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Optimization of Secondary Metabolite Production Using Singular Approximation and Minimum Principle

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Abstract

Optimal control profiles as calculated with two control algorithms, singular approximation and minimum principle, are compared in this article. Switching points were determined using the singular approximation by mathematical calculation. The optimal growth rate was calculated using minimum principle. With an increased number of switching points, the calculated optimal control profiles approached the theoretical optimal control profile as calculated using the minimum principle. With three switching times, the product concentration approached 96% of the theoretical optimal control profile. From these results, optimal control can be achieved with more than a three-switching-point approximation.

Index Entries: Optimization; singular approximation; minimum principle.

Introduction

For most secondary metabolite production, many optimization techniques have been developed to meet the demands of different optimal conditions for cell growth and product formation. Many researchers have proposed a two-stage system to meet these dual demands and have opted for a first stage for maintaining optimal cell growth and a second for optimal product formation.

There are several ways to change from optimal cell growth conditions to those required for product formation; the parameters include pH, temperature, addition of inducer, and substrate concentration *(1–4)*.

Kuriyama et al. *(5)* used a two-stage continuous fermentor for the production of ethanol from yeast. The productivity of ethanol was increased

when the conditions of the first stage were optimized for cell growth and the second stage was optimized for product formation.

Chen et al. *(4)* used a fed-batch fermentor for the production of foreign protein, using a recombinant *Escherichia coli*. The substrate concentration was maintained at a high level during the cell growth period, and the substrate concentration was maintained at a low level during the product formation period. Many researchers working on the fed-batch fermentation process have started with a high substrate concentration and started the addition of substrate after substrate depletion. During the fed-batch operation process, the substrate concentration was controlled at a low level for optimal product formation *(6)*. This kind of operation is representative of the common two-stage fermentation and is used for optimal cell growth and product formation *(2)*.

The effect of pH and temperature on the cell growth and xylitol production was studied by Slininger et al. *(7)*. The optimal conditions for cell growth were maintained during the first stage and then changed to optimal conditions for xylitol production.

The time of switching is important for maximal production of the desired product. In most cases, researchers have used trial-and-error methods to determine the optimal switching time *(8)*. No research has been conducted to compare the differences in performance among the trial-and-error method, singular approximation, and minimum principle.

In the present study, the optimal control profiles with singular approximation control and minimum principles were calculated and the differences in performance compared. Performance variances owing to increased switching times are also presented.

Problem Formulation

For most microorganisms, the optimal conditions for cell growth differ from those required for product formation. Therefore, it is necessary to control the environmental conditions in an optimal state to maximize production.

The specific growth rate of cells is dependent on pH, temperature, substrate concentration, and other environmental variables. The specific production rate is also similarly dependent. The simplest forms of cell growth and product formation rates are expressed by the following differential equations:

$$\frac{d(XV)}{dt} = \mu XV \tag{1}$$

$$\frac{d(PV)}{dt} = \pi XV \tag{2}$$

in which XV is the total cell mass, PV is the total amount of product, and V is the fermentor volume. The specific rates of growth and product forma-

tions, μ and π, are functions of environmental variables, and π is expressed as a function of μ.

When the environmental conditions are used as control variables, it is sometimes difficult to calculate optimal control profiles with the currently developed optimal control strategies. In this case, alternative methods can be used to calculate the optimal control profiles of a fermentation system:

$$\underset{\mu}{\text{Minimize}} \; [-PV(t_f)] \tag{3}$$

The specific cell growth rate was controlled using environmental conditions to maximize the product formation, and the performance index was expressed in the following equation.

$$\pi = \alpha_0 + \alpha_1\mu + \alpha_2\mu^2 + \ldots \tag{4}$$

in which the specific production rate is expressed as a polynomial function of the specific growth rate.

These polynomial functions can be approximated with many piecewise linear functions. When the specific production rate is expressed by a linear function of specific growth rate, the optimization problem is a singular control problem and can be used to calculate optimal control profiles using a singular approximation.

Minimum Principles with Transformed Control Variable (μ)

By using a nonsingular transformation, the proposed singular problem was converted to a nonsingular problem. Pontryagin's minimum principle *(9)* was then applied to calculate optimal control profiles. Instead of using environmental variables, specific growth rate was used as a control variable to maximize product concentration:

$$\frac{dX_1}{dt} = \mu X_1 \tag{5}$$

$$\frac{dX_2}{dt} = \pi(\mu) X_1 \tag{6}$$

The performance index to be maximized is the amount of product present after fermentation and is expressed by the following equation:

$$\underset{\mu}{\text{Minimize}} \; [-X_2(t_f)] \tag{7}$$

The optimal control profiles of a simple transformed optimization system were calculated using analytical approaches using the Gradient Iteration Algorithm, which can be used to calculate specific growth rate to satisfy given optimal conditions *(10)*.

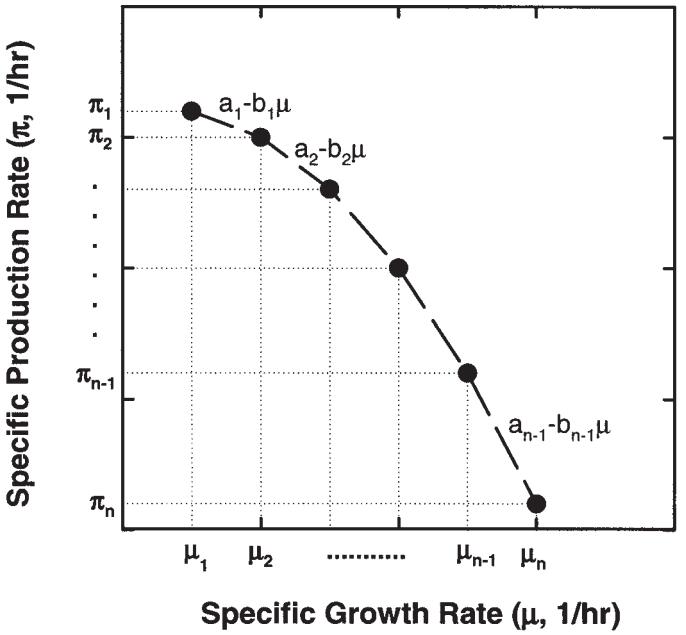

Fig. 1. Linear approximation of the correlation between specific growth rate and specific production rate.

Singular Approximation After Nonsingular Transformation

The singular approximation has been proposed to calculate optimal control profiles using a linear approximation. The switching time was also calculated using a singular approximation:

$$t_1 = t_f - \frac{1}{\mu_1} \ln\left(\frac{a_1}{a_1 - b_1\mu_1}\right) \tag{8}$$

$$t_2 = t_1 - \frac{1}{\mu_2} \ln\left(\frac{a_2}{a_2 - b_2\mu_2}\right) \tag{9}$$

$$t_{n-1} = t_{n-2} - \frac{1}{\mu_{n-1}} \ln\left(\frac{a_{n-1}}{a_{n-1} - b_{n-1}\mu_{n-1}}\right) \tag{10}$$

in which

$\mu = \mu_n$ for $0 \le t \le t_{n-1}$
$\mu = \mu_{n-1}$ for $t_{n-1} \le t \le t_{n-2}$
$\mu = \mu_2$ for $t_2 \le t \le t_1$
$\mu = \mu_1$ for $t_1 \le t \le t_f$

Table 1
Correlation of Specific Growth and Specific Production Rates

μ	π
0.1	0.38
0.2	0.40
0.4	0.32
0.6	0.08

As a result, a series of switching times was calculated, and the corresponding optimal control profiles are shown in Fig. 1.

Proposed Numerical Example

The optimization of fermentation for the maximal production of desired products uses environmental conditions, such as substrate concentration, temperature, and pH, as control variables. It is sometimes difficult to calculate optimal control profiles with conventional optimization schemes. Therefore, several modified algorithms have been proposed to circumvent the mathematical and numerical difficulties. In this article and from the following examples, the efficiency of nonsingular transformation is proposed and performances are optimized with a singular approximation after nonsingular transformation and minimum principle are compared.

The performance index is used to maximize the amount of product after fermentation using the transformed control variable μ. The control variable μ is constrained by the maximum specific growth rate of the cells:

$$\underset{\mu}{\text{Minimize}} \; [-X_2(t_f)] \qquad \text{with } \mu \le \mu_{max} \tag{11}$$

When the fermentation system is expressed as a function of environment variables, this problem can be transformed to one involving the specific growth rate as a control variable. From the experimental results (hypothetical data), the specific growth and specific production rates are given as the substrate concentration changes (Table 1). The specific production rate is expressed as a function of the specific growth rate, as shown in Fig. 2.

From the experimental data, the specific production rate may be expressed as a polynomial function of the specific growth rate:

$$\pi = 0.4 - 2(\mu - 0.2)^2 \tag{12}$$

Minimum Principles

The specific production rate can be expressed as a parabolic function of specific growth rate, as shown in Eq. 12. From the given differential

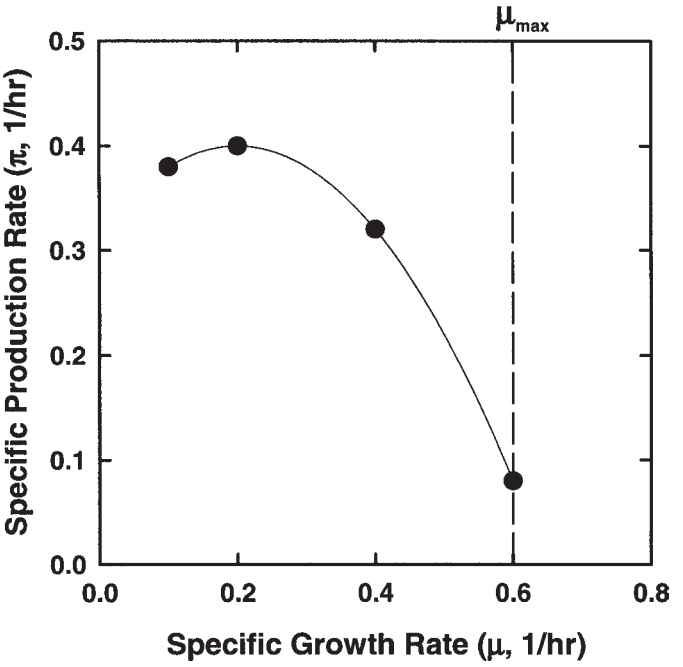

Fig. 2. Change in specific production rate with change in specific growth rate.

equation, the optimal control profiles can be calculated with minimum principle:

$$\frac{dX_1}{dt} = \mu X_1 \tag{13}$$

$$\frac{dX_2}{dt} = [0.4 - 2(\mu - 0.2)^2]X_1 \tag{14}$$

The Hamiltonian of this system is calculated as follows:

$$H = \lambda_1 \mu X_1 + \lambda_2 [0.4 - 2(\mu - 0.2)^2]X_1 \tag{15}$$

Adjoint variables were calculated with the Hamiltonian and state variables, and the final conditions were calculated with transversality conditions:

$$-\dot{\lambda}_1 = \lambda_1 \mu + \lambda_2 [0.4 - 2(\mu - 0.2)^2] \tag{16}$$

$$-\dot{\lambda}_2 = 0 \tag{17}$$

The optimal control condition of the specific growth rate allows that the partial derivative of the Hamiltonian with respect to the control variable (specific growth rate) equals zero:

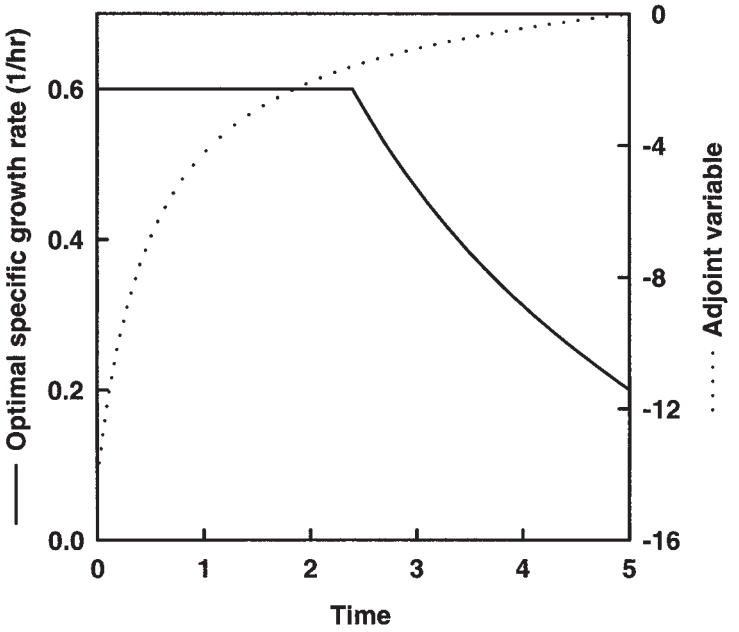

Fig. 3. Optimal specific growth rate (μ) and adjoint variable (λ_1) profiles calculated with minimum principles.

$$\frac{\partial H}{\partial \mu} = \lambda_1 - 4\lambda_2(\mu - 0.2) = 0 \tag{18}$$

From Eq. 17, $\lambda_2(t) = -1$. When this result is inserted into Eq. 18, the correlation between specific growth rate and the adjoint variable is driven as follows:

$$\mu = 0.2 - (\lambda_1/4) \tag{19}$$

From Eqs. 16 and 19, the following first-order differential equation for the final condition is derived:

$$-\dot{\lambda}_1 = 0.2\lambda_1 - \frac{1}{8}\lambda_1^2 - 0.4 \qquad \lambda_1(t_f) = 0 \tag{20}$$

Solving Eq. 20, the adjoint variable may be expressed as a function of time:

$$t_f - t = 5[\arctan(-0.5) - \arctan(0.625\lambda_1 - 0.5)] \tag{21}$$

Therefore, the adjoint variable is expressed as an explicit functional form from Eq. 21:

$$\lambda_1 = 0.8 + 1.6 \times \tan[0.2 \times (t - t_f) + \arctan(-0.5)] \tag{22}$$

The adjoint variable is calculated using Eq. 22 and a numerical solving program (see Fig. 3). From Eqs. 19 and 22, the specific growth rate is then calculated as a function of time:

Table 2
Singular Approximation Coefficient with One Switching Time

Number	μ	π	a_i	b_i	t
1	0.2	0.4	0.56	0.8	3.32
2	0.6	0.08			

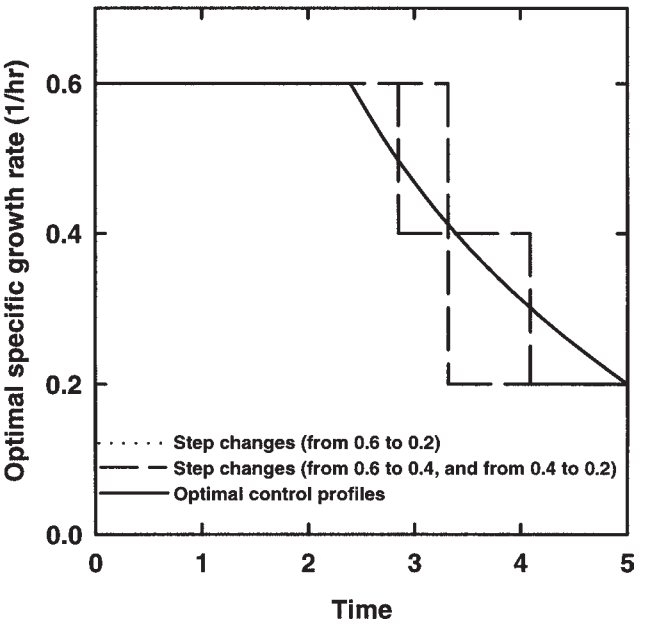

Fig. 4. Optimal specific growth rate profiles with minimum principle and singular approximation.

$$\mu = 0.2 - \frac{1 - e^{0.8(t_f - t)}}{3e^{0.8(t_f - t)} + 5} \tag{23}$$

Since the maximum specific growth rate is 0.6, the calculated specific growth rate using Eq. 23 cannot exceed 0.6. The optimal control profiles of the specific growth rate are shown in Fig. 3.

Singular Approximation

Case 1: One Switching Time (from μ_{max} to μ_{min})

From Eq. 8, switching times were calculated with singular approximation; the results are given in Table 2. When there is only one switching time, from the maximum specific growth rate to the minimum, the results obtained are as given in Table 2. The control profile of the specific growth rate, in the form of a step function, is shown in Fig. 4.

Table 3
Singular Approximation Coefficient and Two Switching Times

Number	μ	π	a_i	b_i	t
1	0.2	0.40	0.48	0.4	4.09
2	0.4	0.32	0.8	1.2	2.813
3	0.6	0.08			

$$t_1 = t_f - \frac{1}{\mu_1} \ln\left(\frac{a_1}{a_1 - b_1\mu_1}\right) = 5 - \frac{1}{0.2} \ln\left(\frac{0.56}{0.56 - 0.4 \times 0.8}\right) = 3.32 \text{ d} \qquad (24)$$

Case 2: Two Switching Times ($\mu_{max} \Rightarrow \mu_{mid} \Rightarrow \mu_{min}$)

When two switching times were used for the optimal control of the fermentation process, the parameters given in Table 3 were calculated. From these results, the specific growth rate changed as a step function, which is presented in Fig. 4.

$$t_2 = t_f - \frac{1}{\mu_1} \ln\left(\frac{a_1}{a_1 - b_1\mu_1}\right) = 5 - \frac{1}{0.2} \ln\left(\frac{0.48}{0.48 - 0.4 \times 0.2}\right) = 4.09 \text{ d} \qquad (25)$$

$$t_1 = t_2 - \frac{1}{\mu_2} \ln\left(\frac{a_2}{a_1}\right) = 4.09 - \frac{1}{0.4} \ln\left(\frac{0.8}{0.48}\right) = 2.813 \text{ d} \qquad (26)$$

Therefore,

$\mu = 0.6/\text{h}$ for $0 \le t \le 2.81$
$\mu = 0.4/\text{h}$ for $2.81 \le t \le 4.09$
$\mu = 0.2/\text{h}$ for $4.09 \le t \le 5$

Discussion and Conclusion

The optimal control outputs with singular approximation and minimum principles are shown in Fig. 5. The cell mass and the product concentration profiles are shown in Fig. 5A and 5B, respectively. With an increased number of switching points, the output profiles of the cell mass and product concentration approached those of optimal control.

The control with singular approximation started with the maximum specific growth rate and ended with the maximum specific production rate. Although the cell mass with one switching point was the highest for the three control types up to 3.8 d, the cell mass with optimal control was the highest at the end of fermentation. Product concentration using singular control was the highest during the fermentation.

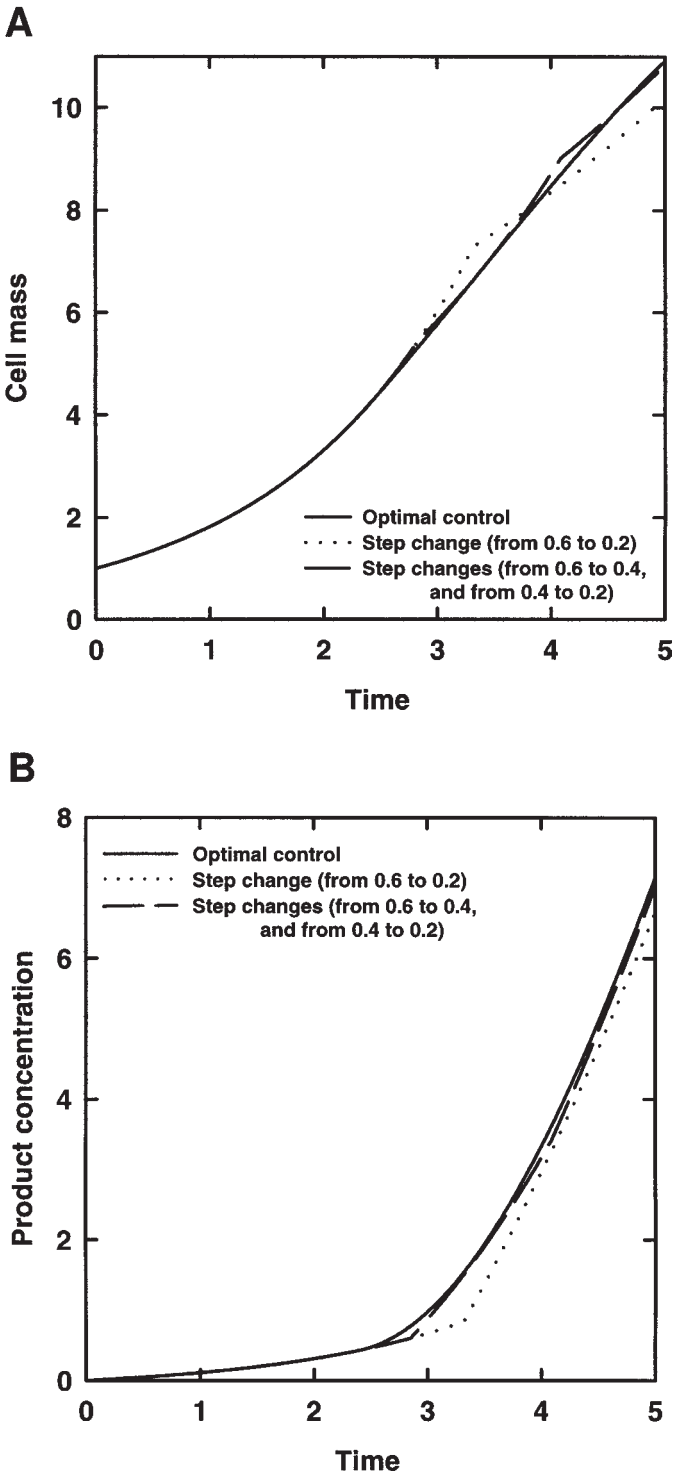

Fig. 5. Cell growth and product formation with applied optimal control profiles. **(A)** Cell mass; **(B)** product concentration.

When the optimization of the fermentation system is difficult, modification methods with mathematical and numerical manipulations have been adopted to make the problem easier. Approximation and transformation methods are commonly applied strategies. Singular approximation and nonsingular transformation methods were applied for the calculation of optimal control profiles, and the performance indices of these were compared herein.

When one switching point approximation is used, the fermentation is the same as in two-stage culture. Two-stage culture is composed of two different steps: the first one involves a cell production period and the second one a product formation period. Since secondary metabolites are produced after cell growth, a two-stage culture is usually used for secondary metabolite production. The performance of a two-stage culture is 92% that of the theoretical optimization. With an increase in switching points, the performance more closely approached its theoretical maximum.

Nomenclature

H = Hamiltonian
P = product concentration (g/L)
t = time (d)
t_f = final time (d)
V = fermentor volume (L)
X = cell concentration (g/L)
X_1 = cell mass (g)
X_2 = product (g)

Greek

λ = adjoint vector
μ = specific growth rate (1/d)
π = specific production rate (1/d)

Subscript

max = maximum

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