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Multipulse Feed Strategy for Glycerol Fed-Batch Fermentation

A Steady-State Nonlinear Optimization Approach

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Abstract

During glycerol fed-batch fermentation, the process could be divided into multiple equal subintervals, and the feed operation was performed in pulse form at the start of each subinterval. Based on the macrokinetic models, the multipulse feed strategy for both glucose and corn steep slurry was determined by a general nonlinear optimization approach to maximize the final glycerol productivity and still control the residual glucose at a low concentration. The experimental results in a 600-mL Airlift Loop Reactor showed that the tested data with this strategy agreed well with the corresponding model prediction, and that the feed mode with nonlinear optimization could improve the glycerol productivity significantly compared with those determined just by limited experimental optimization in previous studies.

Index Entries: Glycerol; multipulse feed; fed-batch fermentation; nonlinear optimization.

Introduction

During the past few decades, glycerol production by fermentation with osmotolerant yeast was widely studied in China to meet the great commercial demand *(1)*. During the fermentation, high glucose concentration will lead to inhibition in the fermentation while low glucose

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concentration will reduce glycerol productivity *(2)*; therefore, the fed-batch process seems more commercially attractive. In addition, the fed-batch fermentation results in a high concentration ratio of the final glycerol to residual glucose, which is also more advantageous for glycerol recovery in the downstream process *(3)*.

For glycerol fed-batch fermentation, the key limited nutrients to be fed include not only glucose but also the corn steep slurry, which is the main source of phosphorus *(4,5)*; thus, it must lead to difficulty in optimizing the two-feed operation during the optimization process. Some previous works *(4–6)* have even investigated the glycerol fed-batch process, in which dry glucose powder was fed in pulse form and the feed amount was optimized based on limited batches of experiments to maintain glucose at a certain constant level, while corn steep slurry was fed just by experience. However, these feed modes were not determined reasonably by biologic analysis or by optimization strategy, and thus, it was difficult to ensure that they were close to an optimal state. To obtain the appropriate feed strategy, physiologic model approaches and dynamic optimization were usually adopted *(7)*. However, most of the metabolic mechanisms are very complicated and difficult to describe and, therefore, with the physiologic model approach it is difficult to obtain precise control. Dynamic optimization generally comes down to an optimal control problem, but it can be solved usually by Pontryagin's maximum principle with the help of a mathematical model; however, this approach will suffer from a singular problem, which is difficult to solve *(2,8,9)*. Furthermore, the feed profile obtained by the dynamic optimization is usually continuous and time varying and, thus, is relatively difficult to be realized since complicated control equipment and operation are needed.

In the present study, to conveniently apply the optimized feed mode to practical glycerol production by fermentation, a suboptimal feed strategy was developed, in which the feed process was divided into multiple subintervals and the feed operation was executed in pulse form at the start of each subinterval. The feed amounts for every subinterval were determined by a general nonlinear optimization approach, and then the strategy was examined by the experimental results in a 600-mL Airlift Loop Reactor.

Nonlinear Optimization Approach for Determining Feed Amounts of Glucose and Corn Steep Slurry in Every Subinterval

Different from the dynamic optimal-control problem *(2)*, here the feed amounts of both glucose and corn steep slurry in every subinterval can be numerically solved by a general nonlinear optimization approach. Thus, the problem is converted to a steady-state optimization.

Variables to Be Optimized

If the process is divided into N subintervals, then total $2N$ variables including the feed volumes of glucose ($V_{FS,1}$, $V_{FS,2}$, …, $V_{FS,N}$) and the solution of corn steep slurry ($V_{FPh,1}$, $V_{FPh,2}$, …, $V_{FPh,N}$) at the start of every subinterval should be optimized.

Constraint Conditions

First, the total feed volume of glucose should be limited by inequality (Eq. 1) so that the reactor can be controlled in a valid worked volume:

$$0 \le \sum_{i=1}^{N} V_{FS,i} \le V_{FS,\max} \tag{1}$$

Second, to avoid significant inhibition, the process concentration of glucose (S) should be constrained below an upper limit (S_{up}). Therefore according to ref. 2, the following inequality was applied:

$$0 \le S \le S_{up} = 40 \tag{2}$$

Third, since a lower concentration of the residual glucose is more advantageous for glycerol separation from the broth in the downstream process, the final glucose concentration S_f was suggested to be controlled less than a certain level of S_{end} (%):

$$0 \le S_f = S(t_f) \le S_{end} \tag{3}$$

In this work, we set $S_{end} = 2$ based on the experimental experience in refs. *2–5*.

State Equations

The macrokinetic models for glycerol production by batch fermentation at constant 35°C was suggested by Xie *(2)* as follows:

$$\frac{dX}{dt} = \mathrm{Exp}(-\frac{t}{k_t}) \cdot (1 + \frac{K_O}{1 + X/K_{do1}}) \cdot \frac{S}{(K_{S1}X + S)\,(1 + S^2/K_{IS})} \cdot \frac{Ph}{K_{Ph1} + Ph} \cdot \mu_{\max} \cdot X \tag{4}$$

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} + \frac{S}{K_{SP}P + S}\frac{Ph}{K_{Ph2} + Ph} \cdot m_t \cdot X + \frac{1}{Y_{P/S}}\frac{dP}{dt} \tag{5}$$

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta\frac{S_0}{(1 + X/K_{do2})^2}\frac{Ph}{K_{Ph3} + Ph}X - \frac{K_{SP}P}{K_{SP}P + S}\frac{Ph}{K_{Ph2} + Ph}m_tX \tag{6}$$

$$-\frac{dPh}{dt} = \frac{1}{Y_{X/Ph}} \cdot \frac{S^2}{K_{S2} + S^2}\frac{dX}{dt} \tag{7}$$

in which X, S, P, and Ph are, respectively, the concentrations of cell, glucose, glycerol, and phosphorus. The kinetic parameters used in this study were estimated based on nine batches of orthogonal experimental data in a 1.5-L Airlift Loop Reactor, as shown in Table 1 *(2)*. For multipulse fed-batch

Table 1
Values of Kinetic Parameters in Eqs. 4–7

Parameter	Value
μ_{max}	111.0
K_t	33.96
K_O	2.174
K_{S1}	652.3
K_{IS}	93.20
$Y_{X/S}$	1.625
m_t	0.05924
K_{SP}	0.1920
$Y_{P/S}$	0.4793
α	0.02310
β	1.061E-03
$Y_{X/Ph}$	0.05450
K_{S2}	152.7
K_{do1}	1.998
K_{do2}	11.39
K_{Ph1}	163.6
K_{Ph2}	14.38
K_{Ph3}	1.088

fermentation discussed here, the following mass balance should be considered between any two closest subintervals:

$$\left.\begin{aligned}
&V_{0,i} = V_{f,i-1} + V_{FS,i} + V_{FPh,i} \\
&X_{0,i} = X_{f,i-1} \cdot (V_{f,i-1}/V_{0,i}) \\
&S_{0,i} = (V_{f,i-1} \cdot S_{f,i-1} + V_{FS,i} \cdot S_{FS})/V_{0,i} \\
&P_{0,i} = P_{f,i-1} \cdot (V_{f,i-1}/V_{0,i}) \\
&Ph_{0,i} = (V_{f,i-1} \cdot Ph_{f,i-1} + V_{FS,i} \cdot Ph_{FS} + V_{FPh,i} \cdot Ph_{FPh})/V_{0,i}
\end{aligned}\right] \tag{8}$$

in which the subscript i represents the sequence number of the subinterval ($1 \leq i \leq N$); S_{FS} and Ph_{FS} are, respectively, the concentrations of glucose and phosphorus in the glucose feed tank; Ph_{FPh} is the phosphorous concentration in the feed tank of corn steep slurry (phosphorous source); and V_{FS} and V_{FPh} are the feed volumes of glucose and corn steep slurry, respectively.

Then, by the Runge-Kutta method, the final glycerol and glucose concentration (P_f, S_f) can be obtained by integrating Eqs. 4–7 in sequence of the subintervals combined with Eqs. 8, and $P_f = P_{f,N}$; $S_f = S_{f,N}$.

Objective Function

The objective is to maximize the final glycerol yield with the constraints (Eq. 1–3). The constraint conditions can be realized by adding punishment functions in the objective function. Thus, the following objective function should be minimized:

$$J(T, F_S, F_{Ph}) = -V_f \cdot P_f + fpun(V_{FS}) + fpun(S_f) + \int_{t_0}^{t_f} fpun(S) \cdot dt \qquad (9)$$

in which $fpun(V_{FS})$, $fpun(S)$, and $fpun(S_f)$ are the punishment functions, respectively, for constraint conditions in Eqs. 1, 2, and 3. They can be determined, respectively, by Eqs. 10, 11, and 12:

$$fpun(V_{FS}) = \begin{Bmatrix} pun_{VFS} \cdot \left(\sum_{i=1}^{j} V_{FS,i} - V_{FS,\max}\right)^2 & \sum_{i=1}^{j} V_{FS,i} > V_{FS,\max} \\ 0 & \sum_{i=1}^{j} V_{FS,i} \le V_{FS,\max} \end{Bmatrix} \qquad (10)$$

$$(i = 1, \ldots, j; j = 1, 2, \ldots, N)$$

$$fpun(S_f) = \begin{Bmatrix} pun_{Sf} \cdot (S_f - S_{\text{end}})^2 & S_f > S_{\text{end}} \\ 0 & S_f \le S_{\text{end}} \end{Bmatrix} \qquad (11)$$

$$fpun(S) = \begin{Bmatrix} pun_S \cdot (S - S_{up})^2 & S > S_{up} \text{ or } S < 0 \\ 0 & 0 \le S \le S_{up} \end{Bmatrix} \qquad (12)$$

In Eqs. 10–12, pun_{VFS}, pun_{Sf}, and pun_S are coefficients for corresponding punishment functions; here they were set as 0.1, 1, and 1, respectively.

Numerical Solution

The nonlinear optimization was executed by the method of Powell *(10)*. The total number of the subintervals (N) is very important to the solution results; the greater N, the closer the solution results (e.g., final glycerol yield) are to that by dynamic optimization with optimal control theory. It was found in previous work *(2)* that when $N > 8$, the final glycerol yield obtained from nonlinear optimization for multipulse feed operation is decreased by only 5% at most compared with that from dynamic optimization with optimal control theory. Therefore, the multipulse feed strategy with nonlinear optimization can be considered as the suboptimal control in feed operation.

The numerical conditions in the present study were as follows: S_0 = 30%; $X_0 = 0.55 \times 10^8$ cells/mL; P_0 = 0%; Ph_0 = 60 μg/mL; S_{FS} = 1.8 g/mL (in this work, glucose was also fed in dry powder form *[4–6]*; therefore, herein S_{FS} is just considered as the density of dry glucose); Ph_{FS} = 0 μg/mL;

Ph_{FPh} = 2000 μg/mL (phosphorous content in the feed tank of 10% [w/v] corn steep slurry); V_0 = 400 mL; $V_{FS,\max}$ = 80 mL (this means the total added glucose is $V_{FS,\max} \cdot S_{FS}$ = 144 g); t_f = 240 h; and N = 10.

Materials and Methods

Microorganism and Media

An osmotolerant yeast, *Candida krusei* (ICM-Y-05), was applied. The mass composition of media for preculture was 10% glucose, 0.3% urea, and 0.3% corn steep slurry, and for batch or fed-batch fermentation was 30% glucose, 0.25% urea, and 0.30% corn steep slurry.

Fed-Batch Fermentation

Seed was precultured aerobically for 12 h, and then was inoculated into the culture media by a ratio of 10%. Dry glucose powder and the solution of corn steep slurry (10%), based on the numerical results, were fed instantly in pulse form at the start of each subinterval. The fermentor was a 600-mL Airlift Internal Loop Reactor with a working volume of 400–480 mL, the aeration rate was 2 vvm, and the culture temperature was controlled at 35 ± 1°C. Since the pH change from 3.0 to 5.0 had no significant effect on glycerol fermentation *(3)*, only the initial pH was adjusted to 4.5 by the addition of 1 *N* HCl, and the later pH could be kept within 3.0–4.0 by the yeast without the addition of any alkaline or acidic solution.

Analyses

Glycerol concentration was determined by periodate-chromotropic acid analysis, glucose concentration was determined by Fehling's test, and cell concentration was measured under the microscope using a hemocytometer *(2)*.

Results and Discussion

After the nonlinear optimization, as described earlier, the feed amounts of glucose and corn steep slurry (phosphorous source) at the start of the 10 subintervals were obtained (see Table 2).

To check whether the multipulse feed operation with nonlinear optimization has the best glycerol productivity, the feed operations optimized experimentally in refs. *4* and *5* were selected as the controls. In ref. *4*, dry glucose powder was fed with 24 g every 24 h during h 24–144, and corn steep slurry (10%, w/v) was fed with 1.4 mL every 24 h during h 24–144 and with 0.7 mL every 24 h during h 144–216. In ref. *5*, glucose was fed after h 24 to maintain the glucose concentration at 25–30%, and corn steep slurry was fed with 1.4 mL every 24 h during h 72–168 and with 0.7 mL every 24 h during h 168–216. All the fed-batch experiments were conducted in duplicate and the total glucose feed amount was 144 g. Figure 1 presents a comparison of the experimental results.

Table 2
Feed Amounts of Glucose ($F_S = V_{FS} \cdot S_{FS}$)
and Corn Steep Slurry (V_{FPh}) at Start of 10 Subintervals
and Culture Volume After Feed Operation (V)

t (h)	F_S (g)	V_{FPh} (mL)	V (mL)
0	52.5	3.0	431.1
24	0.0	0.2	431.3
48	22.6	0.0	443.8
72	47.9	0.0	470.4
96	0.0	0.1	470.5
120	0.1	0.2	470.7
144	13.5	0.0	478.2
168	2.8	0.1	479.8
192	5.1	0.0	482.7
216	0.0	0.0	482.7

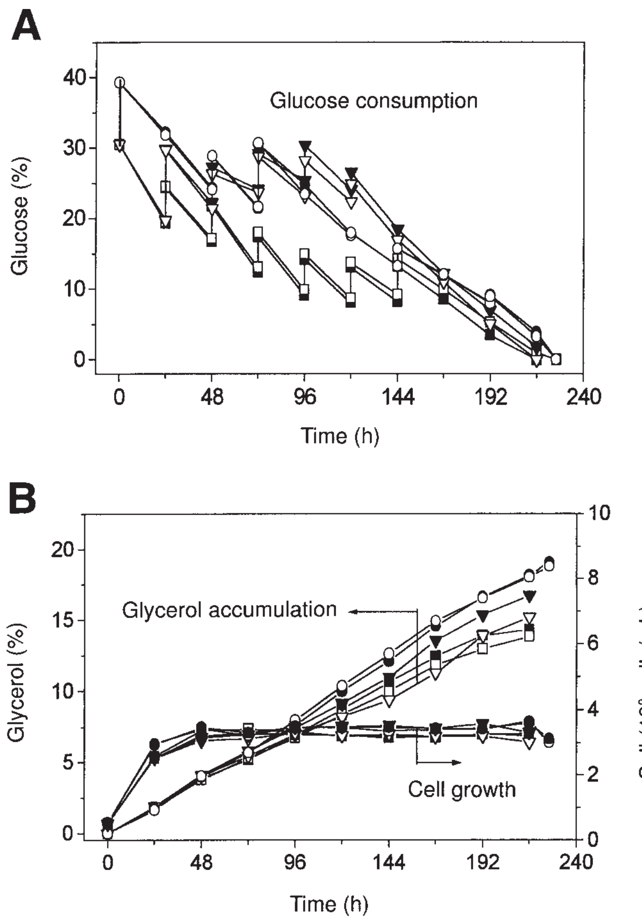

Fig. 1. Time courses of glucose consumption **(A)** and glycerol accumulation and cell growth **(B)** of different feed operations (●, ○: based on Table 2; ■, □: based on ref. *4*; ▼, ▽: based on ref. *5*).

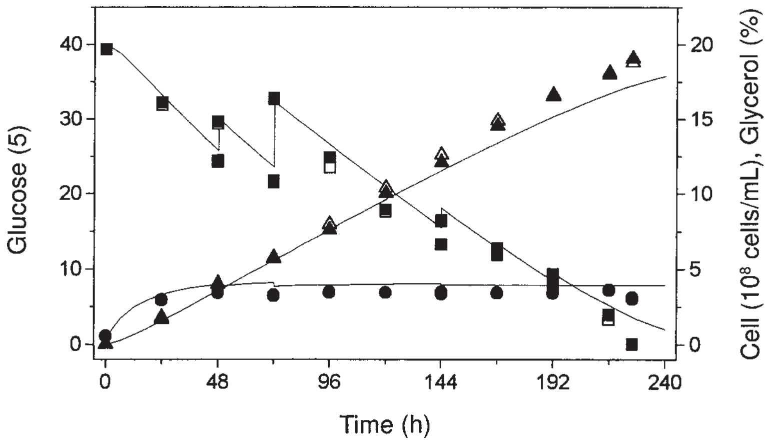

Fig. 2. Comparison of process curves between model prediction and the experimental data in multipulse fed-batch fermentation with nonlinear optimization ($N = 10$). (■, □: tested glucose; ●: tested cell; ▲, △: tested glycerol; ——: model prediction).

From Fig. 1, it was found that among the three feed operations, the feed operation with nonlinear optimization had the greatest glycerol productivity. The final glycerol yields, in the duplicate experiments (1) and (2), for the operation with nonlinear optimization were 19.06 (Exp. 1) and 18.77% (Exp. 2), respectively, while those for operation based on ref. *4* were 14.26 (Exp. 1) and 13.82% (Exp. 2) and for operation based on ref. *5* were 16.73 (Exp. 1) and 15.16% (Exp. 2), respectively.

The feed strategy introduced here belongs to an open-loop control method. Figure 2 shows that the experimental data for feed operation with nonlinear optimization agreed well with the corresponding model prediction in the former stage, whereas in the latter stage, glycerol from experimental results was higher than that from model prediction. The possible reason for this is that the capacity for glycerol accumulation in fed-batch fermentation was intensified compared with that in the batch case, since the osmotolerant yeast in the fed-batch case suffers a longer stage at relatively high osmotic pressure, which is important in order to induce enough relative enzyme to produce glycerol to improve the tolerant ability for high osmotic pressure *(3,11,12)*. However, the model Eqs. 4–7 were derived just from the curve-fitting results of the batch experiments *(2)*; therefore, further study should be undertaken to perfect the mathematical model for the fed-batch fermentation. Nevertheless, the example applied here still suggests that the nonlinear optimization approach to determine the feed strategy should be feasible and promising for future commercial production.

Conclusion

For glycerol fed-batch fermentation, the whole process can be divided into multiple subintervals, and the feed operation for both glucose and corn steep slurry (phosphorous source) can be performed conveniently in pulse form at the start of each subinterval. The feed amounts of glucose and corn steep slurry in every subinterval can be optimized by a general nonlinear optimization approach. Compared with the previous feed operation determined just by limited experimental optimization *(4,5)*, the feed approach proposed herein can improve the final glycerol productivity while the final residual glucose can also be controlled at a low concentration.

Nomenclature

$fpun$ = punishment function
K_{do1} = constant of dissolved oxygen for cell growth (10^8 cells/mL)
K_{do2} = constant of dissolved oxygen for glycerol accumulation (10^8 cells/mL)
K_{IS} = constant for inhibition of glucose in cell growth (10^{-4} [g/mL] · [g/mL])
K_O = ratio of oxygen promotion to cell growth (nondimensional)
K_{Ph1} = saturation constant for phosphorus for cell growth (μg/mL)
K_{Ph2} = saturation constant for phosphorus for glucose consumption (μg/mL)
K_{Ph3} = saturation constant for phosphorus for glycerol production (μg/mL)
K_{S1} = Contois constant for glucose for cell growth (10^{-10} g/mL)
K_{S2} = saturation constant for glucose for phosphorus consumption (10^{-4} [g/mL] · [g/mL])
K_{SP} = coefficient of glycerol as a carbon source compared with glucose (nondimensional)
K_t = attenuation constant for culture time for cell growth (h)
m_t = total maintenance coefficient of glycerol and glucose (h^{-1})
N = total number of the subintervals during multipulse fed-batch fermentation
P = mass concentration of glycerol (%, w/v)
Ph = concentration of phosphorus (μg/mL)
Ph_{FPh} = phosphorous concentration in feed tank of corn steep slurry (μg/mL)
Ph_{FS} = phosphorous concentration in glucose feed tank (μg/mL)
Pun_S = punishment coefficient for constraint on glucose process concentration (nondimensional)
Pun_{Sf} = punishment coefficient for constraint on final glucose concentration (nondimensional)
Pun_{VFS} = punishment coefficient for constraint on total feed amount of glucose (nondimensional)
S = mass concentration of glucose (%, w/v)
S_0 = initial mass concentration of glucose (%, w/v)
S_{FS} = glucose concentration in glucose feed tank (%, w/v)
t = culture time (h)
V = culture volume (mL)
V_{FS} = feed amount of glucose (mL)
V_{FPh} = feed amount of corn steep slurry (mL)
X = cell concentration (10^8 cells/mL)

$Y_{P/S}$ = yield coefficient of glycerol production for glucose consumption (nondimensional)
$Y_{X/Ph}$ = yield coefficient of cell production for phosphorous consumption (10^8 cells/μg)
$Y_{X/S}$ = yield coefficient of cell production for glucose consumption (10^{10} cells/g)

Greek

α = constant associated with cell growth (10^{-10} g/cell)
β = modified constant associated with cell concentration (10^{-8} mL/cell/h)
μ = specific growth rate (h^{-1})

Subscripts

0 = initial state of fed-batch fermentation
end = final state of glucose concentration
f = final state of fed-batch fermentation
i = sequence number of subintervals ($1 \le i \le N$)
max = maximum value or upper limit
up = upper limit

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