f}{dt^j} dt = (-1)^j \int_0^T f \frac{d^j \phi}{dt^j} dt, \tag{3}$$

and the approximation of derivatives from noisy data is avoided. The modulating functions, ϕ, are chosen to be smooth, n times differentiable, and to satisfy the following end conditions to allow for arbitrary initial values for the data, $f(t)$,

$$\frac{d^j}{dt^j}\phi(0) = 0 = \frac{d^j}{dt^j}\phi(T) \qquad j = 0, 1, \ldots, n-1, \tag{4}$$

where n is the order of the system. Pearson and Lee (1985) chose linear combinations of sinusoidal functions to construct orthogonal sets of modulating functions,

$$\Phi = C(1, \cos\omega t, -\sin\omega t, \ldots, \cos L\omega t, -\sin L\omega t)^t, \tag{5}$$

where L is a filtering parameter and $\omega = 2\pi/T$. The matrix C, of size $r \times (2L+1)$ with $r \geq q$, is formulated to satisfy the end conditions and guarantee the linear independence of the modulating functions. There are several methods for obtaining C, including the one by Pearson and Lee (1985), who

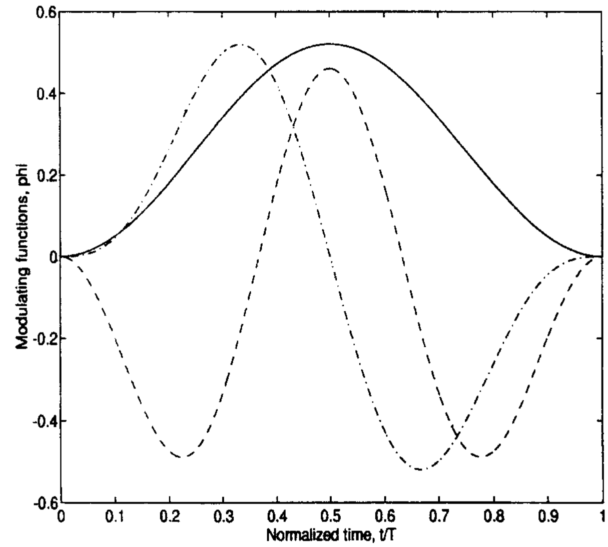

Figure 1. Orthogonal family of modulating functions: Φ for $n = 2$; ϕ_1 (—), ϕ_2 (---), ϕ_3 (-·-).

used Vandermonde matrix identities, and an explicit formulation given in Co and Ungarala (1997). For $L = 2$, $n = 2$, the three modulating functions obtained are shown below (Figure 1),

$$\begin{pmatrix} \phi_1 \\ \phi_2 \\ \phi_3 \end{pmatrix} = \begin{pmatrix} 0.26 & -0.26 & 0 & 0 & 0 \\ -0.12 & -0.23 & 0 & 0.35 & 0 \\ 0 & 0 & -0.4 & 0 & 0.2 \end{pmatrix} \begin{pmatrix} 1 \\ \cos \omega t \\ -\sin \omega t \\ \cos 2\omega t \\ -\sin 2\omega t \end{pmatrix}. \tag{6}$$

Using the Pearson–Lee formulation with trigonometric modulating functions, the estimation problem is now reduced to a linear least-squares problem,

$$C\left(D^{k_1} Z_{\zeta_1}, \ldots, D^{k_p} Z_{\zeta_p}\right) \begin{pmatrix} 1 \\ \vdots \\ 1 \end{pmatrix}_{(p\times 1)} = C\left(D^{k_1} Z_{\psi_1}, \ldots, D^{k_q} Z_{\psi_q}\right) \begin{pmatrix} \alpha_1 \\ \vdots \\ \alpha_q \end{pmatrix}, \tag{7}$$

where

$$Z_f = [C_0(f), C_1(f), S_1(f), \ldots, C_L(f), S_L(f)]^t \tag{8}$$

$$C_m(f) = \int_0^T f(t) \cos m\omega t \, dt \tag{9}$$

$$S_m(f) = -\int_0^T f(t) \sin m\omega t dt \tag{10}$$

$$D = \omega \,\mathrm{diag}(0, \boldsymbol{d}, 2\boldsymbol{d}, \ldots, L\boldsymbol{d}) \qquad \boldsymbol{d} = \begin{pmatrix} 0 & -1 \\ 1 & 0 \end{pmatrix}. \tag{11}$$

Table 1. Model Parameters for the *T. pyriformis* – *E. coli* System

Parameter	Value
μ'	$0.25\ \mathrm{h}^{-1}$
K'	5×10^{-4} mg glucose/mL
Y	3×10^{9} *E. coli* cells/mg glucose or autochthonous substrate
μ	$0.44\ \mathrm{h}^{-1}$
K	5×10^{-5} mg autochthonous substrate/mL
ν	1.8 *E. coli* cells/μm^3 *T. pyriformis* biovolume h
K_p	$\left(7-\frac{0.1}{1+e^{-0.05(t-500)}}\right)\times10^{7}$ *E. coli* cells/mL
X	0.17 μm^3 *T. pyriformis* biovolume/*E. coli* cell
α	5.9×10^{-10} mg autochthonous substrate/μm^3 *T. pyriformis* biovolume
ν_d	$0.022\ \mathrm{h}^{-1}$

The Pearson–Lee method, which uses FFT/DFT methods to evaluate the integrals efficiently, is limited in its application to a batch of time-series data. The method can be extended for on-line estimation by processing one data window of width T at a time, but would require the use of FFT at each sampling instant. Instead, a set of fast and computationally efficient recursion formulas have been developed for a sliding-window estimator by Co and Ungarala (1997). The recursion relations and the estimation algorithm are summarized here. At any sampling time instant, k, the integrals $C_m(f)$ and $S_m(f)$ are evaluated using the trapezoidal rule, that is, $C_{k,m} \approx (\Delta t)\overline{C}_{k,m}(f)$ and $S_{k,m} \approx (\Delta t)\overline{S}_{k,m}(f)$,

$$\overline{C}_{k,m}(f) = \sum_{j=0}^{N-1} f_{(j+K-N)} \cos\left(\frac{2\pi}{N} mj\right) + \frac{1}{2}[f_{(k)} - f_{(k-N)}] \tag{12}$$

$$\overline{S}_{k,m}(f) = -\sum_{j=0}^{N-1} f_{(j+k-N)} \sin\left(\frac{2\pi}{N} mj\right). \tag{13}$$

These summations at k are related to the previous values at $k-1$ by the following matrix equation:

$$\begin{pmatrix} \overline{C}_{k,m}(f) \\ \overline{S}_{k,m}(f) \end{pmatrix} = \begin{pmatrix} c_m & -s_m \\ s_m & c_m \end{pmatrix} \left[\begin{pmatrix} \overline{C}_{k-1,m}(f) \\ \overline{S}_{k-1,m}(f) \end{pmatrix} + \begin{pmatrix} 1 \\ 0 \end{pmatrix} \frac{1}{2} \Delta_N f_{(k-1)} \right] + \begin{pmatrix} 1 \\ 0 \end{pmatrix} \frac{1}{2} \Delta_N f_{(k)}, \tag{14}$$

where, $c_m = \cos(2\pi m/N)$, $s_m = \sin(2\pi m/N)$, $\Delta_N f_{(k)} = f_{(k)} - f_{(k-N)}$. The following recursion formulas are formulated to solve the least-squares problem (Eq. 7) at each sampling instant:

$$G_\zeta(k) = \Theta\left(G_\zeta(k-1) + \frac{1}{2} V_\zeta \Delta_N \zeta_{(k-1)}\right) + \frac{1}{2} V_\zeta \Delta_N \zeta_{(k)} \tag{15}$$

$$G_\psi(k) = \Theta\left(G_\psi(k-1) + \frac{1}{2} V_\psi \Delta_N \psi_{(k-1)}\right) + \frac{1}{2} V_\psi \Delta_N \psi_{(k)}, \tag{16}$$

where

$$G_\zeta(k) = \left[D^{k_1}Z_{\zeta_1}(k), \ldots, D^{k_p}Z_{\zeta_p}(k)\right] \tag{17}$$

$$G_\psi(k) = \left[D^{k_1}Z_{\psi_1}(k), \ldots, D^{k_q}Z_{\psi_q}(k)\right] \tag{18}$$

$$Z_f(k) = [\bar{C}_{k,0}(f), \bar{C}_{k,1}(f), \bar{S}_{k,1}(f), \ldots, \bar{C}_{k,L}(f), \bar{S}_{k,L}(f)]' \tag{19}$$

$$V_\zeta = (D^{k_1}f, \ldots, D^{k_p}f) \tag{20}$$

$$V_\psi = (D^{k_1}f, \ldots, D^{k_q}f)$$

$$f = (1, 1, 0, \ldots, 1, 0)' \text{ of length } 2L+1 \tag{21}$$

$$\Delta_N\zeta(k) = \mathrm{diag}[\Delta_N\zeta_1(k), \ldots, \Delta_N\zeta_p(k)] \tag{22}$$

$$\Delta_N\psi(k) = \mathrm{diag}[\Delta_N\psi_1(k), \ldots, \Delta_N\psi_q(k)] \tag{23}$$

$$\Theta = \mathrm{diag}(1, \theta_1, \ldots, \theta_L)$$

$$\theta_m = \begin{pmatrix} \cos\left(\dfrac{2\pi m}{N}\right) & -\sin\left(\dfrac{2\pi m}{N}\right) \\ \sin\left(\dfrac{2\pi m}{N}\right) & \cos\left(\dfrac{2\pi m}{N}\right) \end{pmatrix}. \tag{24}$$

Estimation Algorithm

1. Initialize the constant matrices C, Θ, V_ζ, V_ψ.
2. Initialize estimation with Pearson–Lee method on a window of N data points.
3. Compute $\Delta_N\zeta(k-1)$, $\Delta_N\zeta(k)$ and $\Delta_N\psi(k-1)$, $\Delta_N\psi(k)$, and update $G_\zeta(k)$ and $G_\psi(k)$.
4. Obtain new estimates $\alpha(k)$ by solving

$$[CG_\zeta(k)]\mathrm{Ones}(p,1) = CG_\psi(k)\alpha(k). \tag{25}$$

Simulation Example

The feeding of ciliated protozoa *Tetrahymena pyriformis* on *Escherichia coli* bacteria and the persistent growth of *E. coli* on the metabolic byproducts of *T. pyriformis* are well-known two-way interactions. For simulation purposes, it is assumed that the three specific growth rates contribute to culture growth through the Monod model,

$$n = \frac{\nu b}{K_p + b} \qquad m = \frac{\mu s}{K + s} \qquad m' = \frac{\mu' s'}{K' + s'}. \tag{26}$$

A representative set of model parameters for the *T. pyriformis–E. coli* system, grown at 25°C in a mineral salts medium with glucose as the rate-limiting allochthonous substrate, is shown in Table 1 (Sambanis et al., 1987). In order to demonstrate the ability of the estimator to track time-varying parameters, the parameter K_p, of the protozoan-specific growth, was gradually reduced from 7×10^7 to 6.9×10^7.

The response of the saprotrophy model (Eqs. 1 and 26) was simulated with initial conditions, $p_0 = 1 \times 10^7$ μm^3/mL, $b_0 = 8 \times 10^6$ cells/mL, $s_0 = 0.006 \times 10^{-3}$ mg/mL, and $s'_0 = 13 \times 10^{-3}$ mg/mL, and operating conditions $D = 0.025$ h^{-1} and $s'_f = 0.05$ mg/mL. The model was integrated for 1,000 h of chemostat operation, and an output sequence was generated using a sampling time of $\Delta t = 0.1$ h. The data were corrupted with white noise: *T. pyriformis* biovolume and *E. coli* density with Gauss $(0, 10^4)$, autochthonous substrate with Gauss $(0, 10^{-8})$, and glucose concentration with Gauss $(0, 10^{-5})$. The damped oscillations of p, b, s, and s' are shown in Figure 2.

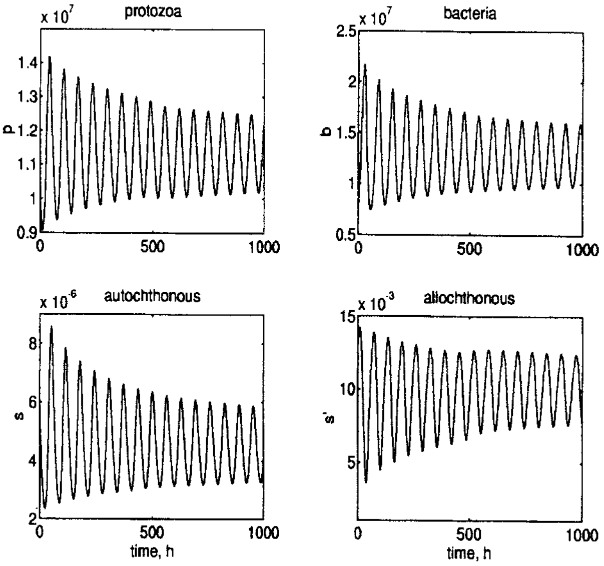

Figure 2. Simulation of saprotrophy model, corrupted with Gaussian noise.

In order to implement the modulating-functions method, the model needs to be expressed in the prescribed affine form. In the first stage of the estimation scheme, the parameters of the saprotrophy model (Eqs. 1) are estimated without using any analytical expressions for the specific growth rates. The model equations are rearranged in the affine form as follows:

$$\frac{db}{dt} + Db = A_1 b - A_2 p \tag{27a}$$

$$\frac{ds}{dt} + Ds = -A_3 b + A_4 p \tag{27b}$$

$$\frac{ds'}{dt} - D(s'_f - s') = -A_5 b \tag{27c}$$

$$\frac{dp}{dt} + Dp = A_6 np - A_7 p, \tag{27d}$$

where A_i are the parameters to be estimated. The three specific growth rates, two yield coefficients, and the two death/maintenance coefficients are calculated from $n = A_2$, $Y = A_1/(A_3 + A_5)$; $m = A_3 Y$; $m' = A_5 Y$; $X = A_6$; $\nu_d = A_7$; and $a = A_4/A_7$. Note that the estimates of n obtained from Eq. 27a are fed to the algorithm for the estimation of A_6 and A_7 in Eq. 27d.

The recursive algorithm is initiated with the Pearson–Lee method with FFT on a window of N samples. The recursion formulas are applied at each sampling instant to solve the

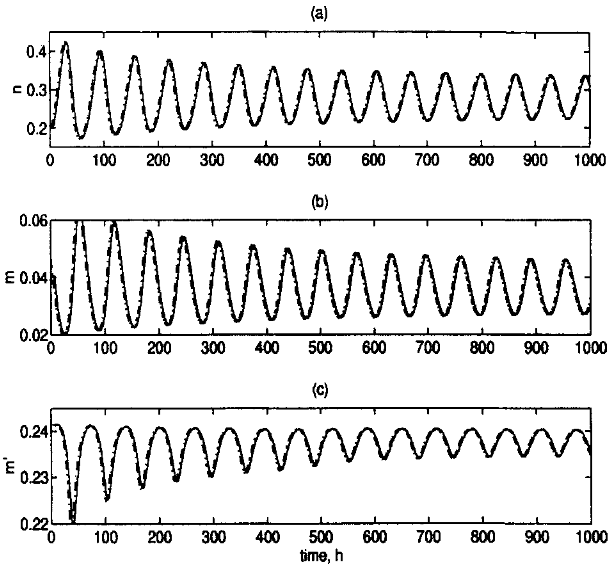

Figure 3. Estimation of (a) specific growth rate of protozoa on bacteria *n*; (b) specific growth rate of bacteria on autochthonous substrate *m*; (c) specific growth rate of bacteria on allochthonous substrate *m'*; prior to adjusting for *T*/2 shift; "true" values (– · –).

least-squares problem for new parameters as the data window is moved forward. A data window of size $T = 7$ h (number of data points $N = 70$) and filtering parameter $L = 6$ were used to estimate the parameters A_i from each of Eqs. 27. The size of the data window is important for the performance of the algorithm. If the window size is too small, the information about the system dynamics may be insufficient, and if the window size is too large, delays are introduced in the estimates. The algorithm essentially treats the parameters as constants inside the data window, and the resulting estimates are average values for the width of the window. Hence, the estimates need to be shifted backwards in time by $T/2$. The estimates of the three specific growth rates n, m, and m' are shown in Figure 3 with a delay of 3.5 h prior to adjusting for the delay. Figure 4 shows the estimates of the other four parameters Y, X, ν_d, and a. It was also observed that the amplitudes of the estimates fail to match those of the actual oscillating parameters as the window size increases, which is another direct consequence of averaging the parameter over T. In actuality, for a time-varying system, the parameters α_j are not constant and hence they cannot be moved out of the integral while modulating. Current research is focused on modeling the time-varying parameter as a polynomial spline inside the moving window (Ungarala and Co, 1996).

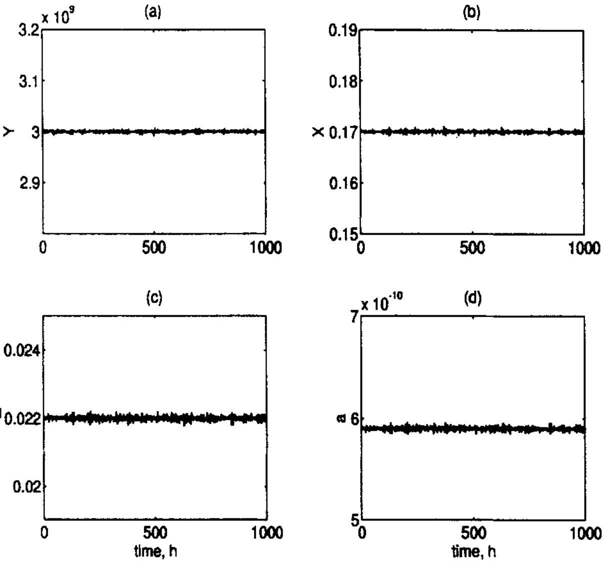

Figure 4. Estimation of (a) bacterial yield coefficient *Y*; (b) protozoan yield coefficient *X*; (c) protozoan death rate ν_d, and (d) autochthonous substrate production constant a; "true" values (– · –).

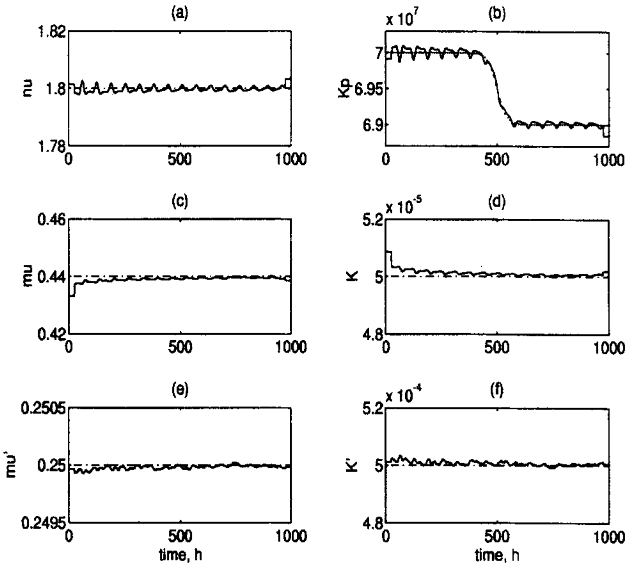

Figure 5. Estimation of Monod law parameters (a) ν; (b) K_p; (c) μ; (d) *K*; (e) μ'; and (f) *K'*; "true" values (– · –).

In the second stage of the estimation scheme, the growth-rate models (Eqs. 26) are identified from the previously obtained estimates of the specific growth rates. The relevant affine forms are

$$nb = \nu b - K_p n \tag{28a}$$

$$ms = \mu s - Km \tag{28b}$$

$$m's' = \mu' s' - K'm'. \tag{28c}$$

With $L = 6$ and $N = 500$, Figure 5 shows the estimates of the three maximum specific growth rates (ν, μ, and μ') and the three saturation constants (K_p, K, and K').

Conclusions

Microbial growth processes are generally nonstationary and difficult to model. In this article, a recursive modulating-functions method was demonstrated to track parameters on-line from a protozoan–bacterial interactions model. The simulation study shows that the specific growth rates can be estimated accurately, independent of the growth model involved. The estimations show good agreement with their actual values, and the variation in the time-varying parameters was followed well. Apart from the $T/2$ delay, in the case of oscillating parameters, it was found that the estimated amplitudes are also a function of the window size. In a second pass of the modulating functions, the relevant growth models are identified from the previous estimates of the specific growth rates. Since the method automatically executes different levels of noise filtering, subsequent identification based on previous estimates results in relatively smooth estimates. The modulating-functions method proves to be a good choice for the identification of nonlinear continuous-time models and has many potential applications in chemical engineering systems, for example, in catalyst deactivation and heat-exchanger fouling.

Literature Cited

Aborhey, S., and D. Williamson, "State and Parameter Estimation of Microbial Growth Processes," *Automatica*, **14**, 493 (1978).

Bastin, G., and D. Dochain, "On-Line Estimation of Microbial Specific Growth Rates," *Automatica*, **22**, 705 (1986).

Canale, R. P., T. D. Lustig, P. M. Kehrberger, and J. E. Salo, "Experimental and Mathematical Modeling Studies of Protozoan Predation on Bacteria," *Biotechnol. Bioeng.*, **15**, 707 (1973).

Co, T. B., and K. Zhong, "Lie Transformations for Parameter Estimation of Continuous-Time Nonlinear Systems," *Proc. Amer. Control Conf.*, **5**, 3053 (1997).

Co, T. B., and S. Ungarala, "Batch Scheme Recursive Parameter Estimation of Continuous-Time Systems Using the Modulating Functions Method," *Automatica*, **33**, 1185 (1997).

Fredrickson, A. G., "Interactions of Microbial Populations in Mixed Culture Situations," *Foundations of Biochemical Engineering, Kinetics and Thermodynamics in Biological Systems*, H. W. Blanch, E. T. Papoutsakis, and G. Stephanopoulos, eds., No. 207, ACS Symp. Ser., American Chemical Society, Washington, DC, p. 201 (1983).

Golden, M. P., and B. E. Ydstie, "Adaptive Extremum Control Using Approximate Process Models," *AIChE J.*, **35**, 1157 (1989).

Habte, M., and M. Alexander, "Protozoan Density and the Coexistence of Protozoan Predators and Bacterial Prey," *Ecology*, **59**, 140 (1978).

Holmberg, A., "On the Practical Identifiability of Microbial Growth Models Incorporating Michaelis-Menten Type Nonlinearities," *J. Math. Biosci.*, **62**, 23 (1982).

Holmberg, A., and J. Ranta, "Procedures for Parameter and State Estimation of Microbial Growth Process Models," *Automatica*, **18**, 181 (1982).

Lubenova, V., "On-Line Estimation of Biomass Concentration and non Stationary Parameters for Aerobic Bioprocesses," *J. Biotechnol.*, **46**, 197 (1996).

Nihtilä, M., and J. Virkkunen, "Practical Identifiability of Growth and Substrate Consumption Models," *Biotechnol. Bioeng.*, **19**, 1831 (1977).

Oliveira, R., E. C. Ferreira, F. Oliveira, and S. Feyo de Azevedo, "A Study on the Convergence of Observer-Based Kinetics Estimators in Stirred Tank Bioreactors," *J. Process Control*, **6**, 367 (1996).

Pearson, A. E., and F. C. Lee, "On the Identification of Polynomial Input-Output Differential Equations," *IEEE Trans. Automat. Contr.*, **AC-30**, 778 (1985).

Preisig, H. A., and D. W. T. Rippin, "Theory and Applications of the Modulating Functions Method—I. Review and Theory of the Method and Theory of the Spline-Type Modulating Functions," *Comput. Chem. Eng.*, **17**, 1 (1993).

Sambanis, A., S. Pavlou, and A. G. Fredrickson, "Coexistence of Bacteria and Feeding Ciliates: Growth of Bacteria on Autochthonous Substrates as a Stabilizing Factor for Coexistence," *Biotechnol. Bioeng.*, **29**, 714 (1987).

Shimizu, H., T. Takamatsu, S. Shioya, and K.-I. Suga, "An Algorithmic Approach to Constructing the On-Line Estimation System for the Specific Growth Rate," *Biotechnol. Bioeng.*, **33**, 354 (1989).

Stephanopoulos, G., and K.-Y. San, "Studies on On-Line Bioreactor Identification: 1. Theory," *Biotechnol. Bioeng.*, **26**, 1176 (1984).

Topiwala, H., and C. G. Sinclair, "Temperature Relationships in Continuous Culture," *Biotechnol. Bioeng.*, **13**, 795 (1971).

Ungarala, S., and T. B. Co, "Recursive Parameter Estimation of Time-Varying Nonlinear Systems," AIChE Meeting, Chicago (1996).

Zhou, Q., and W. R. Cluett, "Recursive Identification of Time-Varying Systems via Incremental Estimation," *Automatica*, **32**, 1427 (1996).

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