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Effect of Incorrectly Estimated Parameters on the Control of Specific Growth Rate in *E. coli* Fed-Batch Fermentation

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An exponential feeding strategy has been frequently used in fed-batch fermentation of recombinant *E. coli*. In this feeding scheme, growth yield and initial cell concentration, which can be erroneously determined, are needed to calculate the feed rate for controlling specific growth rate at the set point. The effect of the incorrect growth yield and initial cell concentration on the control of the specific growth rate was theoretically analyzed. Insignificance of the correctness of those parameters for the control of the specific growth rate was shown theoretically and experimentally.

Key words: fed-batch operation, recombinant *E. coli*, controlled specific growth rate, growth yield, initial cell concentration

INTRODUCTION

Fed-batch operation has been widely used for recombinant *E. coli* fermentation. Specific growth rate is one of the important parameters since the formation of growth inhibitory organic acids such as acetic acid and the expression of recombinant protein depend on the specific growth rate [1-3]. An exponential feeding strategy for controlling the specific growth rate at an appropriate value has been frequently used [4-7]. Growth yield and the initial cell concentration are needed to calculate the feed rate, but they may have some errors. While the correct value of the initial cell concentration can be obtained relatively easily by an accurate measurement, the growth yield may change during the fermentation. The growth yield usually decreases as protein expression is induced. Thus, the growth yield may be incorrectly estimated and, consequently, cause incorrect calculation of the feed rate. Feedback control [8] and several other modified schemes [10, 11] were used to solve this problem. However, we found that the incorrectness of those parameters does not significantly affect the control of the specific growth rate. In this article, insignificance of the correctness of the growth yield and initial cell concentration for the control of specific growth rate has been theoretically analyzed and experimentally shown.

THEORETICAL ANALYSIS

Mass balance equations on cell and substrate for the fed-batch operation are generally described by the following equations.

$$\frac{dX}{dt} = \left[\mu - \frac{F}{V}\right] X \tag{1}$$

$$\frac{dS}{dt} = -\mu\frac{X}{Y} + \frac{(S_0 - S)F}{V} \tag{2}$$

Specific growth rate (μ) can be controlled at a constant value with feed rate determined by the above equations. Feed rate is represented by the following equations when S=0 and dS/dt=0. Substrate concentration should be maintained at near zero for the control of specific growth rate since *E. coli* has very low saturation constant in Monod equation.

$$F = \frac{\mu^* XV}{Y S_0} \tag{3}$$

$$= \frac{\mu^* X_0 V_0}{Y S_0} e^{\mu^* t} \tag{4}$$

where μ^* is a set point of the specific growth rate. This exponential feeding policy has been frequently used in recombinant *E. coli* fermentation, as described earlier. If a false value of the growth yield (Y_F), which is different from true value (Y_T), is incorrectly used, the following equations are obtained from Eqs. (1) and (3).

$$\frac{dX_F}{dt} = \mu^* \left[1 - \frac{X_F}{Y_F S_0}\right] X_F \tag{5}$$

$$\frac{dV}{dt} = \frac{\mu^* X_F V}{Y_F S_0} \tag{6}$$

X_F and V can be calculated by the above equations and feed rate can be expressed by the following equation.

$$F_F = \frac{\mu^* X_F V}{Y_F S_0} \tag{7}$$

Feed solution is supplied with an incorrect feed rate

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represented by Eq. (7) since an incorrect value of the growth yield is used.

Consider the real cell growth in the fermentor when the feed solution is incorrectly supplied according to Eq. (7). The equation for real cell growth is obtained from Eq. (1).

$$\frac{dX_T}{dt}=\left[\mu_T-\frac{F_F}{V}\right]X_T \tag{8}$$

μ_T can be expressed by rearranging Eq. (3).

$$\mu_T=\frac{F_F Y_T S_0}{X_T V} \tag{9}$$

Substitution of Eq. (9) into Eq. (8), another substitution of Eq. (7) into the substituted equation, and rearrangement yield

$$\frac{dX_T}{dt}=\frac{\mu^* X_F}{Y_F S_0}\left[\frac{Y_T S_0}{X_T}-1\right]X_T \tag{10}$$

The above equation describes the cell growth when the incorrect value of the growth yield (Y_F), which is different from the real one (Y_T), is used. Specific growth rate is expressed by the following equation.

$$\mu_T=\frac{1}{X_T}\frac{dX_T}{dt}+\frac{1}{V}\frac{dV}{dt} \tag{11}$$

Substitution of Eqs. (6) and (10) into Eq. (11) and rearrangement give the following equation.

$$\mu_T=\mu^*\frac{X_F}{Y_F}\frac{Y_T}{X_T} \tag{12}$$

The above equation describes the effect of incorrect growth yield on specific growth rate. X_F and X_T can be calculated by Eqs. (5) and (10), and initial conditions for both equations are the same ($X_{F0}=X_{T0}=X_0$).

However, if the initial cell concentration is incorrectly used ($X_{F0}\neq X_{T0}$) and the correct value of the growth yield is used ($Y_F=Y_T=Y$), the following equations are obtained from Eqs. (5), (10) and (12).

$$\frac{dX_F}{dt}=\mu^*\left[1-\frac{X_F}{YS_0}\right]X_F \quad \text{at } t=0,\ X_F=X_{F0} \tag{13}$$

$$\frac{dX_T}{dt}=\mu^*\left[1-\frac{X_T}{YS_0}\right]X_F \quad \text{at } t=0,\ X_T=X_{T0} \tag{14}$$

$$\mu_T=\mu^*\frac{X_F}{X_T} \tag{15}$$

RESULTS AND DISCUSSION

When feeding solution is supplied with a flow rate determined by Eq. (3), specific growth rate is controlled well at the set point and cell concentration is predicted well by Eq. (1) as long as dissolved oxygen and nutrients other than the rate-limiting substrate (carbon source) are not limiting. However, the feed rate can be incorrectly calculated by Eq. (3) if an incorrect value of the growth yield or initial cell concentration is used.

When an incorrect value of the growth yield is used to determine the feed rate, the specific growth rate is represented by Eq. (12). X_F and X_T in this equation can be calculated by Eqs. (5) and (10). It should be noted that Eq. (12) is independent of volume. The effect of incorrect growth yield on specific growth rate is shown in Fig. 1. The nominal values used for computational work are X_0=2.0 (optical density, OD), Y_T=1.2 (OD L/g substrate), and μ^*=0.15 (1/h). It is observed that the specific growth rate approaches the set point even with the incorrect growth yield. In recombinant *E. coli* fermentation, the important reason for controlling the specific growth rate is that high specific growth rate causes acetic acid production, resulting in the inhibition of growth and lower productivity of the recombinant protein. Thus, the control of the specific growth rate is more important in the later phase, in which cell density and growth rate (dX/dt) are higher, than during the early phase of the fermentation. The effect of the growth yield on the control of the specific growth rate is considered to be insignificant since the specific growth rate is controlled well at the set point in the later phase, as shown in Fig. 1. The reason for $\mu_T \approx \mu^*$ in a later phase is that $X_F Y_T/Y_F X_T$ approaches 1 in Eq. (12) with time. X_T and X_F are real cell concentration and incorrectly calculated cell concentration, respectively, when the feed rate is F_F. The amounts of feed substrate can be expressed by $(X_T V-X_0 V_0)/Y_T$ and $(X_F V-X_0 V_0)/Y_F$ in each case. The following equation is obtained since both have the same value.

$$\frac{X_F V-X_0 V_0}{Y_F}\frac{Y_T}{X_T V-X_0 V_0}=1 \tag{16}$$

$X_F V-X_0 V_0$ and $X_T V-X_0 V_0$ approach $X_F V$ and $X_T V$, respectively, with time since $X_F V$ and $X_T V$ increase with time. Therefore, $X_F Y_T/Y_F X_T$ approaches the left-hand side of Eq. (16) and, consequently, 1. During the early phase of fermentation, positive deviation from the set point of the specific growth rate may provide higher productivity than negative deviation since acetic acid production is not so significant in an early phase in which cell densities are low.

However, when an incorrect value of the growth yield is used to determine the feed rate, cell concentration (X_F) is different from the actual cell concentration (X_T) calculated with correct growth yield. Fig. 2 shows the effect of incorrect growth yield on cell concentration. The dashed line indicates actual cell concentration (X_T) in the fermentor. The dotted line represents incorrectly calculated cell concentration

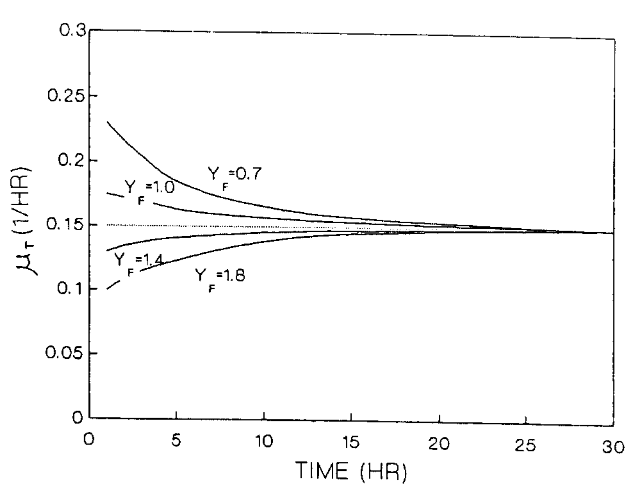

Fig. 1. Effect of incorrect growth yield on specific growth rate. Dotted line represents the set point of the specific growth rate.

(X_F), when the feed rate is calculated with incorrect growth yield (Y_F). A lower value than the real growth yield makes cell concentration underestimated, while a higher one makes it overestimated. Calculated cell concentrations (X_F) with different incorrect growth yields show similar results, implying that the variation of X_F with Y_F is not significant. They closely agree with the cell concentration (solid line) which is controlled with correct growth yield.

Specific growth rate is calculated by Eq. (15) when an incorrect value of initial cell concentration (X_{F0}) is used. Fig. 3 shows the effect of incorrect initial cell concentration on specific growth rate. A similar behavior was observed as in Fig. 1. The specific growth rate approaches the set point during the later phase of the fermentation even with the incorrect initial cell concentration. The effect of incorrect initial cell concentration on cell concentrations is shown in Fig. 4. The dashed line indicates actual cell concentration (X_T) in the fermentor, and the dotted line represents incorrectly calculated cell concentration (X_F), when the feed rate is calculated with incorrect initial cell concentration (X_{F0}). Although they show similar results, they are quite different from the cell concentration (solid line) which is controlled with correct initial cell concentration. The reason for $\mu_T \approx \mu^*$ in the later phase in Fig. 3 is that X_F/X_T approaches 1 in Eq. (15) with time (as shown in Fig. 4).

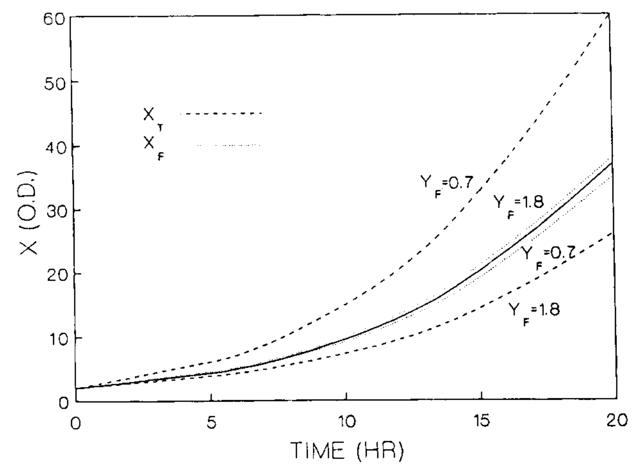

Fig. 2. Effect of incorrect growth yield on cell concentration. Dashed line indicates actual cell concentration (X_T) and dotted line represents incorrectly calculated cell concentration (X_F). Solid line is the cell concentration controlled with correct growth yield.

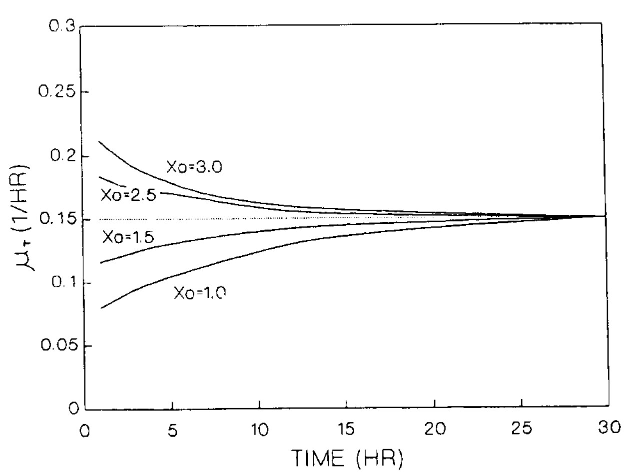

Fig. 3. Effect of incorrect initial cell concentration on specific growth rate. Dotted line represents the set point of the specific growth rate.

CONCLUSIONS

The specific growth rate approaches the desired set

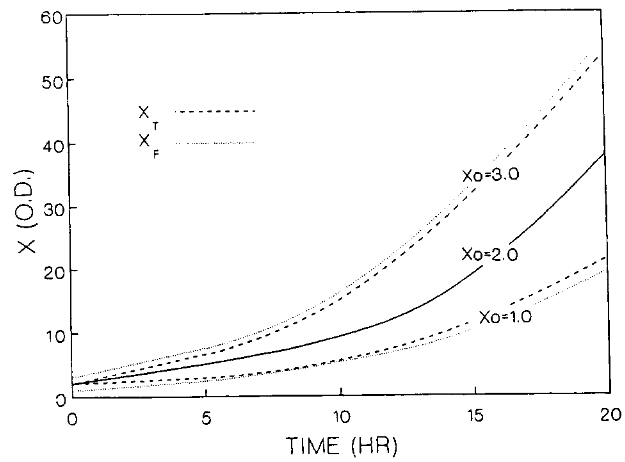

Fig. 4. Effect of incorrect initial cell concentration on cell concentration. Dashed line indicates actual cell concentration (X_T) and dotted line represents incorrectly calculated cell concentration (X_F). Solid line is the cell concentration controlled with correct growth yield.

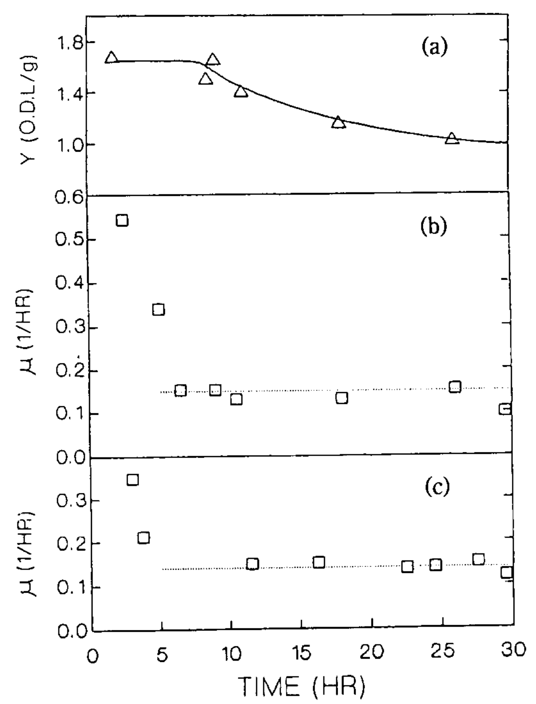

Fig. 5. Insignificance of the correctness of growth yield for the control of specific growth rate. Open symbols represent experimental data and dotted line indicates the set point of μ.
(a) Real growth yield changes with time and the variable Y_T can be expressed by exponential function, $Y_T=0.7e^{-(t-8)/8}+0.95$. Solid line represents calculated values by this equation. (b) Incorrect constant growth yield (Y_F=0.714 O.D.L/g glucose) was used. (c) Correct time-variable growth yield (Y_T) expressed by exponential function was used. $Y_T=0.7e^{-(t-8)/8}+0.95$.

point even with incorrect growth yield or initial cell concentration, although the predicted value of the cell concentration is different from the real one. The correct value of the initial cell concentration can be obtained relatively easily by an accurate measurement. However, the growth yield may be estimated incorrectly, especially if it is time-variable. This analysis shows that incorrectness of the growth yield does not significantly affect the control of the specific growth rate. Fig. 5 shows the insignificance of the correctness of the growth yield for the control of the specific growth rate in recombinant *E. coli* fed-batch operation. Specific growth rate is controlled well at the set point even with the incorrect constant growth yield, although the real growth yield decreases with time.

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