ORIGINAL ARTICLE

Optimal feed rate profiles for fed-batch culture in penicillin production

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Abstract

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The fed-batch optimization of penicillin productivity was applied as an example of optimization algorithm verification. The objective function of this problem was to optimize penicillin productivity by determination of feed rate trajectory. This study compared the optimized results derived from the proposed algorithm and from the iterative dynamic programming. Three decision variables for the proposed algorithm comprised t_s (switching time from exponential to linear feeding schedules), K (constant in feed rate equation), and ε (a multiplier on substrate requirement). Estimation of this set of decision variables employed Markov chain Monte Carlo procedures (the Gibbs parameter sampling and the Metropolis-Hasting algorithm) using an originally given set of initial values. The optimization procedure was divided into two time periods as follows: i) the time period of exponential feeding policy, $t \leq t_s$ and ii) the time period of linear feeding schedule, $t > t_s$. The calculation procedure of the first period of fermentation time had been proposed by integrating Pontryagin's optimum principle and *Luedeking-Piret* equation. The feed rate profile during the later period was obtained from the direct substitution of desired substrate requirement derived from Monod equation. The optimal feed-rate profile corresponded to the values of decision variables as follows [t_s K ε] = [35.937

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0.096 2.087]. The proposed algorithm was appropriate for determination of optimal feed-rate trajectories in any fed-batch problems provided that the product formation rate agrees with a *Luedecking-Piret* model.

Key words : fed-batch, optimization problem, Markov chain Monte Carlo, Pontryagin's optimum principle, iterative dynamic programming

บทคัดย่อ

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การหารูปแบบที่เหมาะสมของสารป้อนเข้ากระบวนการหมักแบบกึ่งต่อเนื่องในการผลิตเพนิซิลิน

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กระบวนการผลิตเพนิซิลินด้วยวิธีการหมักแบบกึ่งต่อเนื่อง เป็นตัวอย่างที่นำมาใช้เพื่อทดสอบขั้นตอนวิธีหาค่าเหมาะสม ซึ่งฟังก์ชันเป้าหมายในการปัญหาโจทย์ตัวอย่างนี้คือ การเพิ่มค่าความสามารถในการผลิตเพนิซิลินให้สูงที่สุดโดยการหารูปแบบที่เหมาะสมของสารป้อนเข้า ในงานวิจัยนี้เปรียบเทียบผลการคำนวณที่ได้จากขั้นตอนวิธีหารูปแบบที่เหมาะสมของสารป้อนเข้า 2 วิธี ได้แก่ วิธีที่นำเสนอ กับวิธี iterative dynamic programming ซึ่งวิธีที่นำเสนอนี้ ประกอบด้วยตัวแปรตัดสินใจ 3 ตัวแปร ดังนี้ t_s (เวลาที่เปลี่ยนรูปแบบอัตราการไหลของสารป้อนเข้าจากแบบเอกซ์โปแนนเชียล เป็นแบบเชิงเส้น) K (ค่าคงที่ในพจน์ของสมการอัตราการไหลของสารป้อนเข้า) และ ε (ตัวคูณสำหรับความต้องการอาหาร) การหาค่าตัวแปรตัดสินใจเหล่านี้ด้วยวิธีมาร์คอฟเชน มอนติคาร์โล (สุ่มหาค่าตัวแปรด้วยวิธี Gibbs และ Metropolis-Hasting) โดยใช้ค่าเริ่มต้นของตัวแปรสภาวะที่กำหนดให้ ขั้นตอนวิธีหาค่าเหมาะสมของสารป้อนเข้าแบ่งออกเป็น 2 ช่วงเวลา ได้แก่ รูปแบบอัตราการไหลของสารป้อนเข้าจากแบบเอกซ์โปแนนเชียล เมื่อ $t \leq t_s$ และรูปแบบอัตราการไหลของสารป้อนเข้าจากแบบแบบเชิงเส้น เมื่อ $t > t_s$ ในช่วงเวลาแรก วิธีที่นำเสนอคือ การใช้ขั้นตอนวิธี Pontryagin's optimum principle โดยใช้สมการเสริมคือ สมการ *Luedeking-Piret* สำหรับช่วงเวลาที่สอง การหารูปแบบที่เหมาะสมของสารป้อนเข้ากระบวนการหมักแบบกึ่งต่อเนื่อง ใช้วิธีการแทนค่าสารป้อนเข้าที่ต้องการโดยตรง ซึ่งค่านี้ประมาณจากการคำนวณโดยใช้สมการของ Monod พบว่าเซตของตัวแปรตัดสนใจซึ่งให้รูปแบบที่เหมาะสมของสารป้อนเข้ามีค่าดังนี้ [t_s K ε] = [35.937 0.096 2.087] จากผลการคำนวณที่ได้แสดงให้เห็นว่าวิธีที่นำเสนอในงานวิจัยนี้ เหมาะสำหรับการหารูปแบบที่เหมาะสมของสารป้อนเข้ากระบวนการหมักแบบกึ่งต่อเนื่อง สำหรับระบบที่ความสัมพันธ์ระหว่างอัตราการผลิตเซลล์กับอัตราการผลิตผลิตภัณฑ์ สอดคล้องกับสมมติฐานของสมการ *Luedeking-Piret*

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Pontryagin's maximum principle (Boltyanskii *et al*., 1956) has been widely applied to a variety of optimization problems. An optimization of feed-rate profiles for fed-batch fermentation processes has been studied extensively using Pontryagin's maximum principle (Staniškis and Levišauskas, 1983; Hong, 1986; Modak *et al*., 1986; Lim *et al*., 1986). However, applications of Pontryagin's maximum principle were reportedly restricted for lower-order fed-batch models due to complexity of the singular control problems, i.e. by introducing more unknown variables such as a vector of adjoint variables. To minimize the complexity, Skolpap *et al*. (2004) applied the Pontryagin's maximum principle integrated with biological observations of fed-batch culture of α-amylase and protease-producing *Bacillus subtilis* ATCC 6051a by reducing the number of equations required. In

our previous study, the *Luedeking-Piret* equation (Luedeking and Piret, 1959) and the experimental results of feed rate were combined with Pontryagin's maximum principle to handle higher-order differential equation models (14 state variables).

The proposed algorithm was verified by optimizing penicillin production that was first studied by Lim *et al*. (1986). Mekarapiruk and Luus (2001) recently applied an iterative dynamic programming with unspecified initial conditions to optimize feed-rate policy. This optimization problem contains four state variables such as biomass, substrate, and penicillin concentrations and feed rate. Originally, the initial condition of the state variables is $\mathbf{x}(0) = [1.5\ 0\ 0\ 7]^T$ in the order of biomass, substrate, and penicillin concentrations and feed rate; however, their study considered the initial volume and the initial substrate concentration as the decision variables to be evaluated together with the feed-rate profile. Regarding their previous work (Mekarapiruk and Luus, 2000) three deterministic candidates were given for the values of the decision variables with a fixed final time at 132 h and equal step size. In this verification one of these candidates, $\mathbf{x}(0) = [1.758\ 0.0\ 0.0\ 5.973]^T$ would be chosen as initial conditions of the decision variables. The proposed optimization algorithm applied Markov chain Monte Carlo procedures (the Gibbs parameter sampling and the Metropolis-Hasting algorithm) for estimation of decision variables using the given set of initial values. The calculation procedure was divided into two time periods: batch and fed-batch fermentation periods. During the batch period the combination of Pontryagin's optimum principle and *Luedeking-Piret* equation was proposed to determine the optimal feed rate profile. In the fed-batch period the feed rate policy was predicted from the direct substitution of desired substrate requirement derived from Monod equation.

Model equations

The optimization non-linear problem for penicillin synthesis consisted of four fed-batch model equations shown in Eqs. (1) to (4) with a set of constants listed in Eqs. (5) to (7) and the objective function stated in Eq. (10). To optimize total penicillin productivity (Eq. (10)), the control variable (u) expressed in Eq. (4) was solved to satisfy a given set of constraints showed in Eqs. (9a) to (9d) by using a given set of initial values of four state variables.

Biomass concentration (X):

$$\frac{dX}{dt} = h_1 X - \left(\frac{X}{500V}\right)u \tag{1}$$

Substrate concentration (S):

$$\frac{dS}{dt} = -\frac{h_1 X}{0.47} - \frac{h_2 X}{1.2} - \left(\frac{0.029S}{0.0001+S}\right)X + \left(1 - \frac{S}{500}\right)\frac{u}{V} \tag{2}$$

Penicillin concentration (P):

$$\frac{dP}{dt} = h_2 X - 0.01P - \left(\frac{P}{500V}\right)u \tag{3}$$

Feed rate (u): Let V is culture volume.

$$\frac{dV}{dt} = \frac{u}{500} \tag{4}$$

where

$$h_1 = 0.11\left(\frac{S}{0.006+S}\right) \tag{5}$$

$$h_2 = 0.0055\left(\frac{S}{0.0001+S(1+10S)}\right) \tag{6}$$

$$h_3 = \left(\frac{0.029S}{0.0001+S}\right) \tag{7}$$

Initial condition of state variables (biomass concentration (X), substrate concentration (S), Penicillin concentration (P), and culture volume (V))

$$\mathbf{x}(0) = [1.5\ 0\ 0\ 7]^T \tag{8}$$

The constraint on feed rate is

$$0 \le u \le 50 \text{ L/h} \tag{9a}$$

The constraints on state variables are

$$0 \le X \le 40 g/L \tag{9b}$$

$$0 \le S \le 25g/L \quad (9c)$$

$$0 \le V \le 10L \quad (9d)$$

Objective function (total penicillin productivity):

$$J = P(T)V(T) \quad (10)$$

Optimization procedure

To optimize penicillin productivity, the estimation of feed rate can be divided into two time segments of fermentation period, i.e., batch period, $t \le t_s$ and fed-batch period, $t > t_s$.

When $t \le t_s$, the feed rate equation was derived from Pontryagin's maximum principle (Boltyanskii *et al*., 1956). The limitation of Pontryagin's maximum principle in optimization of fed-batch culture was three or four-order mathematical equations (Hong, 1986; Lim *et al*., 1986; Modak *et al*., 1986), because this algorithm introduced additional equations, i.e., adjoint equations. Although this fed-batch optimization problem contains four state variables, the equations are rather complicated. The singular control theory alone would introduce more complexity into this problem. Therefore, this study proposed integration of Pontryagin's maximum principle and the *Luedeking-Piret* equation (Luedeking and Piret, 1959). It was claimed that the formation of secondary metabolite, i.e., penicillin, and that of dual-enzyme (Skolpap *et al*., 2004) satisfactorily agrees with the mixed-growth associated model.

1. For $t \le t_s$:

Pontryagin's maximum principle can be derived as follows:

To optimize the penicillin yield based on total penicillin concentration, i.e.

$$J = \left(\frac{PV}{T}\right)_{\max} \quad (11)$$

where

P = penicillin concentration
V = culture volume
T = total time period.

$\frac{PV}{T}$ is a maximum when this term equals to the maximum of the 'local' differential:

$$\left(\frac{PV}{T}\right)_{\max} = \left(\frac{d(PV)}{dt}\right)_{\max} \quad (12)$$

The term, $\frac{d(PV)}{dt}$ is in fact the Hamiltonian function, H. This is a function of state variables y_i and control variable u.

In the present case, the Hamiltonian is given as

$$H(PV,u) = V\frac{dP}{dt} + P\frac{dV}{dt} \quad (13)$$

The usual interpretation of the Pontryagin's maximum principle is by applying additional equations expressed in Eqs. (14a) and (14b).

The *adjoint equations*:

$$\frac{dy_j}{dt} = -\frac{\delta H(y_i,u)}{\delta y_i} \quad \text{when } j > i \quad (14a)$$

where y_i = state variables, i.e., y_1 is biomass concentration.

The *necessary condition for maximum*:

$$\frac{\delta H(y_i,u)}{\delta y_i} = 0 \quad (14b)$$

Since feed rate, u, is the only control variable, the Hamiltonian becomes a function of u only.

However, it is more convenient to express H in terms of biomass concentration, X using the following equations:

Rate of product formation in fed-batch culture (*Luedeking-Piret* equation):

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X - \frac{u}{V}P \quad (15)$$

where α and β = constant for growth- and non growth-associated terms, respectively.

Fed-batch feed rate:

$$\frac{dV}{dt} = u \quad (16)$$

According to Eq. (13), the Hamiltonian becomes:

$$H(PV,u) = V(\alpha\mu + \beta)X - \alpha\, u\, X \quad (17)$$

To find the optimum path, let Eq. (17) equal to zero:

$$u = \frac{dV}{dt} = V_0\left(\frac{\alpha\mu+\beta}{\alpha}\right)e^{\left(\frac{\alpha\mu+\beta}{\alpha}\right)T} \qquad (18)$$

where V_0 = initial culture volume prior to feeding.

In applying Eq. (18) to the present study, it should be noted that the equations used in the derivation above are somewhat simplified analogues of the behavior of penicillin fermentation. Furthermore, the equation does not contain the optimum 'switching time' from exponential to linear feeding policies expressed in Eq. (19) and in Eq. (22), respectively. Consequently, Eq. (18) can be written as Eq. (19) given below:

$$u = u_0 e^{K(t-t_s)} \qquad (19)$$

where

u_0 = feeding rate at switching time (t_s), L/h (in this case u_0 = 50 L/h)
K = constant
t = current fermentation time, h
t_s = switching time h.

The global optimum values of the parameters (t_s and K) need to be determined since they depend on the speed of biomass production before u reaches its maximum value.

2. For $t > t_s$:

1) Determine μ at current time, t

During exponential phase the growth rate can be represented as:

$$\mu = \frac{\ln\left(\frac{X_f V_f}{X_t V_t}\right)}{(t_f - t)} \qquad (20)$$

where

μ = specific growth rate, h^{-1}
t and t_f = current time and final time, respectively, h
X_t and V_t = biomass concentration (g/L) and volume at the current time (L), respectively
X_f and V_f = biomass concentration (g/L) and volume at final time (t_f) (L), respectively.

2) Determine a desired remaining substrate in a fermentor (S_D) for a future time interval

Since h_1 in Eq. (5) is equivalent to the Monod equation (Monod, 1949), it can be re-arranged as

$$S_D = \frac{\mu K_S}{\mu_m + \mu} \qquad (21)$$

where

S_D = desired remaining substrate concentration, g/L
μ_m = maximum specific growth rate, 0.11 h^{-1}
K_S = saturation constant, 0.006 g/L.

3) Determine a future feed rate u

S_D is substituted in Eq. (2), which can be algebraically rearranged as follows:

$$u = \frac{V}{\left(1-\frac{P}{500}\right)}\left\{\varepsilon\frac{S_D - S}{\Delta t} + \frac{h_1 X}{0.47} + \frac{h_2 X}{1.2} + h_3 X\right\} \qquad (22)$$

where

t = time interval used in optimization algorithm, h
ε = a multiplier on substrate requirement.

Therefore, during this fermentation period ($t > t_s$) t_s and ε are two decision variables.

Optimization results and discussion

The optimal feed-rate profile yielded from the proposed algorithm corresponded to the values of decision variables as follows: [t_s K ε] = [35.937 0.096 2.087].

Figures 1 to 5 illustrate the comparison of optimized result using this proposed algorithm, optimized result reported by Mekarapiruk and Luus (2001), and verified result of Mekarapiruk and Luus (2001) with a given set of optimal u.

The verified result of objective function reported by Mekarapiruk and Luus (2001) was about one-third of their reported value with the same set of given u (Figure 1).

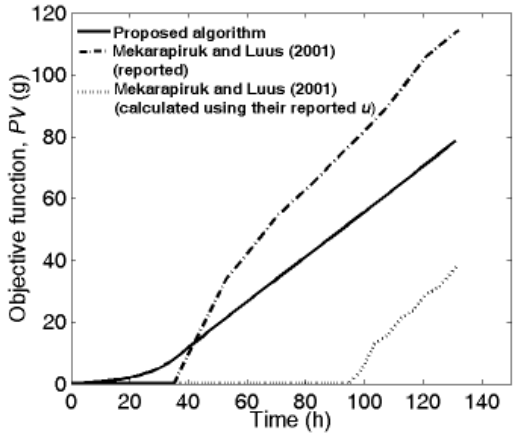

Figure 1. Time profiles of penicillin productivity.

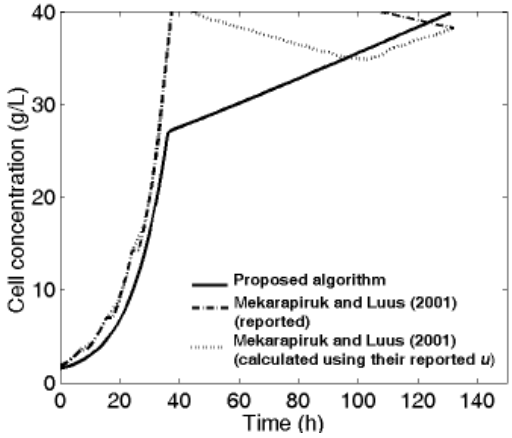

Figure 2. Time profiles of cell concentration.

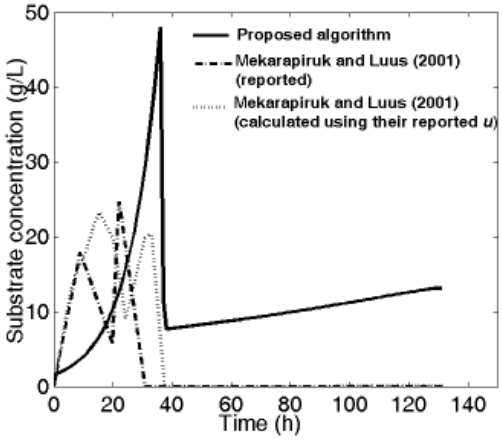

Figure 3. Time profiles of substrate consumption.

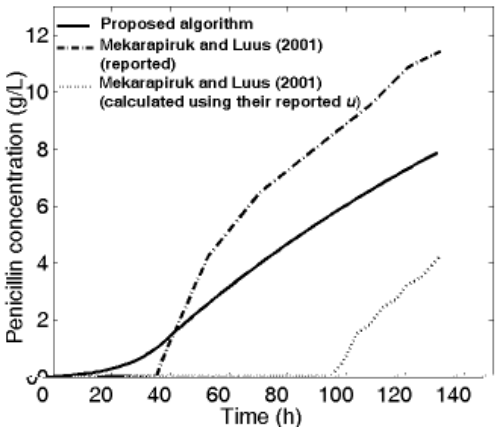

Figure 4. Time profiles of penicillin production.

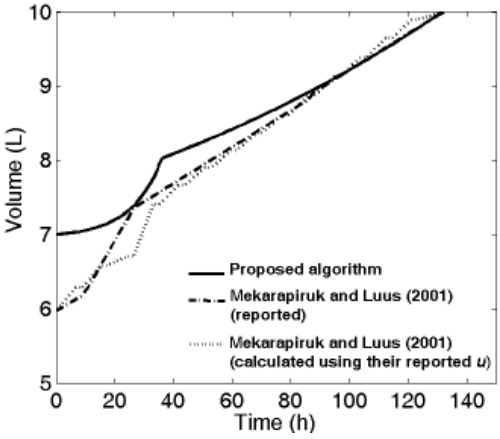

Figure 5. Time profiles of culture volume in a fermentor.

The result of biomass production profile reported by Mekarapiruk and Luus (2001) and the verified result coincided until fermentation time reached 44 h (Figure 2). After 44 h, the cell concentration result reported by Mekarapiruk and Luus (2001) remained constant at the maximum 40g/L until 107.8 h and then slightly dropped towards the end of fermentation while the verified result gradually dropped until 110 h and then it rose again. During $t \leq t_s$ (= 35.937 h) the optimized result of cell production yielded from this proposed algorithm increased exponentially which was similar to others. After the calculation procedure was switched at t_s cell concentration increased linearly until it reached 38.646 g/L at the end of fermentation.

The substrate consumption profiles of both optimized and verified results of Mekarapiruk and Luus (2001) were comparable; however, they peaked at different times. The profile of substrate concentration uptake determined by the proposed algorithm increased exponentially (Figure 3). After $t > t_s$ the substrate concentration drastically declined and then it gradually increased again.

All of the product formation profiles as shown in Figure 4 were similar to the objective function profiles illustrated in Figure 1. Evidently, product formation was growth associated.

All volume profiles were comparable (Figure 5). It confirmed that the set of optimal u yielded the same set of volume increase.

Conclusion

Evidently, the given set of optimal u was correctly reported by Mekarapiruk and Luus (2001) due to the same profiles of volume increase. However, the optimized results of profiles of biomass, substrate, and penicillin concentrations and objective function reported by Mekarapiruk and Luus (2001) could not be verified with the same given set of optimal u.

The proposed algorithm can be applied for determination of optimal feed-rate profiles in any fed-batch problems provided that the production rate of the secondary metabolite resembles a *Luedecking-Piret* type dependence on growth and biomass concentration.

Nomenclature

H = Hamiltonian function
K_S = saturation constant, 0.006 g/L
P = penicillin concentration, g/L
S = substrate concentration, g/L
S_D = desired remaining substrate concentration, g/L
t = current fermentation time, h
t = time interval used in optimization algorithm, h
T = total time period, h
t_f = final fermentation time,h
t_s = switching time from exponential to linear feeding policies, h
u = feed rate, L/h
u_0 = feeding rate at switching time (t_s)
V = culture volume, L
V_0 = initial culture volume prior to feeding, L
V_f = volume at final time, L
V_t = volume at the current time, L
X = biomass concentration, g/L
X_f = biomass concentration at final time, g/L
X_t = biomass concentration at current time, g/L
y_i = state variables

Greek symbols

α = constant for growth-associated term in *Luedeking-Piret* equation
β = constant for non growth-associated term in *Luedeking-Piret* equation
μ = specific growth rate, h^{-1}
μ_m = maximum specific growth rate, 0.11 h^{-1}
ε = a multiplier on substrate requirement
K = constant in feed rate equation (Eq. (19))

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