생촉매 반응기에서의 활성도 추정

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Activity Estimation in a Biocatalyst Reactor

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ABSTRACT

In immobilized cell reactors, effective cell mass is a very important parameter which must be estimated during operation for control and regeneration of biocatalyst. In this report, the effective cell mass in immobilized cell reactor was studied using a sequential estimation method. An immobilized yeast reactor was operated in batch recycle mode. The states of the immobilized cell reactor could be estimated from the process data using an extended Kalman filter.

INTRODUCTION

The application of immobilized living cells as biocatalysts represents a new and rapidly growing trend in biotechnology. Considerable research on immobilized cell system has been conducted over the past several years (1,2,3). One of the important application of immobilized cell culture is the production of alcohol from renewable resources (4,5). For the application of immobilized cell system, the system should be understood. Especially identification of states of the cells and stability of biocatalysts during operation is an important factor. For an immobilized cell reactor, effective cell mass should be estimated for the optimal control of the bioreactor, especially the flow rate control and regeneration of the cell.

It is very difficult to estimate the effective cell mass accurately, for porous catalyst systems, because too many factors influence the result (6). Internal and external mass transfer can not be accurately considered, because diffusivities of substrate are different and difficult to measure, and the partition coefficient should be incorporated. In the case of an immobilized enzyme, the amount of initial charge is known and the enzyme remains unchanged except being deactivated by shear or heat. But the system becomes more complicated in the case of immobilized cells; initial cell mass is very difficult to measure, a dense cell layer exists in the support matrix, and the dead cells cannot be distinguished from the live cells. Also the cells change with the nutritional and environmental conditions (7). Even though the total mass in biocatalysts can be measured, it has no meaning because the reaction is determined by the effective cell mass only. Product or substrate concentrations can be obtained by on-line or off-line measurements but effective cell mass cannot be measured, instead this should be estimated from possible mesurements. By employing on-line and/or off-line measurements, sequential estimation of the effective cell mass is possible.

The optimal control and regeneration of an immobilized cell system require the updated estimates of the system state variables and parameters based on the limited number of measurements containing errors. Random errors and noises are always present in the process and the measurements. To meet the demands of an effective state estimation from discrete or continuous measurements, sequential estimation algorithms have been developed (8). This sequential estimation theory has been applied to chemical and biochemical engineering processes. Gavalas et al (9,10) applied the Cox filter and extended Kalman filter to a plug flow reactor with first order irreversible reaction and catalyst decay and got good results. Wells (11) showed an application of extended Kalman estimator to a well-stirred reac-tor. Kiparissides et al (12) included this method for an optimal stochastic control of a continuous latex reactor. Recently this method was applied to a biochemical engineering processes. Jefferis et al (13) used a digital filtering technique for the fermentation process analysis. They estimated the cell density and the growth rate from the periodic measurements of turbidity. Suvcek et al (14) applied the extended Kalman filter to a continuous culture. The state variables and the constants in the Monod equation were estimated by this method. Stephanopoulos and San (15,16) applied this filter method to a fermentor for the on-line bioreactor

identification using elemental balance equations. Yoo et al (17,18) applied the sequential parameter estimation method to the optimal control of fed batch culture.

In this paper, a sequential estimation of effective cell mass and state variables were used to identify the immobilized cell reactor system.

SEQUENTIAL ESTIMATION METHOD

The extended Kalman filter technique is well established as a powerful sequential estimator and has been applied to many areas. The extended Kalman filter determines the estimates of the states which minimize the least squares of an objective function (8). The system equations are expressed by the difference equations

$$x_{k+1} = f(x_k, u_k) + w'_k \quad (1)$$

where, f_k is a vector function of the state x_k and the control u_k, and x_k is a state vector of the system. w'_k is a zero mean, white Gaussian noise process that may represent either actual input disturbance or inaccuracies in the system model. The observation equations are represented by the relation

$$z_k = h_k(x_k) + v_k \quad (2)$$

where z_k is an observation vector, h is also a vector function of the state variables and v_k is a zero-mean, white Gaussian noise process assumed to be independent of w'_k. The respective covariances of w'_k and v_k are Q_k and R_k. The estimate of x through time k is denoted by $x_{k/k}$. $P_{k/k}$ denotes the covariance of the error in this estimate. Then the extended Kalman filter is represented by the following equations;

prediction

$$\hat{x}_{k/k-1} = f_{k-1}(\hat{x}_{k-1/k-1}, u_{k-1}) \quad (3)$$

correction

$$\hat{z}_{k/k-1} = h_k(\hat{x}_{k/k-1}) \quad (4)$$

$$P_{k/k-1} = \phi_{k-1} P_{k-1/k-1} \phi_{k-1}^T + Q_{k-1} \quad (5)$$

$$\hat{x}_{k/k} = \hat{x}_{k/k-1} + w_k (z_k - \hat{z}_{k/k-1}) \quad (6)$$

$$P_{k/k} = (I - w_k H_k) P_{k/k-1} \quad (7)$$

where

$$\phi_{k-1} = \left. \frac{df_{k-1}}{dx_{k-1}} \right|_{\hat{x}_{k-1/k-1}} \quad (8)$$

$$H_k = \left. \frac{dh_k}{dx_k} \right|_{\hat{x}_{k/k-1}} \quad (9)$$

$$w_k = P_{k/k-1} H_k^T (H_k P_{k/k-1} H_k^T + R_k)^{-1} \quad (10)$$

From these equation (3-10), states of the variables can be predicted and corrected.

EXPERIMENTS

5g of sodium alginate was dissolved in a 250 ml water and 5 g of lyophilized Baker's yeast was rehydrated in 100 ml of water. The above solution were mixed well and the mixture was pumped into 500 ml of 0.05 M $CaCl_2$ solution through a No. 20 syringe at a temperature below 5 °C. The droplets were suspended for 2 hours to allow gelation. Beads of 2.0 mm diameter were obtained. The beads containing yeast were filled into a glass column (25 mm ø x 200 mm H) and the complete nutrient medium was passed through the column for 48 hours to activate the cells and to increase the number of cells inside the beads.

The composition of the nutrient medium was; glucose 100g, $(NH_4)_2SO_4$ 21.4g, $MgSO_4 7H_2O$ 0.29 g, yeast extract 1.14 g, KH_2PO_4 3g, K_2HPO_4 1 g, $CaCl_2$ 2.75 g in 1000 ml of nutrient solution. All the experiments were done in an aseptic condition and the chemicals used were reagent grade. The temperature of the reactor was maintained at 30 °C. A batch recycle reactor was used to study the immobilized cell reaction scheme.

The experimental set up is shown in Figure 1. The medium was changed after activation to a production medium which contained a known amount of glucose and 0.114 %(w/v) of yeast extract. Yeast extract was added to medium to maintain the cell viability during alcohol production. Samples were taken from the reservoir and the concentration of glucose and ethanal were measured.

Glucose was analyzed enzymatically by a glucose analyzer, ethanol was analyzed by gas chromatography. To measure the cell mass in the beads, 20 beads were taken intermittently during the experiment and dissolved in 0.1 M sodium citrate solution. After being dissolved, centrifuged, washed, it was dried in the oven over night at 90 °C. Total cell mass in the column was

obtained by multiplying the number of beads by the cell mass in the bead.

Table 1. shows the experimental conditions of the immobilized yeast reactor.

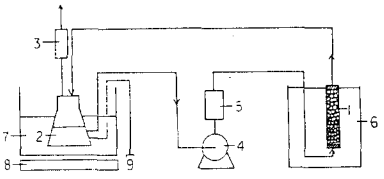


Figure 1. Experimental system of batch recycle reactor.
1. immobilized yeast reactor 2. reservoir 3. condenser 4. pump 5. flow meter 6. constant temperature water bath 7. low temperature water bath 8. magnetic stirrer 9. sampling line

Table 1. Experimental condition of immobilized yeast reactor

condition	Exp. No.1	Exp. No.2
initial substrate concen. S_i (g/l)	100	130
gel volume (ml)	70	65
reservoir volume (ml)	1500	1000
flow rate (ml/hr)	1100	1100
total cell mass (g)	4.86	4.33
cell mass in gel (g/l of gel)	69.4	66.6
cell mass in reactor (g/l)	109.0	71.0

* cell mass in reactor volume was calculated to employ the kinetic expression as in equation (11).

$$\text{cell mass in reactor} = \text{total cell mass} \times \frac{\text{reservoir + gel volume}}{\text{gel volume}}$$

SIMULATION

It was reported that high concentrations of substrate and product inhibit the production of alcohol (19). The following equation was used by Aiba et al (20) for the alcohol production kinetics of the yeast.

$$r = V_m \frac{S}{K_s + S} \frac{K_p}{K_p + P} X \qquad (11)$$

$$P - P_i = Y_{P/s} (S_i - S) \qquad (12)$$

where r is the reaction rate, P and P_i is the product concentration and initial product concentration respectively, S and S_i is the substrate concentration and initial substrate con-centration, x is the cell mass, $Y_{P/s}$ is the product yield from substrate and V_m, K_s and K_p are constants. For the differential reactor studied here, the material balance equation was derived (21) and can be expressed as follow ;

$$r = \frac{V_R + V_T}{V_R} \frac{dP}{dt} \qquad (13)$$

where V_R and V_T are the reactor volume and the reservoir volume respectively. By combining equation (11) and equation (13).

$$\frac{dP}{dt} = \frac{V_R}{V_R + V_T} V_m \frac{S}{K_s + S} \frac{K_p}{K_p + P} X$$

X is considered as a state variable and its derivative is set equal to zero.

$$\frac{dX}{dt} = 0 \qquad (15)$$

Equation (14) and (15) were used for the simulation. The value of constants V_m, K_s and K_p are the same as in a Aiba et al's work (20,22,23). These physiological constants in an immobilized cell are assumed to be the same as in a free cell. The first simulation was tried for the constant cell mass system. Data from the experiments were used to simulate the immobilized yeast reactor. Product concentrations were obtained by solving the above equations and 5% of noise was included in the measurements of P using a random number generator. After a long period of operation, the cells slowely decay due to a shortage of nutrients (23). So the next simulation was performed for the decaying cell mass system. The deactivation of the cell was simulated using the equation

$$x = x_i \exp(-k_d (t - t_d)), \quad t > t_d = d \qquad (16)$$

Where X_i in the initial effective cell mass and k_d is the deactivation constants, and t_d is the time after which decay occurs. For the decaying cell mass system, the true value of X was calculated from equation (16) and the effective cell mass was estimated by solving the extended Kalman filter equations (14) and (15). V_T= 2000 ml, V_R= 50 ml, k_d= 0.0078 and t_d= 50 hours were used for this simulation. To obtain a discrete version of equation (14) and (15), the Euler method was used. Equation (14) and (15) were integrated with small intervals (ΔT = 0.02 hour) and the extended Kalman filter was operated every 0.5 hour. For this study, following values of P , Q and R_k were used.

$$P_{0/-1} = \begin{pmatrix} 0.0025 & 0 \\ 0 & 0.25 \end{pmatrix}$$

$$Q = \begin{pmatrix} 0.0001 & 0 \\ 0 & 0.0025 \end{pmatrix}$$

$$R_k = 0.0025$$

RESULTS AND DISCUSSION

The cell concentrations in the gel was, after being activated, 67 - 70 mg/ml of gel and this result is almost same as reported by Ryu et al (22). Figure 2(a) shows the experimental measurements of the total cell mass during operation for the first experiment. The yield, $Y_{p/s}$, in equation (12) was 0.50 from the experiment. The cell mass was calculated from equation (14) to fit the experimental measurements. Using the parameter values of the free cell system, model equation fitted the measurements curve well. Therefore the same model and parameter values were used for further study. The effective cell mass calculated was 21 g/l in this case. By comparing with the measured cell mass 109 g/l, the efficiency of the immobilized cell system(effective cell mass/total cell mass) was found to be 19 %.

The true value of effective cell mass calculated above was used for simulations. Figure 2(b) shows the corrected values of product concentration measurement. The corrected values of the product concentration followed the true value well as shown in Figure 2(b). The estimated effective cell mass converged to the true value (22 g/l) from initial guess (30 g/l) as shown in Figure 2(c). By increasing the number of measurements, the estimated value converged rapidly to a true value.

Similar results were obtained for the second experiment as shown in Figure 3(a). Measured cell mass was 71 g/l and the effective cell mass was estimated to be 16.5 g/l. Therefore the efficiency was 23 % in this case. Also almost same results were obtained from the simulations as shown in Figure 3(b) and (c).

For the decaying system, the sequential estimation method was again used to estimate the effective cell mass as shown in Figure 4(a) and 4(b), and this method gave good results.

It seems that the equation(11) can be used directly to estimate the effective cell mass by knowing S,P and the reaction rate. When many data are taken in a short time, the reaction rate is sometimes positive or negative due to the measurement errors, which makes the estimated value of X meaningless. When a small number of data are taken, the reaction rate expressed as dS/dt (or dP/dt) becomes inaccurate. But this sequential method eliminates the above problems. For a packed bed reactor which is commonly used an extended Kalman filter equations are to be modified, which is left for further study.

CONCLUSIONS

Immobilized yeast cells were packed in a column and operated in batch recycle mode to study the immobilized cell system. Effective cell mass was calculated to be 19 to 23 % of the cell mass in the immobilized cell system for alcohol production. Simulation results show that the effective cell mass could be estimated sequentially from the measurement of product concentration for the system with or without cell decaying. By increasing the number of measurements, a rapid convergence was observed.

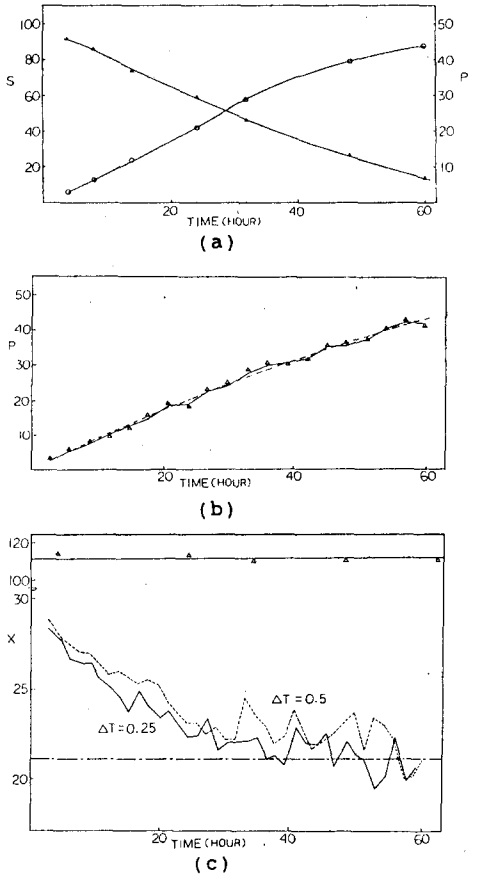
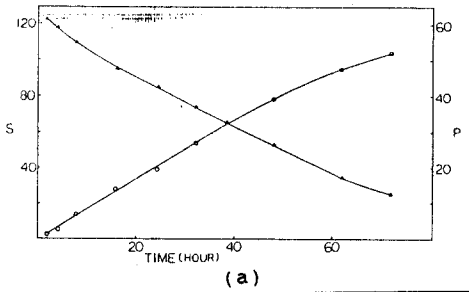
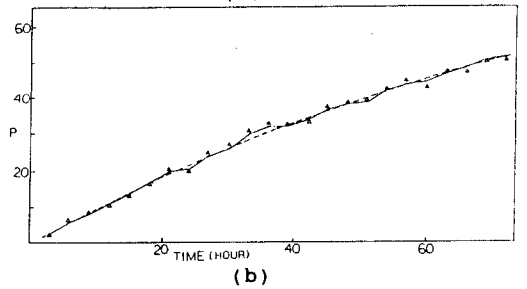


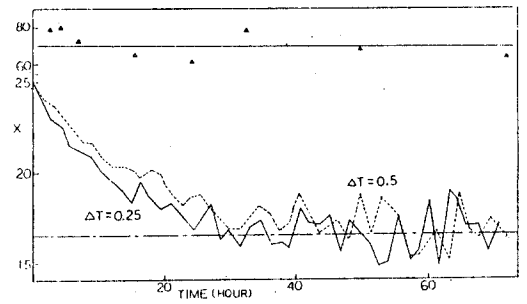
Figure 2. Experimental and simulation results of Exp. No.1
(a) experimental measurements with simulation results
Δ ; experimental measurement of S(g/l)
o ; experimental measurement of p(g/l)
——; result from curve fitting
(b) estimation of state P
---; true values
Δ ; simulated measurement
——; corrected value
(c) measurements of cell mass and estimation of the effective cell mass (x_{true}= 21 g/l, x_{guess}= 30 g/l)
Δ ; cell mass measurement
——; true effective cell mass
---; corrected effective cell mass (ΔT=0.50 hour)
——; corrected effective cell mass (ΔT=0.25 hour)



(a)

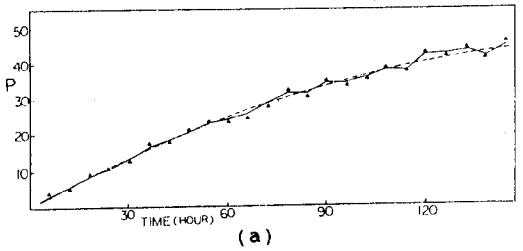


(b)

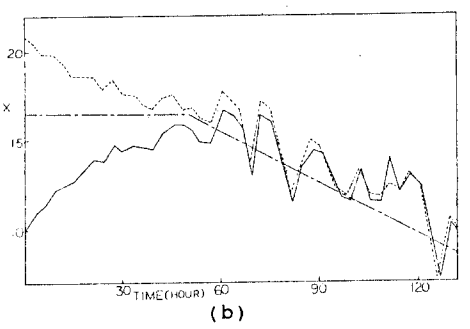


(c)

Figure 3. Experimental and simulation results of Exp. No.2
(a) experimental measurement with simulation results
▲ ; experimental measurement of S(g/l)
o ; experimental measurement of P(g/l)
——; result from curve fitting
(b) estimation of state P
---; true value
▲ ; simulation measurement
——; corrected value
(c) measurements of cell mass and estimation of the effective cell mass
(x_{true}=16.5 g/l, x_{guess}= 25 g/l)
▲ ; cell mass measurement
—·—; true effective cell mass
---; corrected effective cell mass
(ΔT=0.50 hour)
——; corrected effcctive cell mass
(ΔT=0.25 hour)



(a)



(b)

Figure 4. Simulation results of decaying system
(a) estimation of state P
---; true values
▲ ; simulated measurement
——; corrected value
(b) estimation of effective cell mass
(x_{true}= 16.5 g/l T = 0.50 hour)
—·—; true effective cell mass
---; corrected effective cell mass
(x_{guess}= 21 g/l)
——; corrected effective cell mass
(x_{guess}= 10 g/l)

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