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

Tai Hyun Park*, Sung Kwan Yoon¹, and Whan Koo Kang²

Department of Genetic Engineering, Sung Kyun Kwan University, Suwon, Korea
¹Lucky Biotech Research Institute, P.O. Box 10, Daejeon, Korea
²Department of Chemical Engineering, Han Nam University, Daejeon, Korea

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.

\[ \frac{dX}{dt} = \frac{\mu - F}{V} X \]  
\[ \frac{dS}{dt} = -\frac{\mu}{Y} X + \frac{(S_0 - S)F}{Y} \]

Specific growth rate (\( \mu \)) 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.

\[ F = \frac{p^* XV}{Y S_0} \]
\[ = \frac{\mu^* X_0 V_0}{Y S_0} e^{\mu^* t} \]

where \( \mu^* \) 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 (\( Y_v \)), which is different from true value (\( Y_F \)), is incorrectly used, the following equations are obtained from Eqs. (1) and (3).

\[ \frac{dX}{dt} = \mu^* \left[ 1 - \frac{X_0}{Y_F S_0} \right] X_F \]
\[ \frac{dV}{dt} = \frac{\mu^* X_F V}{Y_F S_0} \]

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

\[ F_F = \frac{\mu^* X_F V}{Y_F S_0} \]

Feed solution is supplied with an incorrect feed rate...
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).

\[
\frac{dX_t}{dt} = \left[ \mu_T - \frac{F_t}{V} \right] X_t \tag{8}
\]

\( \mu_T \) can be expressed by rearranging Eq. (3).

\[
\mu_T = \frac{F_t Y_t S_0}{X_t V} \tag{9}
\]

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

\[
\frac{dX_r}{dt} = \frac{\mu_T X_r}{Y_t S_0} \left[ \frac{Y_t S_0}{X_t} - 1 \right] X_t \tag{10}
\]

The above equation describes the cell growth when the incorrect value of the growth yield \( (Y_T) \), 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.

\[
\mu_t = \mu^* \frac{X_r}{Y_t} \frac{Y_t}{X_t} \tag{12}
\]

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

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

\[
\frac{dX_T}{dt} = \mu^* \left[ 1 - \frac{X_T}{Y_T S_0} \right] X_T \quad \text{at } t = 0, \quad X_T = X_{T0} \tag{13}
\]

\[
\frac{dX_T}{dt} = \mu^* \left[ 1 - \frac{X_T}{Y_T S_0} \right] X_T \quad \text{at } t = 0, \quad X_T = X_{T0} \tag{14}
\]

\[
\mu_T = \mu^* \frac{X_T}{X_T} \tag{15}
\]

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). \( X_T \) 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 g substrate), and \( \mu^* = 0.15 \) (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 \( X_T Y_T Y_S X_T \) approaches 1 in Eq. (12) with time. \( X_T \) and \( X_T \) are real cell concentration and incorrectly calculated cell concentration, respectively, when the feed rate is \( F_T \). The amounts of feed substrate can be expressed by \( (X_T - X_T) Y_T \) and \( (X_T - V - X_T V_T)/Y_T \) in each case. The following equation is obtained since both have the same value.

\[
\frac{X_T V_T - X_T V_T}{Y_T} = 1 \tag{16}
\]

\( X_T V_T - X_T V_T \) and \( X_T - X_T V_T \) approach \( X_T V_T \) and \( X_T \), respectively, with time since \( X_T V_T \) and \( X_T \) increase with time. Therefore, \( X_T Y_T Y_S X_T \) approaches the right-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 \( (X_T) \) 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...