On-Line Estimation of the Specific Growth Rate in the Bacitracin Fermentation Process

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An on-line estimation of the specific growth rate of an aerobic fermentation batch process was conducted based on measurements of oxygen or carbon dioxide concentrations in exhaust gas, or the concentrations of dissolved oxygen in the liquid phase in the fermentor. A simple linear model used was upgraded with a mechanism for a time-varying forgetting factor to enable good tracking of estimated parameters as well during rapid and large changes in the process. Because the measuring methods used are independent of each other and can identify the process well, mutual control of individual sensors is also possible in industrial reactors. The results from the model agree well with experimentally obtained values for bacitracin fermentation in an 80-m^3 and 1.5-m^3 batch bioreactor. The model provides a good basis for robust adaptive regulation of aerobic batch processes.

Introduction

Microbiological processes in which microorganisms use a substrate for growth, and in some instances for the production of economically promising products, are important sources of different biological products, such as biomass, primary and secondary metabolites, enzymes, and proteins. Fermentation processes in batch reactors can be monitored through certain measurable physical variables without excessive risk of medium contamination. In general, the product is not removed until the process is completed. Due to necessary biological transformation, the production of bacitracin as a secondary metabolite is a highly interactive and interlinked system with demanding complex dynamics, and for that reason it is not a well-understood process. The development of reliable, robust, and high-performance systems to enable control of the fermentation process is a good approach to a significant upgrading of production capability. Limiting factors in research on the optimization of industrial fermentative production, such as reliability of operation, possibility of contamination, and difficulties in transferring the results from pilot plants to industrial fermentors, led us to use experimental modeling, that is, identification of process dynamics. Identification enables the building of mathematical models of the process by on-line measuring of physical variables and provides a good method for acquiring the necessary information for monitoring and optimization of the process.

Adaptive control of bioreactors has a variety of advantages over other control systems, the primary one being that it is not necessary to know the process kinetics and complicated structured process models (Boškovič, 1996). Rather, this control method uses measurable process parameters to continuously adapt to the variable process properties. Since the bioprocess behavior is nonlinear and dynamic, during modeling it is necessary to use techniques such as an extended Kalman filter or an extended Luenberger state observer (Stephanopoulos and San, 1984; Bastin and Dochain, 1990; Gauthier et al., 1992; Zhang et al., 1994; Loeblein and Perkins, 1999). In general, the design of stable estimators for bioprocess remains a complex task that must be studied for each particular process (Charbonnier and Cheruy, 1996).

The production of bacitracin is an aerobic process. Bastin and Dochain (1990) suggested the use of an asymptotic observer, which enables process state estimation based on partial measurement of state variables. However, due to its slow convergence this tool is unable to track rapid processes. Cazzador and Lubenova (1995) proposed a nonlinear observer on the basis of the balance equation for oxygen uptake rate (OUR), which takes into account the dynamics of specific

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growth rate. Estler (1995b) suggested separate estimations of parameters, especially of the specific growth rate of the process and of the process state, thus achieving unbiased convergence and making possible the estimation of rapidly changing process parameters. He estimated a specific growth rate solely from the measurements of the partial pressure of oxygen in the fermentor's exhaust gas. To estimate a specific growth rate and biomass concentration and determine a proper strategy for lipase production control, Charbonnier and Cheruy (1996) used a model based only on the measurement of the partial pressure of released carbon dioxide in the fermentor's exhaust gas.

This article illustrates identification of the bacitracin fermentation process by separate estimations of process parameters and process state, such as specific growth rate and biomass production, on the basis of measurements of the partial pressure of oxygen and/or carbon dioxide in the exhaust gas and/or dissolved oxygen in the liquid phase in the fermentor. In certain phases of the fermentation process, changes in specific growth rate are large and rapid, therefore ignoring that its dynamics may cause an incorrect and biased estimation. This was avoided by upgrading the least-squares method with a time-varying forgetting factor. Since on-line measuring methods used for individual physical variables are independent, in addition to good process identification, there is also the possibility of reciprocal control of individual sensors. This is especially important for industrial bioreactors from the point of view of model reliability, as is the case here.

Process Description

On-line measurements of oxygen and carbon dioxide concentrations in the fermentor's exhaust gas and dissolved oxygen concentration in the broth were conducted during bacitracin fermentation in an industrial 80-m^3 batch reactor. Bacitracin fermentation with noncontinuous feeding was performed in a pilot 1.5-m^3 batch reactor. The concentration of oxygen in the fermentor's exhaust gas was measured using an IJS MK 100 gauge operating on the principle of oxygen diffusion between actual and reference states. Carbon dioxide in the fermentor's exhaust gas was measured via IR absorption using a Siemens Ultramat 22 P gauge. The measurement of carbon dioxide concentration is more demanding than that of oxygen concentration because carbon dioxide is more soluble, and also it reacts with water, forming HCO_3^-, the concentration of which depends on the broth pH value. An Ingold polarographic electrode was used to measure the concentration of dissolved oxygen in the broth (Rehm and Reed, 1993). The uncertainty in these measurements originates mainly from the time variation of electrical resistance and changes in other membrane surface properties, the persistence of air bubbles on the electrode, and the effect of any possible addition of surface-active substances, such as antifoaming agents. Figure 1 illustrates the time variation of carbon dioxide and oxygen concentrations in the exhaust gas, as well as that of oxygen dissolved in the broth.

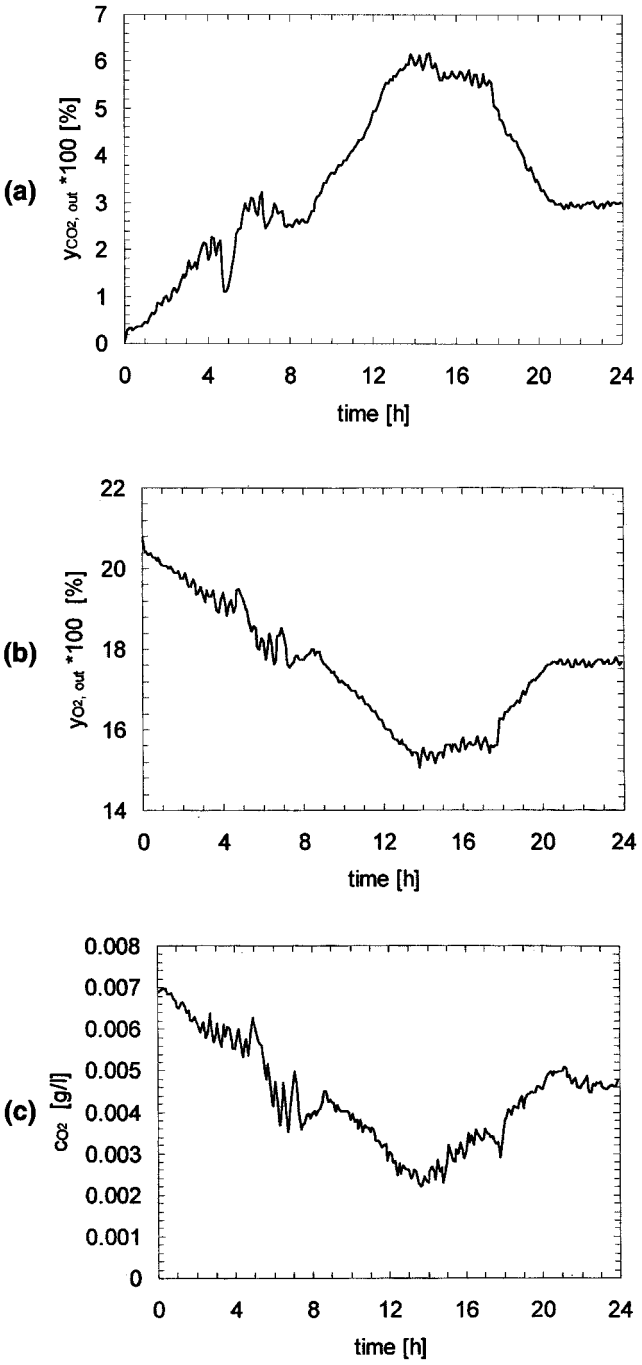

Figure 1. Time variation of (a) carbon dioxide concentration in the fermentor's exhaust gas; (b) oxygen concentration in exhaust gas; (c) dissolved oxygen concentration in the liquid phase in the fermentor.

Estimation of the Specific Growth Rate of the Batch Process

Even if several process variables are able to be measured, they need to be carefully selected when software sensors are utilized. An excessively high level of correlation between measured variables can lead to a divergence of parameter estimates in the model, or render impossible the reconstruction of required state variables.

In bacitracin fermentation, the respiratory coefficient (RQ), Eq. 1, which represents the ratio of released carbon dioxide

to oxygen consumed,

$$\mathrm{RQ} = \frac{\text{mol } CO_2 \text{ formed}}{\text{mol } O_2 \text{ consumed}}, \tag{1}$$

has a value of approximately one. Bastin and Dochain (1990) showed that in these instances, with an asymptotic observer, a reconstruction of the process state with simultaneous oxygen and carbon dioxide measurements is not possible, because these two types of measurement do not yield independent information on the process state. When the recursive least-squares method is used, however, one of the conditions for process identification is the requirement for noncorrelating input signals. In the opposite case, the estimation algorithm becomes a singular one, or it only estimates the dynamics of the measurement error (Isermann et al., 1992).

The fermentation process in a batch reactor for a given case can be described with the following dynamic Bastin and Dochain (1990) model:

$$\frac{d}{dt}\begin{bmatrix} c_X \\ c_{O_2} \\ c_{CO_2} \end{bmatrix} = \begin{bmatrix} 1 \\ k_C \\ k_{CO_2} \end{bmatrix} c_X \mu - \begin{bmatrix} 0 \\ Q_{O_2 in} - Q_{O_2 out} \\ Q_{CO_2 out} - Q_{CO_2 in} \end{bmatrix}, \tag{2}$$

where

$$Q_{O_2} = \frac{\dot{m}_{O_2}}{V_b} \tag{3}$$

and

$$Q_{CO_2} = \frac{\dot{m}_{CO_2}}{V_b} \tag{4}$$

are the oxygen and carbon dioxide mass ratios with regard to the liquid volume in the fermentor. According to Zabriskie and Humphrey (1978), the OUR can be included as follows

$$\mathrm{OUR}(t) = \left[Y_{O_2/X}\, \mu(t) + k_{\mathrm{main},O_2} \right] c_X(t). \tag{5}$$

The oxygen yield coefficient, $Y_{O_2/X}$, and oxygen maintenance rate, k_{main,O_2}, are constant and need not be known. The specific growth rate, $\mu(t)$ is an unknown time-varying parameter. $\mathrm{OUR}(t)$ is measured indirectly, on-line, while the biomass concentration $c_X(t)$ cannot be measured on-line.

Equations 2 and 5 yield the balance equation for OUR

$$\frac{d\,\mathrm{OUR}(t)}{dt} = \mu(t)\mathrm{OUR}(t) + c_X(t) Y_{O_2/X} \frac{d\mu(t)}{dt} = \left(\mu(t) + \frac{\dfrac{d\mu(t)}{dt}}{\mu(t) + \dfrac{k_{\mathrm{main},O_2}}{Y_{O_2/X}}} \right) \mathrm{OUR}(t). \tag{6}$$

Computer implementation requires discretization. A first-order forward Euler approximation was used

$$\frac{\mathrm{OUR}(n+1) - \mathrm{OUR}(n)}{\Delta t} = \mu(n)\mathrm{OUR}(n) \left[1 - \frac{\dfrac{\mu(n+1)}{\mu(n)} - 1}{\Delta t \left[\mu(n) + \dfrac{k_{\mathrm{main},O_2}}{Y_{O_2/X}} \right]} \right]. \tag{7}$$

The magnitude of the specific growth rate is not limited. In the treated case it can also be negative since we measure and determine the net specific growth rate, which also includes the specific rate of endogenous metabolism, substrate consumption for the maintenance of the cell structure, and other complex phenomena. The dynamics of the reduction in cell yield is not the same as the dynamics of the observed net growth rate. Using up one substrate and introducing a second is a shock to the population of microorganisms, which tends to be reflected in a reduction in the growth of the population within the time period of approximately one generation, or 30 min. At the end of the biosynthesis of the bacitracin, the specific rate of cell lysis becomes significant. As a result of this, at low growth rates one can often observe a decrease in the cell yield (Rehm and Reed, 1993).

It is obvious that the second term in the brackets of Eq. 7 becomes very large if

$$\frac{k_{\mathrm{main},O_2}}{Y_{O_2/X}} \approx -\mu(n),$$

therefore taking dynamics directly in the form of an Eq. 7 into account in the estimation algorithm can cause inconsistent estimation. Cazzador and Lubenova (1995) took the dymamics of μ into account in their proposed adaptive estimator on the basis of the balance equation of OUR. Simulation's results showed good global performance and stability of the estimator, but as the estimators are nonlinear, an optimal tuning cannot be easily derived (Cazzador and Lubenova, 1995).

Large industrial fermentors require a reliable and stable estimation of the specific growth rate at which parameter estimations must be unbiased and consistent even in the case of their significant variations. It was shown by Roux et al. (1996) that we can adequately estimate the process of fermentation with the linear model, and at the same time keep a reasonable trade-off between the model structure complexity and its effectiveness (Carrier and Stephanopoulos, 1998). Estler (1995b) proposed a simple model for the estimation of specific growth rate on the basis of a measurement of the partial pressure of oxygen in the exhaust gas of the fermentor. In his model he assumes that μ varies very slowly and that its dynamics are negligible in the OUR balance equation. This hypothesis might prove to be problematic, because it can result in biased estimations of μ when the latter is subject to significant variations.

It was shown by Banerjee and Pearson (1995) that the nonlinear model can be considered as a series of linear models. A continuous nonlinear process can be adequately modeled as linear over a particular operating region (Carrier and Stephanopoulos, 1998). We only need to determine an appropriate mechanism that can adapt the linear model to the temporary operation range, over which the linearization is sufficient. In this case, we have selected a linear model, in which, as in to Estler (1995a), the dynamic part in brackets was omitted in Eq. 7

$$\frac{\mathrm{OUR}(n+1)-\mathrm{OUR}(n)}{\Delta t}=\mu(n)\mathrm{OUR}(n) \tag{8}$$

and the nonlinear dynamics of $\mu(n)$ was included in the model by weighting a recursive algorithm with a variable forgetting factor $\lambda(n)$. This enables the model to also track the process during large changes, when linearization is no longer satisfactory.

The justification for the compensation of the dynamic part of Eq. 7 with the use of a time-varying forgetting factor can be verified with an analysis of the behavior of the expression in the brackets in Eq. 7. If $\mu(n)$ is constant or varies slowly

$$\frac{\mu(n+1)}{\mu(n)}-1=0, \tag{9}$$

and Eq. 7 becomes equal to Eq. 8. However, since $\mu(n)$ varies very quickly in certain phases of the process of bacitracin fermentation, the left side of Eq. 9 does not always equal zero. Deviations are corrected by the variable forgetting factor, which enables rapid adaptation of the estimated parameter to the actual circumstances of the process in the case of greater model error $e(n)$ by reducing the values of $\lambda(n)$.

The proposed method is adequate if in all cases, including large dynamic changes in the process, the time variation of the left side of Eq. 9 is in agreement with the variation of model error $e(n)$ as a result of operation of time-varying $\lambda(n)$, which means that the variation for the left side of Eq. 9 must have the characteristics of white noise with a mean value of zero. The least-squares algorithm compensates for such disturbances—in our case, parameter nonlinearities in a linear model—without a danger of biased estimation.

The specific growth rate can be expressed as

$$\mu(n)=\frac{\hat{a}(n)-1}{\Delta t}, \tag{10}$$

where $\hat{a}(n)$ is a parameter estimated with equation

$$\mathrm{OUR}(n+1)=\hat{a}(n)\mathrm{OUR}(n) \tag{11}$$

using the recursive least-squares method with a variable forgetting factor. The parameters are calculated according to equation (Isermann et al., 1992)

$$\hat{\boldsymbol{\theta}}(n+1)=\hat{\boldsymbol{\theta}}(n)+\frac{\underline{\underline{\Gamma}}(n)\boldsymbol{\psi}(n+1)}{\lambda(n)+\boldsymbol{\psi}^T(n+1)\underline{\underline{\Gamma}}(n)\boldsymbol{\psi}(n+1)}\cdot e(n+1), \tag{12}$$

where $e(n+1)$ is model error

$$e(n+1)=\mathrm{OUR}(n+1)-\boldsymbol{\psi}^T(n+1)\hat{\boldsymbol{\theta}}(n), \tag{13}$$

and the covariance matrix $\underline{\underline{\Gamma}}(n+1)$ is calculated using the following recursive equation

$$\underline{\underline{\Gamma}}(n+1)=\left(\underline{\underline{\Gamma}}(n)-\frac{\underline{\underline{\Gamma}}(n)\boldsymbol{\psi}(n+1)\boldsymbol{\psi}^T(n+1)\underline{\underline{\Gamma}}(n)}{\lambda(n+1)+\boldsymbol{\psi}^T(n+1)\underline{\underline{\Gamma}}(n)\boldsymbol{\psi}(n+1)}\right)\cdot\frac{1}{\lambda(n+1)}. \tag{14}$$

In our case, the model (Eq. 11) is of the first order, therefore the parameter vector is $\hat{\boldsymbol{\theta}}(n)=\hat{a}(n)$, the data vector is $\boldsymbol{\psi}(n+1)=-\mathrm{OUR}(n)$, and the covariance matrix $\underline{\underline{\Gamma}}(n+1)$ has a dimension of 1. The trace of the covariance matrix, $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$, gives the degree of confidence in the parameter estimations. The smaller the $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$ in static processes, the greater the probability that the parameter estimations are correct. In dynamic processes, where estimated parameters vary all the time, a constant decrease in the value of $\underline{\underline{\Gamma}}(n+1)$ must be prevented, since only parameters estimated in this manner are capable of tracking the process. The time-varying forgetting factor $\lambda(n)$ prevents the constant reduction in the value of $\underline{\underline{\Gamma}}(n+1)$.

The mechanism of regulating the forgetting factor is based on model error $e(n)$. If this is small, it either means that the estimation is correct or that the parameter does not vary. In either case, $\lambda(n)$ must be ≈ 1. If $e(n)$ is large, $\lambda(n)$ must be small to allow a rapid adaptation of the parameters. The calculation of $\lambda(n)$ is based on constant information content of the estimator and was proposed by Fortescue et al. (1981)

$$\lambda(n+1)=1-\frac{e^2(n+1)}{\Sigma_0}\cdot\left(1-\frac{\boldsymbol{\psi}^T(n+1)\underline{\underline{\Gamma}}(n)\boldsymbol{\psi}(n+1)}{\lambda(n)+\boldsymbol{\psi}^T(n+1)\underline{\underline{\Gamma}}(n)\boldsymbol{\psi}(n+1)}\right), \tag{15}$$

where Σ_0 is a scalar representing the information content of the estimator and is the only parameter in the calculation that needs to be determined empirically. It depends on process dynamics, sample time, and measurement noise (Isermann et al., 1992). In the identification of bacitracin fermentation, Σ_0 was determined empirically in at least one measurement. The obtained value is generally applicable for the chosen measurable signal if the sample time is not changed and if the characteristics of the measuring sensor are not changed. The sample time t must be selected such that no loss of information on the process behavior (too long t) or singularity in the covariant matrix $\underline{\underline{\Gamma}}(k+1)$ (too short t) occurs. In our case the suitable sampling interval for the bacitracin fermentation process in an 80-m^3 fermentor was $\Delta t=0.1$ h.

To ensure a rapid convergence of parameter $\hat{a}(n)$, the estimating algorithm was started with the nonrecursive least-squares method, by which it obtained reliable initial values for the recursive procedure.

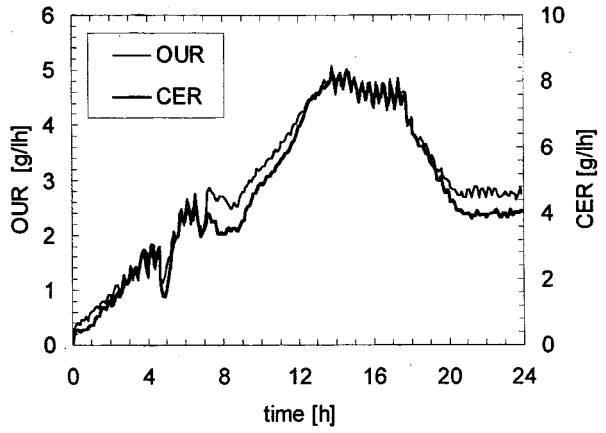

Figure 2. Time variation of OUR and CER for bacitracin fermentation in an 80-m^3 fermentor.

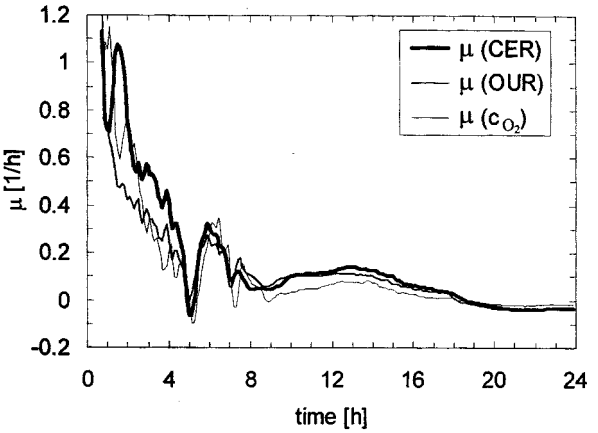

Figure 3. Specific growth rate μ based on OUR, CER, and c_{O_2}.

Analogously with the OUR, a balance equation for carbon dioxide excretion rate (CER) can be written in aerobic fermentation

$$\mathrm{CER}(t) = \left(Y_{CO_2/X}\,\mu(t) + k_{\mathrm{main},CO_2}\right)c_X(t). \qquad (16)$$

According to a similar logic with Eqs. 7 and 8 and Eqs. 10 through 15, in which OUR(t) is replaced by CER(t), the measurement of CER can serve for estimating the specific growth rate.

In calculating the OUR, Estler (1995a) assumed that the variation of oxygen concentration in the broth dc_{O_2}/dt is negligible in comparison with the variation of the oxygen transfer rate, and that the oxygen concentration in the broth is in a quasi-stationary state. On the basis of oxygen and carbon dioxide concentration measurements in the exhaust gas, OUR and CER can be calculated as

$$\mathrm{OUR} = \frac{M_{O_2}}{V_b M_{\mathrm{air}} 100}\left[\dot{m}_{\mathrm{air,in}}\,y_{O_2,\mathrm{in}} - \dot{m}_{\mathrm{air,out}}\,y_{O_2,\mathrm{out}}\right] \qquad (17)$$

$$\mathrm{CER} = \frac{M_{CO_2}}{V_b M_{\mathrm{air}} 100}\left[\dot{m}_{\mathrm{air,out}}\,y_{CO_2,\mathrm{out}} - \dot{m}_{\mathrm{air,in}}\,y_{CO_2,\mathrm{in}}\right]. \qquad (18)$$

Figure 2 illustrates the variations of OUR and CER assuming a 20.96% oxygen ratio and a negligible carbon dioxide ratio in the air entering the fermentor. There is a high level of correlation between OUR and CER, from which it follows that RQ is approximately 1.

Although the time variation of oxygen concentration in the broth $c_{O_2}(t)$ is negligible when determining OUR, because its order of magnitude is of the same order as the noise present during oxygen concentration measurement in the exhaust gas, it is sufficiently large on another level to characterize the dynamics of the fermentation process. Therefore, the concentration of oxygen dissolved in the broth $c_{O_2}(t)$ was also used as an independently measured parameter.

Results

Specific growth rate μ was estimated on-line using the model, Eq. 8, and the recursive least-squares method with a time-varying forgetting factor, Eqs. 10 through 15, whereby the OUR, CER, or concentrations of oxygen dissolved in the liquid phase c_{O_2} were used as a measurable signal of the model, as shown in Figure 3. The recursive algorithm converges between the second and fourth hour of the process. After this, the estimates consistently follow the development of the process, including the characteristic minimums that occur approximately at the fifth and seventh hours. After the eighth hour, when the production of the secondary metabolites begins, μ becomes settled, except during the technologically important interval at about the end of the eighteenth hour, when the concentration of the biomass begins to drop. Determining μ on the basis of OUR and CER gives good agreement, but the estimation of μ on the basis of c_{O_2} is slightly off. The problems with measuring c_{O_2} are mainly in the time delay of the process and the dynamics of the measuring sensor (Sergantanis and Karim, 1998).

Figure 4 shows the typical variations of $\lambda(n)$ and $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$ between the second and twenty-fourth hour of bacitracin fer-

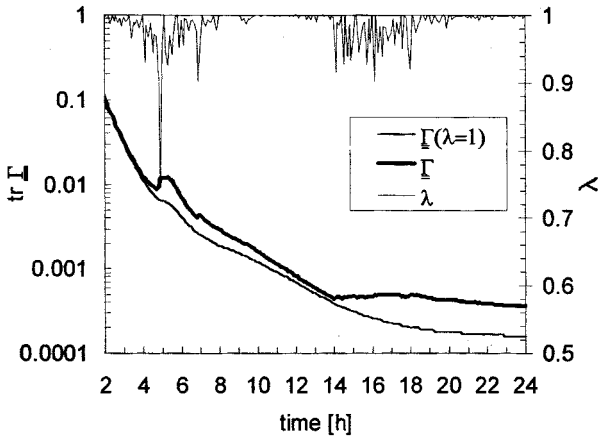

Figure 4. Forgetting factor $\lambda(n)$, $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$ for this forgetting factor and $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$ at constant $\lambda = 1$ during the estimation of specific growth rate on the basis of CER measurement in bacitracin fermentation in an 80-m^3 fermentor.

mentation. The signal being measured is CER. To illustrate the operation of the forgetting factor mechanism $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$ for the same set of measurements, but with $\lambda = 1$, is also shown. It can be seen that at $\lambda = 1$ $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$ decreases exponentially, which prevents rapid changes in parameter estimations. And conversely, the time-dependent decrease in the forgetting factor between the fourth and the eighth, and the fourteenth and the eighteenth hour, marks greater changes in the process and prevents constant reduction in the covariance matrix value, which enables the estimated parameters to follow the process dynamics.

The variation of LS values in Eq. 9, the model error $e(n)$, and their mean values are shown in Figure 5. It can be seen that in slow variation of CER a linear model (Eq. 8) is sufficient for the $\mu(n)$ estimation, since both the model error $e(n)$ and LS of Eq. 9 are small and the forgetting factor $\lambda(n) \approx 1$. When the fermentation process becomes more dynamic, model error $e(n)$ increases. Since the model could not follow the process satisfactorily with the existing estimated parameters, $\lambda(n)$ decreased and, consequently, the trace of the covariance matrix $\mathrm{tr}\underline{\underline{\Gamma}}(n+1)$ increased.

Figure 5 also shows that the variation of the differences between the models (Eqs. 7 and 8), which is represented by the LS of Eq. 9, is very similar to the variation of model error $e(n)$. The correlation factor between the two values from the second to the twenty-fourth hour is 0.7257. The LS of Eq. 9 has a mean value of -0.0018 and $e(n)$ has a mean value of -0.025. In Figure 5 it is also possible to see mean values in the five intervals of the process that have dynamics of varying intensity. Even in cases of larger changes in the process, the mean values do not deviate much from the value of 0. The largest deviation of the mean value of $e(n)$ occurs during the interval between the fourteenth and the 17.8th hour by 8.6% with reference to the estimated parameter. During the first 20 hours the standard deviations of $e(n)$ and $(\mu(n+1)/\mu(n)) - 1$ are very similar. The difference appears during the last interval when μ changes slightly and when the model error is mainly the result of the noise of the measurement, not the dynamics of the process. In separate regions it is possible to see differences from the normal distribution, mainly between the eighth and tenth hours, but the distributions of both values are sufficiently similar, so that we can justify the presupposition according to which the dynamics of the process are included in $e(n)$ by means of the forgetting factor. This is consistent with the idea of using the forgetting factor mechanism, which corrects the estimates of a linear model on the basis of the model error $e(n)$ on-line, with respect to conditions in the process.

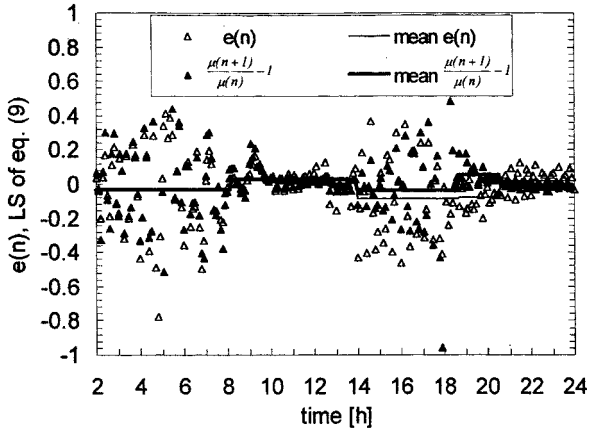

Figure 5. $e(n)$, $[\mu(n+1)/\mu(n)] - 1$ and their main values in estimating the specific growth rate on the basis of CER measurement in bacitracin fermentation in an 80-m^3 fermentor.

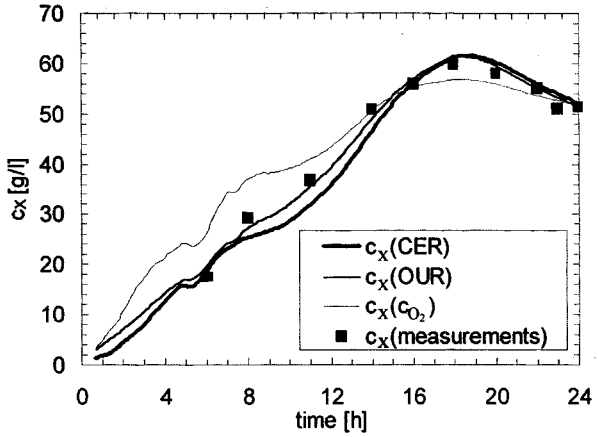

Figure 6. Time variation of biomass concentration c_X in an 80-m^3 fermentor.

Biomass concentration $c_X(t)$ can be obtained by integrating the first term of Eq. 2:

$$c_X(n) = c_X(n-1)\exp[\mu(n)\Delta t]. \tag{19}$$

Equation 19 requires a knowledge of the biomass concentration at the beginning of the calculation. Because of the sensitivity of Eq. 19 to the initial values of c_X, we analyzed a larger number of the 80-m^3 fermentor batches, and thus obtained a reliable interval of initial values. Figure 6 illustrates the time variation of biomass concentration.

Good agreement with experimental values, as well as the variation of $c_X(n)$ obtained from the measurements of oxygen and carbon dioxide in the exhaust gas, indicate very promising possibilities of using these types of software sensors. The variation of biomass growth $c_X(n)$ obtained from independent measurement of the oxygen dissolved in broth c_{O_2} matches the directions of biomass growth obtained from OUR and CER. The inaccuracy inherent in using c_{O_2} as the measured signal originates mainly from the problems with measuring dissolved oxygen in an 80-m^3 industrial fermentor due to the inhomogeneous broth and the sensitivity of the sensor to the changes in the pH of the broth.

The reliability of the model in estimating a specific growth rate during rapid and dictated changes was tested using bacitracin fermentation with noncontinuous feeding in a pilot batch reactor. As shown in Figure 7, the specific growth rate increases during feeding because biomass grows again. The proposed model can also track the process well during rapid changes.

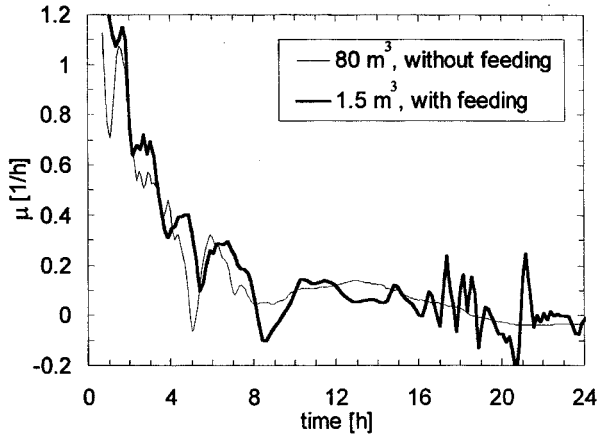

Figure 7. Specific growth rate μ in bacitracin fermentation with feeding in a pilot batch reactor and without feeding in an industrial fermentor.

Conclusions

In the proposed method, specific growth rate is estimated using a linear model, which is supplemented by a time-varying forgetting factor mechanism, which encompasses dynamics, nonlinearities, and the unreliability of measurements in the process. On the basis of either OUR, CER, or c_{O_2} measurement, the estimation algorithm provided consistent estimates and was able to rapidly track changes in the bacitracin fermentation process both in an industrial 80-m^3 fermentor and in a pilot 1.5-m^3 fermentor. Since the methods used for measuring carbon dioxide and oxygen concentrations in the exhaust gas, as well as oxygen dissolved in the broth, are mutually independent, independent software sensors were obtained. In addition to good process identification, there is also the possibility of reciprocal control of individual sensors. From the point of view of reliability of operation of industrial fermentors, which is the case here, this is quite important.

The developed software sensors are used as additional tools for process monitoring and fault detection in the industrial production of bacitracin, and are a good foundation for robust adaptive control of aerobic batch processes.

Notation

k_i = kinetic coefficient of species i
M = molecular weight, g/mol
$\dot{m}$ = mass flux, g/h
n = discrete time step
Q = gaseous flow rate, g/(Lh)
V_b = volume of the liquid phase, L
y_i = mole fraction of species i in air
Δt = sample time, h

Superscripts and subscripts

air = air
in = input
out = output

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