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OPTIMAL STATE AND PARAMETER ESTIMATION FOR A FED–BATCH INDUCED FOREIGN PROTEIN BIOREACTOR

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An extended Kalman filter was used for on-line estimation of system states and parameters for a fed-batch induced foreign protein bioreactor. Simulation work was carried out to test the feasibility of the filter. The extended Kalman filter worked well for state and parameter estimation. The ratio of the covariance matrices reflecting model uncertainty to measurement noise significantly influenced the filter performance. An iterative version of the extended Kalman filter algorithm was even more effective in estimating model parameters and states. When the model parameters to be estimated are assumed constant, application of a static estimation algorithm was extremely effective. Estimates were significantly less noisy than estimates using the dynamic extended Kalman filter.

1. Introduction

Biological systems can be characterized as highly uncertain processes, with the system dynamics changing from process to process. Therefore, on-line state and parameter estimation should be carried out to obtain current information about the processes and to improve process productivity. Model-based state and parameter estimation also gives information necessary for process optimization.

The Kalman filter is an optimal recursive data processing algorithm. It combines all available measurement data, plus prior knowledge about the system and measuring devices, to produce an optimal estimate of the state variables. The Kalman filter can be augmented so that model parameters are also estimated (Maybeck, 1982).

There has been significant effort in mathematical modeling of the dynamics of foreign protein production by recombinant bacteria. Structured and unstructured models have been developed to describe host-vector foreign protein production systems. Lee and Ramirez (1992) have developed a mathematical model which includes both inducer and glucose effects on the specific growth rate and the foreign protein production rate. This model was successfully applied to the host-vector system of *E. coli*, D1210, and plasmid, pSD8.

Deterministic models usually have several problems for direct application to real processes. First of all, the mathematical models are not perfect, since the models

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were developed to represent the dominant or critical model of system response. Many effects are knowingly left unmodeled. Secondly, the dynamic systems are driven not only by the system's control inputs, but also by disturbances, many of which are not measurable. Finally, sensors do not provide perfect and complete data about a system due to measurement noise (Maybeck, 1982). To compensate for both model uncertainties and the measurement noise, optimal estimates of process state and model parameters can be obtained via extended Kalman filtering.

Shimizu and Takamatsu (1989) proposed an algorithm for the on-line estimation of the specific growth rate in fermentation processes utilizing macroscopic balances and extended Kalman filtering. In the range of low cell density, the cell concentration was measured by spectrophotometry. However, for high cell densities, a macroscopic balance model was used. Oxygen uptake rate was used for on-line estimation of the specific growth rate in baker's yeast fermentation (Wu *et al.*, 1985). Park *et al.* (1983) have estimated the specific growth rate on-line for a glutamic-acid fermentation by using carbon dioxide evolution rate measurements. Bellgardt *et al.* (1986) applied an extended Kalman filter for state and parameter estimation of yeast fermentation. Park and Ramirez (1990) applied Kalman filtering for state estimation and used a sequential least squares method to estimate model parameters for a fed-batch bioreactor using yeast to produce a secreted foreign protein.

1.1. Extended Kalman Filter

The Kalman filter is an optimal linear estimator and is an optimal recursive data processing algorithm. The Kalman filter uses model predictions and process measurements to obtain optimal estimation of process states. At time t_{i-1}, a state is assumed to have the Gaussian distribution whose mean and covariance are $\overline{\mu}$ and σ^2, respectively. The next state values can be predicted by integrating the state equations over one sampling time. Because the model is not perfect, the prediction has a larger covariance. This means that the confidence level of the next state is decreased. However, system outputs can be measured directly from sensors with a mean, $\overline{\mu}_S$, and covariance, σ_S^2. Information from those two sources is combined by applying Bayes's rule. The resulting covariance becomes smaller than that of the model above.

The basic idea of the extended Kalman filter is to relinearize about each estimate $\hat{\boldsymbol{x}}(t)$. In this manner, deviations from the reference (nominal) trajectory are minimized which enhances the applicability of linearized perturbation techniques.

The nonlinear uncertain system is given by

$$\dot{\boldsymbol{x}}(t) = \boldsymbol{f}\Big[\boldsymbol{x}(t), \boldsymbol{u}(t), t\Big] + \boldsymbol{G}(t)\boldsymbol{w}(t) \tag{1}$$

where $\boldsymbol{w}(t)$ is a zero-mean white Gaussian noise process with a covariance kernel

$$\boldsymbol{E}\Big\{\boldsymbol{w}(t)\boldsymbol{w}^T(t+\tau)\Big\} - \boldsymbol{Q}(t)\delta(\tau) \tag{2}$$

The initial condition $\boldsymbol{x}(t_0)$ is assumed to be a Gaussian random n-vector with mean $\hat{\boldsymbol{x}}_0$ and covariance $\boldsymbol{P}_0$. The discrete-time measurements can be modeled as a known nonlinear function of the state corrupted with noise,

$$\boldsymbol{z}(t_i) = \boldsymbol{h}\Big[\boldsymbol{x}(t_i), t_i\Big] + \boldsymbol{v}(t_i) \tag{3}$$

where $\boldsymbol{h}$ is a known m-vector function of the state and time, and $v(t_i)$ is a white Gaussian noise sequence of mean zero and covariance kernel

$$\boldsymbol{E}\Big\{\boldsymbol{v}(t_i)\boldsymbol{v}^T(t_j)\Big\} = \begin{cases} \boldsymbol{R}(t_i), & t_i = t_j \\ 0, & t_i \neq t_j \end{cases} \tag{4}$$

At a measurement time t_i, the measurements $\boldsymbol{z}(t_i, w_j) = \boldsymbol{z}_i$ become available. The estimate is updated by computing the Kalman filter gain $\boldsymbol{K}(t_i)$,

$$\hat{\boldsymbol{x}}(t_i^+) = \hat{\boldsymbol{x}}(t_i^-) + \boldsymbol{K}(t_i)\Big\{\boldsymbol{z}_i - \boldsymbol{h}\Big[\hat{\boldsymbol{x}}(t_i^-), t_i\Big]\Big\} \tag{5}$$

$$\boldsymbol{K}(t_i) = \boldsymbol{P}(t_i^-)\boldsymbol{H}^T\Big[t_i; \hat{\boldsymbol{x}}(t_i^-)\Big]\Big\{\boldsymbol{H}\Big[t_i; \hat{\boldsymbol{x}}(t_i^-)\Big]\boldsymbol{P}(t_i^-)\boldsymbol{H}^T\Big[t_i; \hat{\boldsymbol{x}}(t_i^-)\Big] + \boldsymbol{R}(t_i)\Big\}^{-1} \tag{6}$$

$$\boldsymbol{P}(t_i^+) = \boldsymbol{P}(t_i^-) - \boldsymbol{K}(t_i)\boldsymbol{H}\Big[t_i; \hat{\boldsymbol{x}}(t_i^-)\Big]\boldsymbol{P}(t_i^-) \tag{7}$$

where $\boldsymbol{P}$ is the state covariance matrix and $\boldsymbol{H}[t_i; \hat{\boldsymbol{x}}(t_i^-)]$ is the m-by-n measurement matrix defined as

$$\boldsymbol{H}\Big[t_i; \hat{\boldsymbol{x}}(t_i^-)\Big] = \left.\frac{\partial \boldsymbol{h}[\boldsymbol{x}, t_i]}{\partial \boldsymbol{x}}\right|_{\boldsymbol{x}=\hat{\boldsymbol{x}}(t_i^-)} \tag{8}$$

The estimate and covariance matrices are propagated forward to the next sampling time t_{i+1} by integrating

$$\dot{\hat{\boldsymbol{x}}}(t \mid t_i) = \boldsymbol{f}\Big[\hat{\boldsymbol{x}}(t \mid t_i), \boldsymbol{u}(t), t\Big] \tag{9}$$

$$\dot{\boldsymbol{P}}(t \mid t_i) = \boldsymbol{F}\Big[t; \hat{\boldsymbol{x}}(t \mid t_i)\Big]\boldsymbol{P}(t \mid t_i) + \boldsymbol{P}(t \mid t_i)\boldsymbol{F}^T\Big[t; \hat{\boldsymbol{x}}(t \mid t_i)\Big] + \boldsymbol{G}(t)\boldsymbol{Q}(t)\boldsymbol{G}^T(t) \tag{10}$$

using the initial conditions provided by

$$\hat{\boldsymbol{x}}(t_i \mid t_i) = \hat{\boldsymbol{x}}(t_i^+) \tag{11}$$

$$\boldsymbol{P}(t_i \mid t_i) = \boldsymbol{P}(t_i^+) \tag{12}$$

Here $\boldsymbol{F}[t; \hat{\boldsymbol{x}}(t \mid t_i)]$ is the n-by-n state Jacobian matrix defined by

$$\boldsymbol{F}\Big[t; \hat{\boldsymbol{x}}(t \mid t_i)\Big] = \left.\frac{\partial \boldsymbol{f}[\boldsymbol{x}, \boldsymbol{u}(t), t]}{\partial \boldsymbol{x}}\right|_{\boldsymbol{x}=\hat{\boldsymbol{x}}(t \mid t_i)} \tag{13}$$

1.2. Iterative Linearized Kalman Filter

The basic idea of the iterative linearized Kalman filter is to relinearize about each estimate $\hat{\boldsymbol{x}}(t)$. At measurement time t_i, the measurement $\boldsymbol{z}(t_i, w_j) = \boldsymbol{z}_i$ becomes available. The estimate is updated iteratively by relinearizing about the current estimate.

$$\begin{aligned}\hat{\boldsymbol{x}}(t_i^+)^{i+1} = \hat{\boldsymbol{x}}(t_i^-) + \boldsymbol{K}(t_i)\Big\{ & \boldsymbol{z}(t_i) - \boldsymbol{h}\Big[\hat{\boldsymbol{x}}(t_i^-)^i, t_i\Big] \\ & - \boldsymbol{H}\Big[t_i, \hat{\boldsymbol{x}}(t_i^+)^i\Big]\Big(\hat{\boldsymbol{x}}(t_i^-) - \hat{\boldsymbol{x}}(t_i^+)^i\Big)\Big\}\end{aligned} \tag{14}$$

$$\begin{aligned}\boldsymbol{K}(t_i) = \boldsymbol{P}(t_i^-)\boldsymbol{H}^T\Big[t_i; \hat{\boldsymbol{x}}(t_i^+)^i\Big]\Big\{ & \boldsymbol{H}\Big[t_i; \hat{\boldsymbol{x}}(t_i^+)^i\Big]\boldsymbol{P}(t_i^-)\boldsymbol{H}^T\Big[t_i; \hat{\boldsymbol{x}}(t_i^+)^i\Big] \\ & + \boldsymbol{R}(t_i)\Big\}^{-1}\end{aligned} \tag{15}$$

$$\boldsymbol{P}(t_i^+) = \boldsymbol{P}(t_i^-) - \boldsymbol{K}(t_i)\boldsymbol{H}\Big[t_i; \hat{\boldsymbol{x}}(t_i^+)^i\Big]\boldsymbol{P}(t_i^-) \tag{16}$$

where $\boldsymbol{H}(t_i; \hat{\boldsymbol{x}}(t_i^+)^i]$ is the m-by-n measurement matrix

$$\boldsymbol{H}\Big[t_i; \hat{\boldsymbol{x}}(t_i^+)^i\Big] = \left.\frac{\partial \boldsymbol{h}\Big[\boldsymbol{x}, t_i\Big]}{\partial x}\right|_{\boldsymbol{x}=\hat{\boldsymbol{x}}(t_i^+)^i} \tag{17}$$

The iteration with a starting value $\hat{\boldsymbol{x}}(t_i^+)^1 = \hat{\boldsymbol{x}}(t_i^-)$ proceeds until $|\hat{\boldsymbol{x}}(t_i^+)^l - \hat{\boldsymbol{x}}(t_i^+)^{l-1}| \leq \epsilon$ is reached. The bound ϵ must be suitably chosen.

A steady-state value, $\boldsymbol{K}_{SS}$, cannot be used in this system, because the measurement matrix is not constant. Therefore, the last term of eqn. (14) cannot be neglected.

1.3. A Static Parameter Estimation Algorithm

When a parameter, which is considered as a constant, is estimated by applying dynamic estimation algorithms, the estimates are usually noisy. This noise level can be attenuated by using a static estimation algorithm. Noisy estimates are related to a constant model parameter vector $\boldsymbol{\theta}$ by

$$\boldsymbol{y} = \boldsymbol{\theta} + \boldsymbol{v} \tag{18}$$

where $\boldsymbol{y}$ are the model parameters calculated from the extended Kalman filter and $\boldsymbol{v}$ is an m vector representing parameter identification uncertainty. It is desired to obtain the best estimate of θ, denoted $\hat{\theta}$, such that

$$J = \frac{1}{2}(\boldsymbol{\theta} - \overline{\boldsymbol{\theta}})^T \boldsymbol{M}^{-1}(\boldsymbol{\theta} - \overline{\boldsymbol{\theta}}) + (\boldsymbol{y} - \boldsymbol{\theta})^T \boldsymbol{R}^{-1}(\boldsymbol{y} - \boldsymbol{\theta}) \tag{19}$$

is minimum where $\boldsymbol{R}$ is a symmetric positive definite matrix and $\boldsymbol{M}$ is the current covariance of the parameters from extended Kalman filtering. This is accomplished by setting

$$\frac{\partial \boldsymbol{J}}{\partial \hat{\boldsymbol{\theta}}} = 0 \tag{20}$$

Therefore, the best least-square error estimate of $\boldsymbol{\theta}$ becomes

$$\hat{\boldsymbol{\theta}} = \overline{\boldsymbol{\theta}} + \boldsymbol{P}\boldsymbol{R}^{-1}(\boldsymbol{y} - \overline{\boldsymbol{\theta}}) \tag{21}$$

where

$$\boldsymbol{P}^{-1} = \boldsymbol{M}^{-1} + \boldsymbol{R}^{-1} \tag{22}$$

Thus, the new estimate is equal to the old one plus a linear correction term based on the difference between a new Kalman filter value and the old static estimate.

2. Application of the Extended Kalman Filter to a Recombinant Bacterial System

Estimation algorithms were applied to the fed-batch system model (Lee and Ramirez, 1992) for the induced production of a foreign protein. The reactor system is shown in Fig. 1. The system has two control variables, glucose and inducer feeding rates. There are five important state variables: reactor volume, cell concentration, glucose concentration, protein concentration, and inducer concentration. The glucose and inducer feeding rates are controlled to follow the optimal trajectories (Fig. 2). Two measurement systems were incorporated into the system. The glucose concentration can be measured on-line by a YSI 2000 system from Yellow Springs Instrument Co., Inc. The cell concentration can be measured on-line by spectrometry (Lee, 1992). Simulation work is performed in this study to find the influence of the covariance matrices, $\boldsymbol{Q}$ and $\boldsymbol{R}$. The influences of the unmeasured disturbances, the incorrect initial state estimates, and the incorrect model parameters were also considered. The extended Kalman filter is compared to the iterative linearized Kalman filter and the static estimation algorithm is tested.

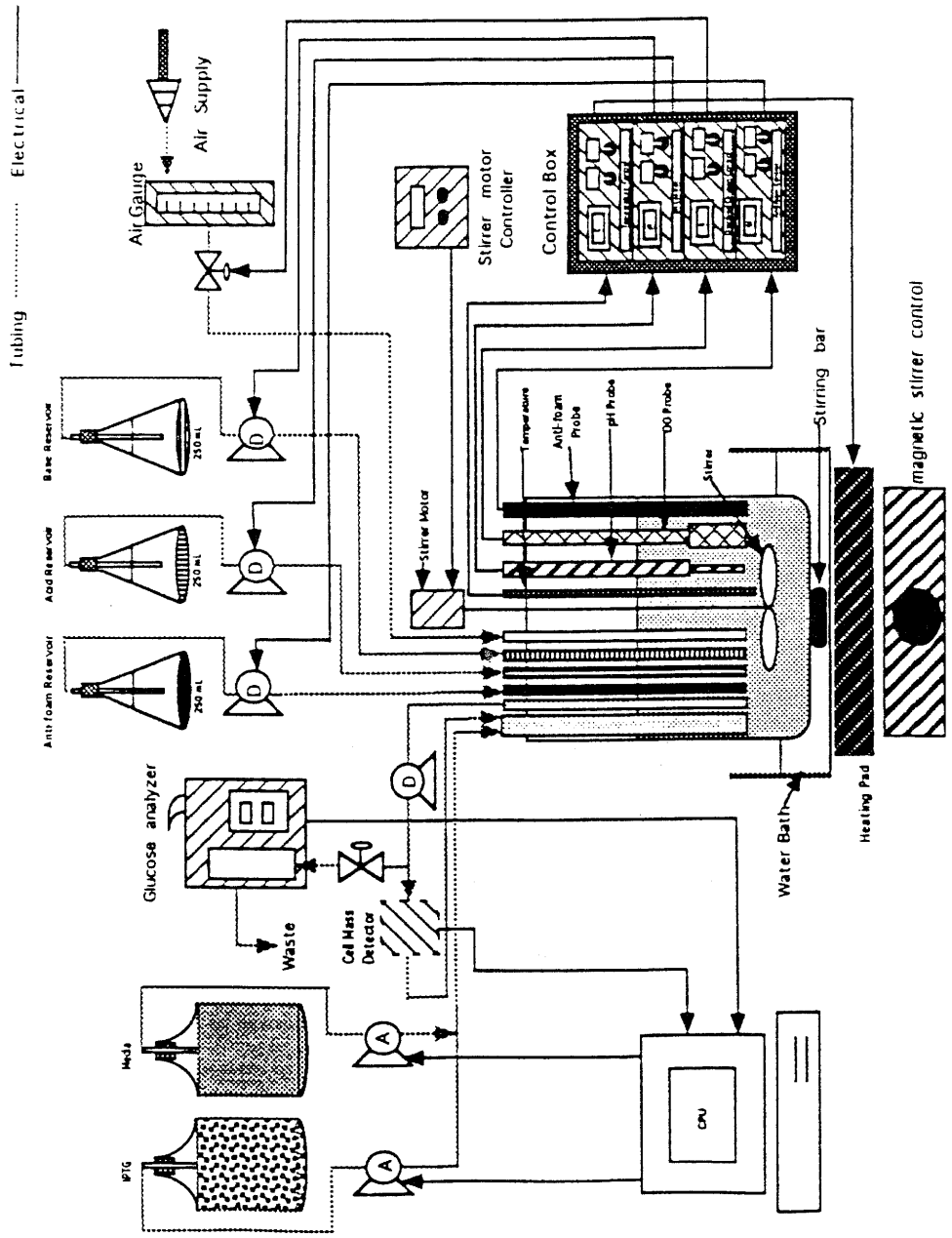

Fig. 1. Bioreactor system.

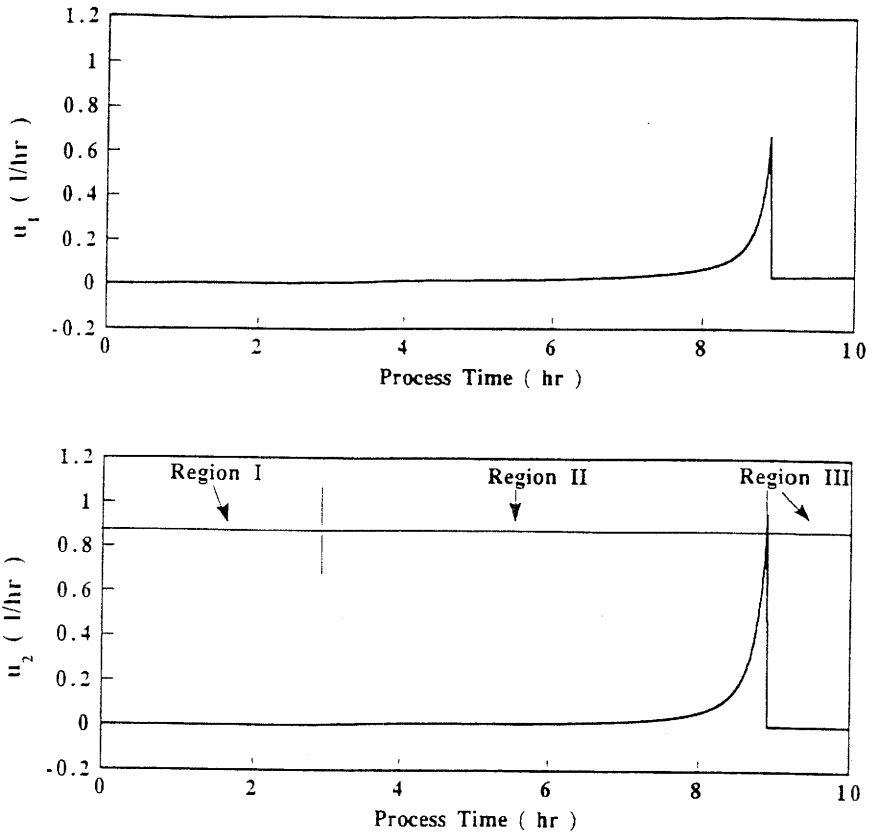

Fig. 2. Optimal bang-singular-bang control policies for the glucose and inducer feeding rates: (a) glucose feeding rate, (b) inducer feeding rate.

2.1. A Stochastic Model

The deterministic model of Lee and Ramirez (1992) can be expressed in a state variable form as

$$\begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \\ \dot{x}_4 \\ \dot{x}_5 \\ \dot{x}_6 \\ \dot{x}_7 \end{pmatrix} = \begin{pmatrix} 0 \\ \mu(x_3,x_5,x_6,x_7)x_2 \\ -Y^{-1}\mu(x_3,x_5,x_6,x_7)x_2 \\ R_{fp}(x_3,x_5)x_2 \\ 0 \\ -k_1(x_5)x_6 \\ k_2(x_5)(1-x_7) \end{pmatrix} + \begin{pmatrix} 1 & 1 \\ -\frac{x_2}{x_1} & -\frac{x_2}{x_1} \\ \frac{C_n^f}{x_1} - \frac{x_3}{x_1} & -\frac{x_3}{x_1} \\ -\frac{x_4}{x_1} & -\frac{x_4}{x_1} \\ -\frac{x_5}{x_1} & \frac{C_i^f}{x_1} - \frac{x_5}{x_1} \\ 0 & 0 \\ 0 & 0 \end{pmatrix} \begin{pmatrix} u_1 \\ u_2 \end{pmatrix} \quad (23)$$

where x_1 is the reactor volume, x_2 the cell density, x_3 the limiting nutrient concentration, x_4 the foreign protein concentration, x_5 the inducer concentration, x_6 the shock rate effect, x_7 the recovery rate effect, u_1 the glucose feeding rate,and u_2 the inducer feeding rate.

Lee and Ramirez (1992) have determined the specific growth rate, μ, the foreign protein production rate, R_{fp}, and shock and recovery parameters, k_1 and k_2, for a recombinant bacteria system. The *E. coli* host-vector system produces the foreign protein, β-galactosidase. Isopropylthiogalactoside (IPTG) was used as the inducer. Since it is found that there is growth inhibition by substrates such as glucose at high concentrations, the specific growth rate was formulated as

$$\mu = \frac{\mu_{\max} x_3}{K_{C_N} + x_3 + \frac{x_3^2}{K_S}} \left\{ x_6 + x_7 R_R(x_5) \right\} \tag{24}$$

where $\mu_{\max}$ and K_{C_N} are Monod type constants and K_S is a substrate inhibition constant. The recovery ratio, $R_R(x_5)$, is defined as

$$R_R(x_5) = \frac{K_{C_I}}{K_{C_I} + x_5} \tag{25}$$

The foreign protein production rate is described as

$$R_{fp} = \left(\frac{f_{\max} x_3}{K_{C_N} + x_3 + \frac{x_3^2}{K_S}} \right) \left(\frac{f_{I_O} + x_5}{K_I + x_5} \right) \tag{26}$$

where $f_{\max}$ and K_I are Monod type constants and f_{I_O} is a constant.

The shock and recovery parameters, k_1 and k_2, are defined as

$$k_1 = \frac{k_{11} x_5}{K_{IX} + x_5} \tag{27}$$

$$k_2 = \frac{k_{22} x_5}{K_{IX} + x_5} \tag{28}$$

where k_{11}, k_{22}, and K_{IX} are constants.

The initial conditions for the system are

$$x_1(0) = 1\,\mathrm{L} \tag{29}$$

$$x_2(0) = 0.1 \tag{30}$$

$$x_3(0) = 40\,\mathrm{g/L} \tag{31}$$

$$x_4(0) = 0\,\mathrm{g/L} \tag{32}$$

$$x_5(0) = 0\,\mathrm{g/L} \tag{33}$$

$$x_6(0) = 1 \tag{34}$$

$$x_7(0) = 0 \tag{35}$$

For this system, the measurements are unique functions of the cell density (x_2), the glucose concentration (x_3), and the protein concentration (x_4)

$$\begin{pmatrix} z_1 \\ z_2 \\ z_3 \end{pmatrix} = \begin{pmatrix} h_2(x_2) \\ h_3(x_3) \\ h_4(x_4) \end{pmatrix} \tag{36}$$

where h_i is the measurement function and z_i the digital signal from the sensing system which is a computer recognizable digital number.

2.2. An Optimal Control Policy

The system has two control variables, glucose and inducer feeding rates. Optimal control laws that maximize the profitability of the fed-batch reactor system can be obtained by applying Pontryagin's maximum principle (Lee and Ramirez, 1994). A bang-singular-bang (minimum-singular-minimum) control policy was identified in that work. The glucose concentration is always kept on an optimal singular arc yielding an optimal feeding policy shown in Fig. 2(a). For the bang-singular-bang control policy (Fig. 2(b)), the inducer control policy can be divided into three sub-regions. In the first region, inducer is not added in order to increase the numbers of cells. In the second region, inducer is added in an exponential way to produce the maximum amount of foreign protein. In the last region, minimum control effort was used in order to minimize the costs associated with this control variable.

2.3. Parameter and State Estimations

The model parameters can be determined by an off-line method (Lee and Ramirez, 1992). But, for the purpose of on-line optimization, an on-line parameter estimation method needs to be used. An extended Kalman filter can be used to estimate model parameters and state variables at the same time. In many applications, the robustness of the Kalman filter yields adequate state estimation despite parameter uncertainties. Sometimes it is necessary to add pseudonoise to the filter model to cause a heavier weighting of real world measurements, thereby decreasing sensitivity to erroneously assumed parameter values (Maybeck, 1982). For our simulation work, the parameters and states are estimated simultaneously by using the extended Kalman filter for estimation.

2.4. Simultaneous Estimation of System Parameters and States

The parameters to be estimated are assumed to be new states. For this system, the most important unknown parameters are $\mu_{\max}$, k_{l1}, and $f_{\max}$. The parameter $f_{\max}$ is estimated best by an off-line method (Lee and Ramirez, 1992). The parameters, $\mu_{\max}$ and k_{l1}, are considered auxiliary state variables, x_8 and x_9, respectively. The

two assumed states are incorporated into the system equation set (23) as follows:

$$\dot{x}_8 = 0 \tag{37}$$

$$\dot{x}_9 = 0 \tag{38}$$

The model says that the new states (parameters) are constant or at best slowly varying so that a quasi-steady-state assumption can be used.

With new system state variables, a new formulated state equation set is constructed as

$$\boldsymbol{F}\left[t; \hat{\boldsymbol{x}}(t \mid t_i)\right] = \left.\frac{\partial \boldsymbol{f}[\boldsymbol{x}, \boldsymbol{u}(t), t]}{\partial \boldsymbol{x}}\right|_{\boldsymbol{x}=\hat{\boldsymbol{x}}(t \mid t_i)}$$

$$= \left.\begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ f_{21} & f_{22} & f_{23} & 0 & f_{25} & f_{26} & f_{27} & f_{28} & 0 \\ f_{31} & f_{32} & f_{33} & 0 & f_{35} & f_{36} & f_{37} & f_{38} & 0 \\ f_{41} & f_{42} & f_{43} & f_{44} & f_{45} & 0 & 0 & 0 & 0 \\ f_{51} & 0 & 0 & 0 & f_{55} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & f_{65} & f_{66} & 0 & 0 & f_{69} \\ 0 & 0 & 0 & 0 & f_{75} & 0 & f_{77} & 0 & f_{79} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}\right|_{\boldsymbol{x}=\hat{\boldsymbol{x}}(t \mid t_i)} \tag{39}$$

where the f_{ij} functions are given in the Appendix. Also, the measurement matrix is

$$\boldsymbol{H}\left[t_i; \hat{\boldsymbol{x}}(t_i^-)\right] = \left.\frac{\partial \boldsymbol{h}[\boldsymbol{x}, t_i]}{\partial \boldsymbol{x}}\right|_{\boldsymbol{x}=\hat{\boldsymbol{x}}(t_i^-)}$$

$$= \left.\begin{pmatrix} 0 & \frac{\partial h_2}{\partial x_2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{\partial h_3}{\partial x_3} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{\partial h_4}{\partial x_4} & 0 & 0 & 0 & 0 & 0 \end{pmatrix}\right|_{\boldsymbol{x}=\hat{\boldsymbol{x}}(t_i^-)} \tag{40}$$

2.5. Observability of the System

Based on the matrices, $\boldsymbol{F}[t; \hat{\boldsymbol{x}}(t \mid t_i)]$ and $\boldsymbol{H}[t_i; \hat{\boldsymbol{x}}(t_i^-)]$, the following observability matrix, $\boldsymbol{m}$, is constructed

$$\boldsymbol{m} = \left[\boldsymbol{F}^T\boldsymbol{H}^T \mid (\boldsymbol{F}^T)^2\boldsymbol{H}^T \mid \cdots \mid (\boldsymbol{F}^T)^{n-1}\boldsymbol{H}^T\right] \tag{41}$$

$$\text{rank}\,[\boldsymbol{m}] = n \tag{42}$$

It can be easily shown that the matrix $\boldsymbol{m}$, is a full rank matrix. The system is therefore observable.

3. Simulation

The purpose of this simulation work is to confirm the feasibility of the on-line estimation of both parameters and states. To achieve this goal, the following issues were considered:

1. The influence of ratio of the system Gaussian noise covariance matrix, $\boldsymbol{Q}$, to the measurement noise covariance matrix, $\boldsymbol{R}$,
2. The influence of unmeasured process disturbances,
3. The influence of incorrect estimates of the initial state,
4. The influence of errors in model parameters,
5. A comparison between the extended Kalman filter and the iterative linearized Kalman filter,
6. The application of the static parameter estimation algorithm.

Even though the extended Kalman filter was derived by assuming a Gaussian distribution for the measured variables, uniformly distributed random noise was added to simulate the noisy measurements of the processes. This was done because the actual process noise was assumed to have a distribution between that of uniform and Gaussian. Thus, a uniformly distributed noise was apt to test the filter algorithm more severely. Noise, consisting of random numbers between 0.1 and -0.1, was added to the measurements. The standard deviation, σ, of the noise added to the cell concentration (OD) was 0.064. The standard deviations of the noise added to the glucose and protein concentrations, were 0.064 g/L.

The parameters, $\mu_{\max}$ and k_{11}, are closely related to each other, because the parameter, k_{11}, is determined based on the value of the parameter, $\mu_{\max}$. A strategy to estimate them on-line is that, in the region without any inducer, the maximum growth rate is estimated, and then the shock and recovery constant is estimated in the region with inducer addition.

3.1. Influence of Noise Covariance Matrices, Q and R

The covariance matrices, $\boldsymbol{Q}$ and $\boldsymbol{R}$, of zero-mean white Gaussian noise were assumed as diagonal matrices. The physical interpretation of the diagonal matrices, $\boldsymbol{Q}$ and $\boldsymbol{R}$, is that the measurement noise is statistically independent and that the model uncertainties are diagonally dominant. The matrix $\boldsymbol{G}(t)$, is assumed as an identity matrix. The ratio of the values of diagonal elements, Q_{ii} and R_{ii}, are very important in calculating the Kalman gains (Maybeck, 1982).

Case I: $Q_{ii}/R_{ii} = 0.1$

The deterministic nonlinear model equation was integrated with the optimal control policy to simulate the actual process. Random numbers were added to the measurement model to simulate real stochastic measurements.

In this case, the ratio of Q_{ii} to R_{ii} is set at 0.1. The measurement uncertainty is larger than the model uncertainty. An estimator in cases $Q/R < 1$ will weigh the model's prediction, in contrast to the cases $Q/R > 1$ which will weigh the noisy measurements. This estimator ($Q_{ii}/R_{ii} = 0.1$) will depress the noise from the measurement system. Figures 3 through 7 show the simulation results. Figure 3 shows changes in the cell concentration during fermentation. The dotted line represents the true cell concentration based on the nonlinear model's prediction which in this case is almost equivalent to that of the optimal estimate (the bold solid line). Before injecting the inducer, the cell concentration increased exponentially. After injection (region II in Fig. 2), the specific growth rate decreased, reflecting the metabolic burden due to the injection of the inducer. During the final times of operation in region II, the concentration of cells inside the bioreactor decreased due to the dilution effect arising from the addition of glucose and inducer exponentially. As soon as the minimum control effort started (region III), the cell concentration increased since dilution is eliminated. The noisy measurements are also shown in Fig. 3 as the light solid line. The extended Kalman filter works well in estimating the cell concentration profile.

The glucose level inside the reactor is shown in Fig. 4. The glucose level is kept at its optimal concentration of 40 g/L. The glucose concentration is well estimated.

Figure 5 shows the protein level inside the bioreactor. In the first bang-bang period of inducer feeding, foreign protein production rate is minimized in order to maximize cell growth rate. After two hours, the estimated protein level became more noisy due to inducer injection.

Figure 6 shows the estimation results for the parameter $\mu_{\max}$, diagonal components of covariance matrix, and components of Kalman gain matrix. Figure 6(a) shows that the estimated values of the model parameter scatters around the expected value. By decreasing the values of Q_{ii}, fluctuations can be depressed. However, important updated innovations might be neglected, and an incorrect initial estimate will never be improved by the filter. Increasing the values of Q_{ii} will cause increased noise levels in the parameter estimations. This noise problem in constant parameter estimation can be mitigated by use of a static estimation algorithm discussed later. Figure 6(b) shows the changes in three important diagonal compo-

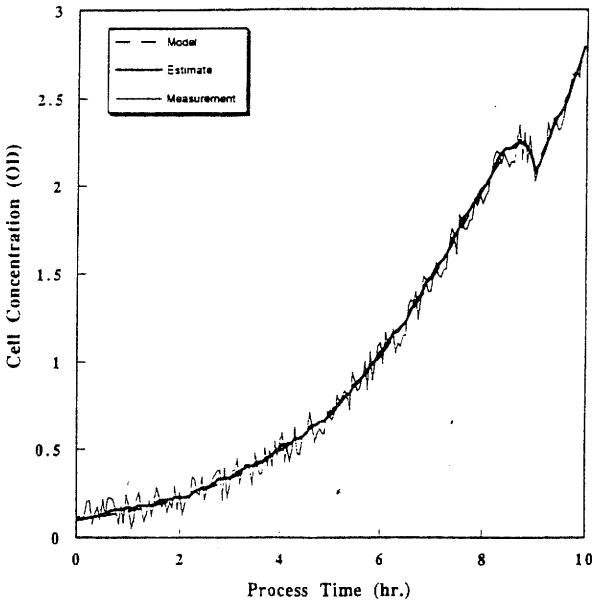

Fig. 3. Simulation result of cell concentration when $Q_{ii} = 0.1$ and $R_{ii} = 1$.

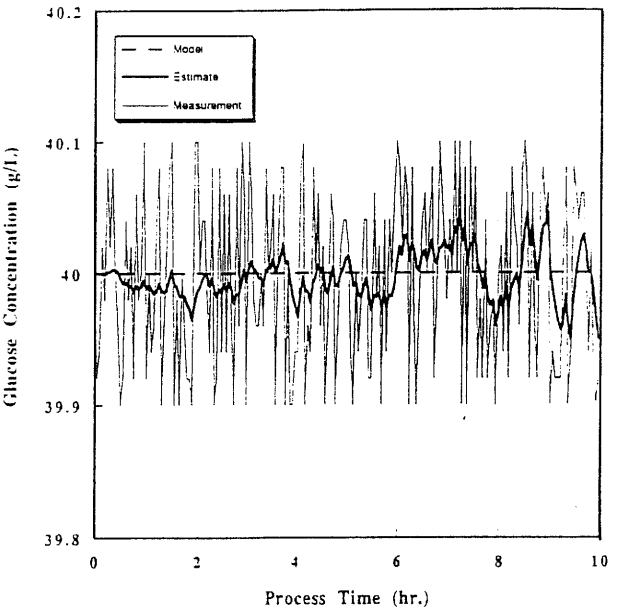

Fig. 4. Glucose level inside the bioreactor when $Q_{ii} = 0.1$ and $R_{ii} = 1$.

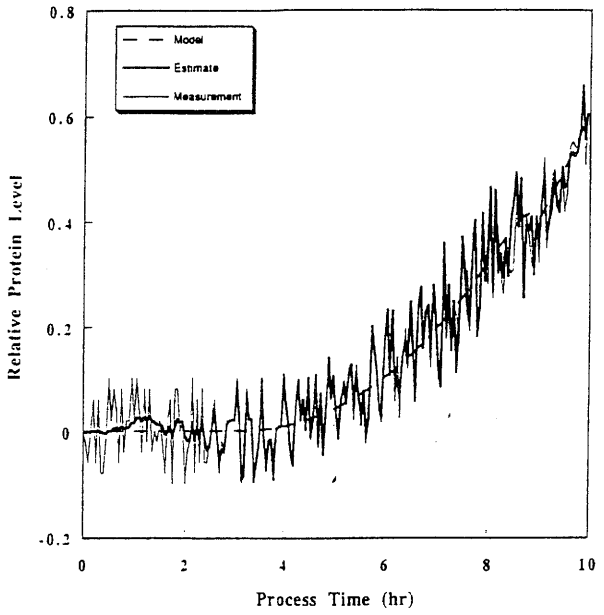

Fig. 5. Protein concentration inside the bioreactor when $Q_{ii} = 0.1$ and $R_{ii} = 1$.

nents of the covariance matrix P_{22} (biomass related), P_{33} (glucose concentration related), and P_{44} (protein concentration related). The covariance value of the protein related components, P_{44}, was larger than those of the other covariance components, P_{22} and P_{33}. Thus, the estimate of protein level is more uncertain than that of either cell mass or glucose concentration. Figure 6(c) shows values of various components of the Kalman gain matrix. As expected, the protein related component, K_{43}, of the Kalman gain matrix had larger value than the other components, K_{21} (biomass related) and K_{32} (glucose concentration related). Therefore, the extended Kalman filter weighted the protein measurement relatively more than the model prediction due to the larger uncertainty in that state covariance term.

Case II: $Q_{ii}/R_{ii} = 10$

In this case, the ratio of Q_{ii} to R_{ii} is set to 10. This was accomplished by keeping the same degree of noise in the measurements but increasing the model uncertainty. The measurement uncertainty is smaller than the model uncertainty. Therefore, the estimates will have less filtering of the measurements and will more closely track the noisy measurements in comparison with the cases, $Q_{ii}/R_{ii} < 1$. This estimator reflects the fact that the measurements are relatively better than the model predictions.

Figure 7 shows the results of estimating the parameter, $\mu_{\max}$ selected diagonal components of the covariance matrix, and components of the Kalman gain matrix when $Q_{ii}/R_{ii} = 10$. Figure 7(a) shows that the estimated values of the maximum specific growth rate parameter scatter around the expected value. Comparing with Fig. 6(a), the noise level is larger than that in Case I. Figure 7(b) shows the changes

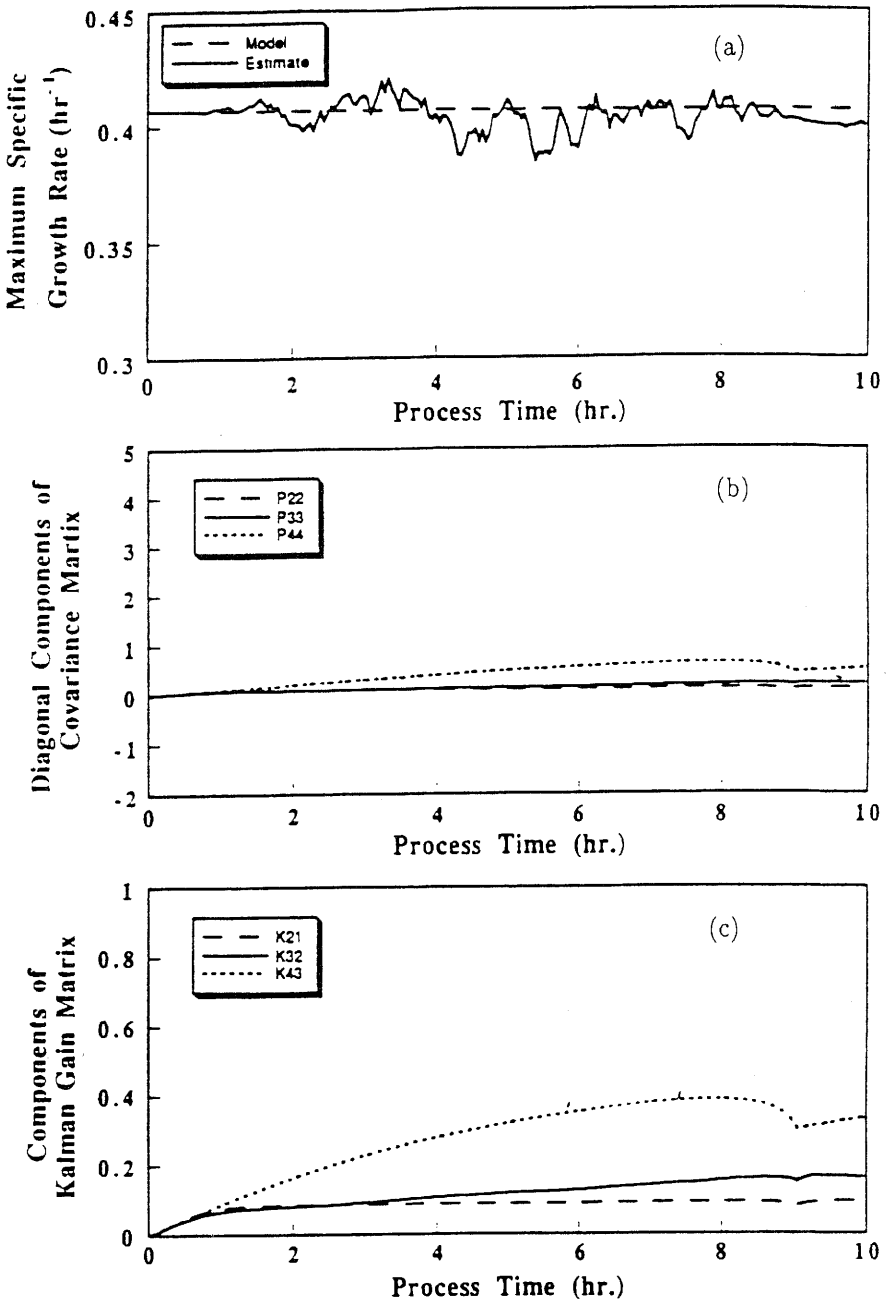

Fig. 6. Simulation results when $Q_{ii} = 0.1$ and $R_{ii} = 1$:
(a) estimation of parameter, $\mu_{\max}$,
(b) diagonal components of covariance matrix,
(c) components of Kalman gain matrix.

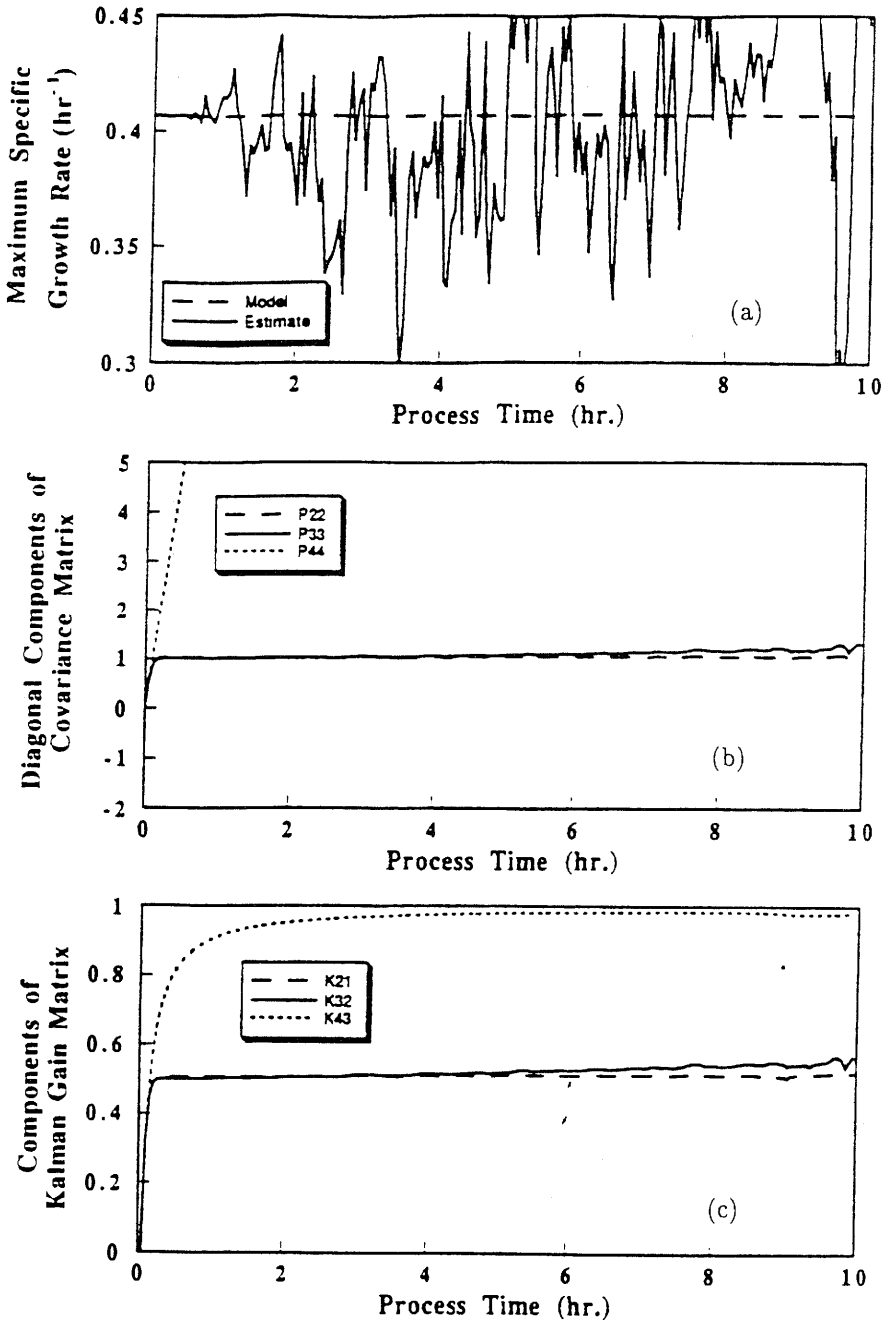

Fig. 7. Simulation results when $Q_{ii} = 10$ and $R_{ii} = 1$:
(a) estimation of parameter, $\mu_{\max}$,
(b) diagonal components of covariance matrix,
(c) components of Kalman gain matrix.

in three important diagonal components, P_{22}, P_{33}, and P_{44}, of the covariance matrix. The values of the covariance matrix components are larger than those in Case I. Thus, the estimates of cell mass and glucose concentrations, and protein concentration have more uncertainty than the estimates of Case I. Figure 7(c) shows components of the Kalman gain matrix. As expected, the important three components of the Kalman gain matrix, K_{21}, K_{32}, and K_{43} are larger than those in Case I. Therefore, the measurements are more important in determining optimal estimates than the model predictions.

3.2. Comparison Between Extended Kalman Filter and Iterative Linearized Kalman Filter

The iterative extended Kalman filter algorithm is compared to the extended Kalman filter in Figs. 8 and 9. In Fig. 8, a ten times iteration algorithm was used. The results show that the iterative version of the extended Kalman filter algorithm was more effective in estimating the parameters and states, even though the extended Kalman filter performed well. These simulations used a Q_{ii}/R_{ii} ratio of 0.1.

3.3. Application of the Static Parameter Estimation Algorithm

The static estimation algorithm can be applied again to minimize noisy estimates of system parameters assuming that the parameters are constant.

When the static estimation filter is applied to the results of the parameter estimates from the extended Kalman filter, parameter estimation noise can be decreased significantly as shown in Fig. 10. This is a very effective approach to parameter identification when the parameters are expected to be relatively constant.

4. Conclusions

An extended Kalman filter was applied to estimate the parameters and states of a fed-batch bioreactor. Simulation work on various cases was carried out to test the feasibility of the filter. The influence of the covariance matrices, Q and R, on the filter performance was studied. The ratio of Q to R is an important factor in performance. Cases when $Q/R < 1$ will track the model prediction, while cases when $Q/R > 1$ will track the noisy measurements.

An iterative version of the extended Kalman filter algorithm gives better estimates than the extended Kalman filter for the same Q and R design parameters. When the parameters to be estimated can be assumed constant, then the application of a static estimation algorithm is very effective in eliminating model parameter oscillations.

Acknowledgments

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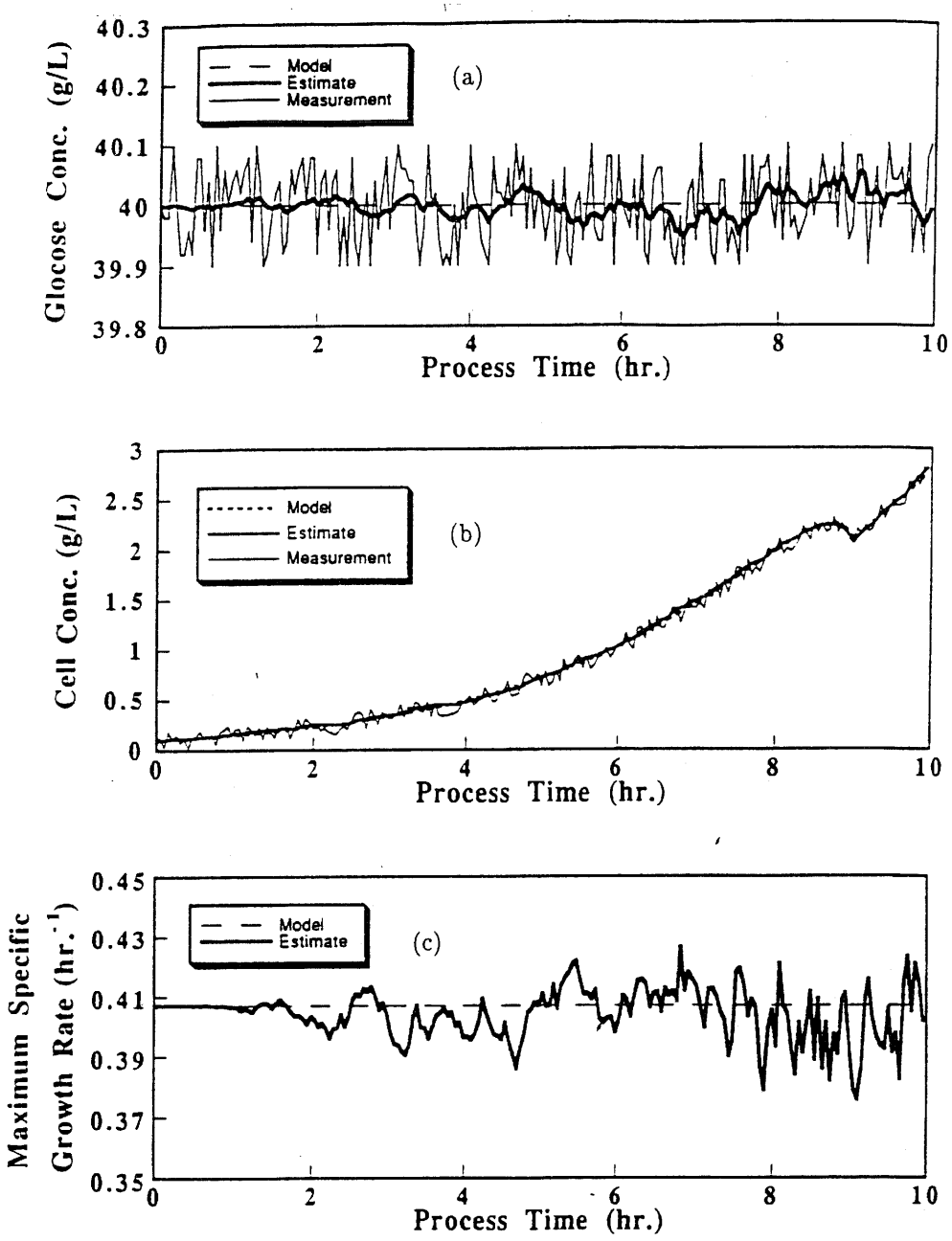

Fig. 8. Iterative extended Kalman filter (10 times).

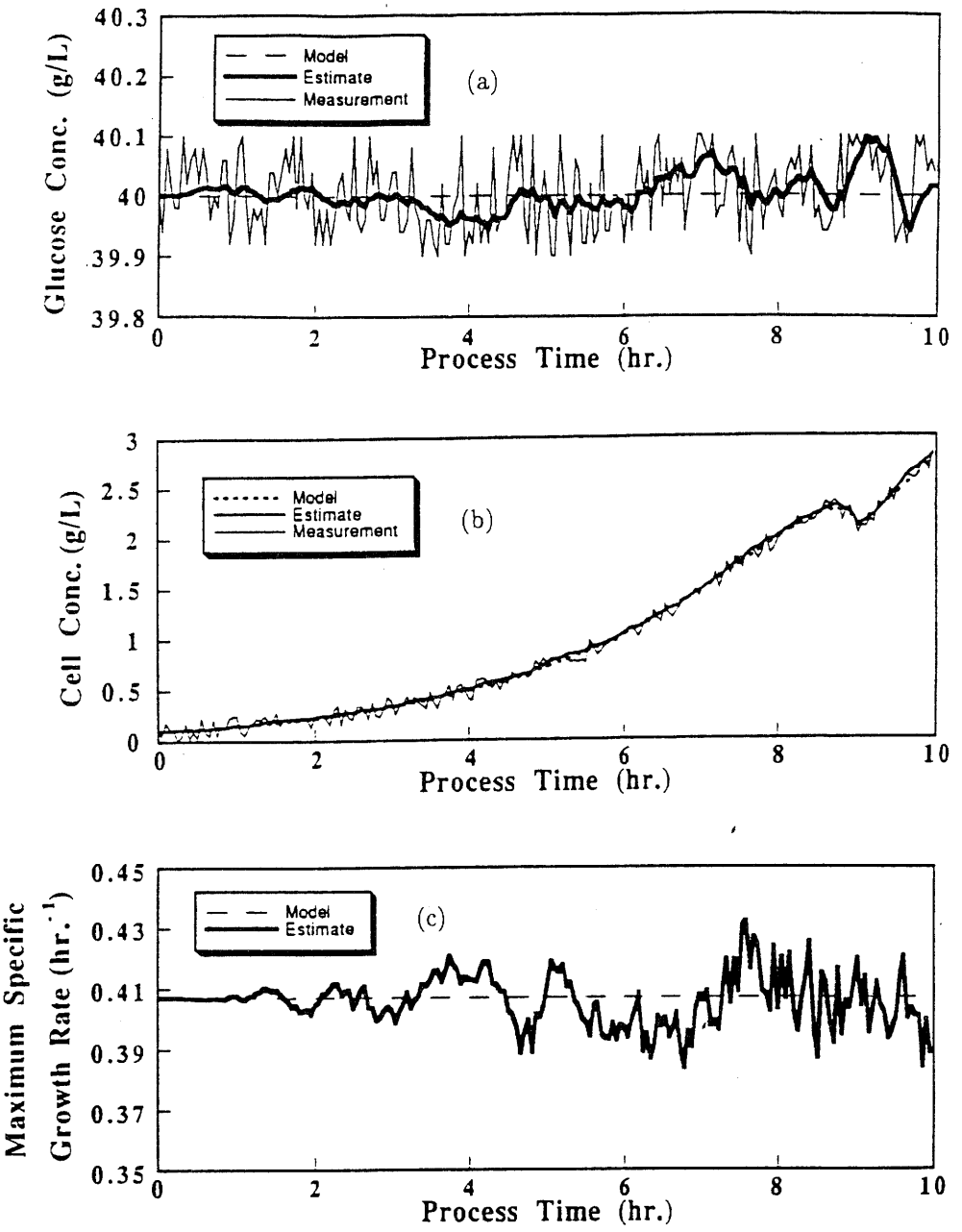

Fig. 9. Extended Kalman filter.

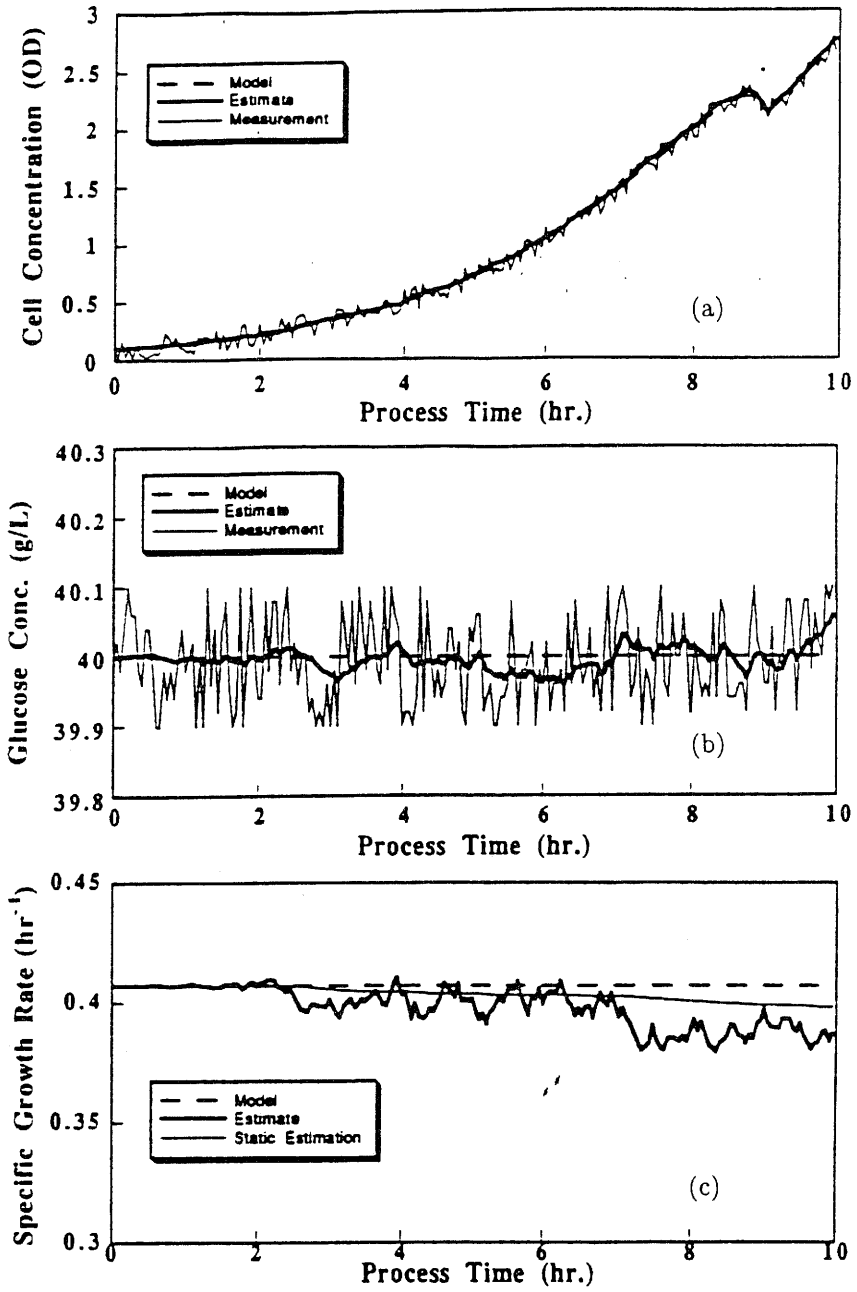

Fig. 10. Static estimation algorithm: (a) cell mass, (b) glucose concentration, (c) maximum specific growth rate. The thick solid line shows the estimates from the extended Kalman filter. The thin solid line denotes the results of the static estimation.

Nomenclature

C_i^f	inducer level of feed, g/L
C_n^f	nutrient level of feed, g/L
$\boldsymbol{f}[x(t), \boldsymbol{u}(t), t]$	a nonlinear system function of states and controls defined in eqn. (1)
f_{I_O}	a constant defined in eqn. (26)
$f_{\max}$	maximum protein production rate defined in eqn. (26)
$\boldsymbol{F}[t; \hat{\boldsymbol{x}}(t \mid t_i)]$	a partial derivative matrix defined in eqn. (13)
$\boldsymbol{G}(t)$	defined in eqn. (1)
$\boldsymbol{H}$	a measurement matrix in eqn. (18)
h_i	a measurement function shown in eqn. (36)
$\boldsymbol{h}[x(t_i), t]$	a nonlinear measurement function of states defined in eqn. (3)
$\boldsymbol{H}[t_i, \hat{\boldsymbol{x}}(t_i^-)]$	a partial derivative matrix defined in eqn. (8)
$\boldsymbol{J}$	an objective function defined in eqn. (19)
k_1	shock rate constant defined in eqn. (27)
k_{11}	a constant defined in eqn. (27)
k_2	recovery rate constant defined in eqn. (28)
k_{22}	a constant defined in eqn. (28)
K_{C_I}	a constant defined in eqn. (25)
K_{C_N}	a constant defined in eqn. (24)
K_I	a constant defined in eqn. (26)
K_{IX}	a constant defined in eqn. (27) and eqn. (28)
K_S	a substrate inhibition constant defined in eqn. (24)
$\boldsymbol{K}(t_i)$	Kalman filter gain defined in eqn. (5)
$\boldsymbol{P}(t)$	a covariance shown in eqn. (7)
$\boldsymbol{Q}$	a covariance kernel defined in eqn. (2)
$\boldsymbol{R}$	a covariance kernel shown in eqn. (4)
R_{fp}	foreign protein production rate defined in eqn. (26)
R_R	recovery ratio defined in eqn. (25)
t	time, hr
$\boldsymbol{u}(t)$	a vector of control variables in eqn. (1)

u_1	glucose feeding rate, L/h
u_2	inducer feeding rate, L/h
$v(t_i)$	a zero-mean white Gaussian noise measurement in eqn. (3)
$w(t)$	a zero-mean white Gaussian noise process in eqn. (1)
$x(t)$	a vector of state variables in eqn. (1)
$\hat{x}(t)$	estimate of state variables in eqn. (9)
x_1	culture volume, L
x_2	cell mass, g/L
x_3	nutrient level inside reactor, g/L
x_4	protein level inside reactor, g/L
x_5	inducer level insider reactor, g/L
x_6	shock rate effect
x_7	recovery rate effect
x_8	a new state variable (maximum specific growth rate, $\mu_{\max}$)
x_9	a new state variable (recovery rate effect constant, k_{11})
y	model parameters calculated by extended Kalman filtering
Y^{-1}	inverse of growth yield coefficient (produced cell mass/consumed nutrient)
z	measurement from sensor
$z(t_i)$	measurement vector
θ	static parameter vector
μ	specific growth rate, h^{-1}
$\mu_{\max}$	maximum specific growth rate, h^{-1}

Appendix

$$f_{21} = \frac{x_2(u_1+u_2)}{x_1^2}, \qquad f_{22} = \mu - \frac{u_1+u_2}{x_2}$$

$$f_{23} = \frac{\partial \mu}{\partial x_3}\, x_2, \qquad f_{25} = \frac{\partial \mu}{\partial x_5}\, x_2$$

$$f_{26} = \frac{\partial \mu}{\partial x_6}\, x_2, \qquad f_{27} = \frac{\partial \mu}{\partial x_7}\, x_2$$

$$f_{28} = \frac{\partial \mu}{\partial x_8}\, x_2$$

$$f_{31} = -\frac{(C_n^f - x_3)u_1 - x_3 u_2}{x_1^2}, \qquad f_{32} = -Y^{-1}\mu$$

$$f_{33} = \frac{\partial(-Y^{-1}\mu)}{\partial x_3}\, x_2 - \frac{u_1 + u_2}{x_1}, \qquad f_{35} = \frac{\partial(-Y^{-1}\mu)}{\partial x_5}\, x_2$$

$$f_{36} = \frac{\partial(-Y^{-1}\mu)}{\partial x_6}\, x_2, \qquad f_{37} = \frac{\partial(-Y^{-1}\mu)}{\partial x_7}\, x_2$$

$$f_{38} = \frac{\partial(-Y^{-1}\mu)}{\partial x_8}\, x_2$$

$$f_{41} = \frac{x_4(u_1 + u_2)}{x_1^2}, \qquad f_{42} = R_{fp}$$

$$f_{43} = \frac{\partial R_{fp}}{\partial x_3}\, x_2, \qquad f_{44} = -\frac{u_1 + u_2}{x_1}$$

$$f_{45} = \frac{\partial R_{fp}}{\partial x_5}\, x_2$$

$$f_{51} = \frac{x_5 u_1 - (C_i^f - x_5)u_2}{x_1^2}, \qquad f_{55} = -\frac{u_1 + u_2}{x_1}$$

$$f_{65} = \frac{\partial(-k_1)}{\partial x_5}\, x_6, \qquad f_{66} = -k_1$$

$$f_{69} = \frac{\partial(-k_1)}{\partial x_9}\, x_6$$

$$f_{75} = \frac{\partial k_2}{\partial x_5}\,(1 - x_7), \qquad f_{77} = -k_2$$

$$f_{79} = \frac{\partial k_2}{\partial x_9}\,(1 - x_7)$$

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