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Independent Exponential Feeding of Glycerol and Methanol for Fed-Batch Culture of Recombinant *Hansenula polymorpha* DL-1

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Abstract

As a novel feeding strategy for optimizing human epidermal growth factor (hEGF) production with a recombinant *Hansenula polymorpha* DL-1 using the methanol oxidase (*MOX*) promoter in *H. polymorpha* DL-1, independent exponential feeding of two substrates was used. A simple kinetic model considering the cell growth on two substrates was established and used to calculate the respective feeding rates of glycerol and methanol. In the fed-batch culture with methanol-only feeding, the optimal set point of specific growth rate on methanol was found to be 0.10 h^{-1}. When the fed-batch cultures were conducted by the independent feeding of glycerol and methanol, the actual specific growth rate on glycerol and methanol was slightly lower than the set point of specific growth rate. By the uncoupled feeding of glycerol and methanol the volumetric productivity of hEGF increased from 6.4 to 8.0 mg/(L·h), compared with methanol-only feeding.

Index Entries: *Hansenula polymorpha*; human epidermal growth factor; fed-batch culture; exponential feeding; mixed feed.

Introduction

Methylotrophic yeasts including *Hansenula polymorpha* have been found economically appealing as an alternative high-yield expression

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system for heterologous proteins. There are recent reviews describing the general features of the expression systems and the relevant achievements on the production of recombinant proteins *(1–5)*. The advantages of these systems include cell growth to high densities on inexpensive, defined media; the very strong and tightly regulated methanol-induced promoter; efficient posttranslational modification; and secretion of expressed proteins. The most common culture strategy adopted in the production of recombinant proteins using methylotrophic yeasts is a two-phase (growth phase/production phase) fed-batch culture *(5)*. In the growth phase, glycerol or glucose, which allows high cell growth rate, but by which expression of a foreign gene is not efficiently induced, is used for cell growth in batch and/or fed-batch operations. Then methanol feeding is started to induce the expression of a foreign gene.

Mixed substrate feeding in the induction phase has been implemented to improve the productivity of recombinant protein by many researchers *(6–13)*. In those approaches, two carbon sources, methanol and a higher carbon source such as glycerol, are fed in the induction phase of fed-batch culture. It is important to control the higher carbon source level in the induction phase because the carbon source must be supplemented in quantities sufficient to enhance the cell growth while not repressing both the foreign gene expression and metabolic pathway of methanol. For feeding of the supplementary carbon source, feeding using a mixture form with methanol *(6–11)* and constant feeding of the carbon source while keeping methanol concentration constant using a methanol sensor *(12,13)* have been studied. However, there have been limited reports on optimal feeding rate of mixed carbon sources through kinetic analysis of the heterologous protein production based on a theoretical model. Recently, Zhang et al. *(14)* reported a model that described the relationship between specific growth rate and methanol concentration, and the relationships between specific methanol and ammonium consumption rates and specific growth rate under methanol-limited growth conditions. However, their model was applicable only for methanol feeding and not for the feeding of mixed carbons. D'Anjou and Daugulis *(10,11)* established a simple model capable of predicting cell growth and methanol utilization during the mixed-substrate fed-batch fermentation of Mut^s recombinant *Pichia pastoris*. The model was used to design an exponential feeding strategy for mixed-substrate fed-batch fermentation at a constant specific growth rate. However, the model was used only for a mixture of two substrates with a predetermined composition of methanol/glycerol feed. Hellwig et al. *(13)* reported in detail the effects of supplementary glycerol feeding while maintaining a constant methanol concentration during the induction of a Mut^+ strain of *P. pastoris*. The glycerol cofeeding rate needed to be further optimized within the strategy.

A recombinant *H. polymorpha* strain *(15)* producing human epidermal growth factor (hEGF) was used throughout the present study. The strain was obtained by using an expression vector *(16)* containing an autonomously replicating sequence in *H. polymorpha* (*HARS36*) and an auxotrophic

marker of *H. polymorpha*. We have reported the improvement of the hEGF productivity using an optimal strategy of pH control *(17)*. EGF is a polypeptide consisting of 53 amino acids with three internal disulfide bonds. It has several biologic functions for cells such as stimulation of protein transport, activation of the synthesis of extracellular macromolecules, and increased cell proliferation *(18)*. These properties have led to its development for several therapeutic applications such as wound and burn healing, healing of corneal surface abrasion, and the treatment of gastric ulcers *(18)*. The gene encoding hEGF has been expressed in various hosts *(19–22)*.

In this article, we report on a new exponential feeding strategy for two substrates playing different roles in the expression of hEGF gene. Independent feeding of two substrates in the fed-batch culture was introduced to increase the productivity of hEGF. In the independent feeding strategy, a supplementary carbon source such as glycerol was fed exponentially, as was methanol, into a bioreactor. Through the fed-batch cultures adopting an independent exponential feeding strategy, the effects of higher carbon sources such as glycerol and glucose on cell growth and the gene expression were determined.

Materials and Methods

Strain and Media

Mut$^+$ *H. polymorpha* UR2/CPY$^-$ *(15)* was used for the production of hEGF. A leucine and uracil double auxotrophic mutant of *H. polymorpha* DL1 (leu2-ura3-) was derived from DL-1 (American Type Culture Collection 26012). For gene integration and selection of a transformant with multiple hEGF expression cassettes in the chromosome, on expression vector that had been previously reported *(16)* was constructed. It possessed *Hansenula LEU2* and *Sc-URA3* as selection markers, methanol oxidase (*MOX*) promoter, *MOX* terminator, a *Hansenula* autonomously replicating sequence (*HARS36*), and *Saccharomyces* prepro alpha-factor sequence. The strain was stored at –70°C in 30% (w/w) glycerol solution. To obtain inoculum, the frozen strain was transferred to YPD agar plates, which were stored at 4°C for a maximum of 1 mo. Growth medium consisted of 10 g/L of yeast extract, 20 g/L of bactopeptone, and 20 g/L of glycerol. Production medium used in the batch cultures consisted of 10 g/L of yeast extract, 20 g/L of bactopeptone, and 20 g/L of methanol.

Batch Cultures

Batch cultures were performed in a KF-2.5 L bioreactor (Korea Fermentor, Incheom, Korea) equipped with temperature, pH, and dissolved oxygen (DO) control systems. The bioreactor culture had a working volume of 1 L. In the bioreactor culture, the agitation speed was 500 rpm, temperature was controlled at 37°C, and the aeration rate was 1 L/min. The pH of culture broth was controlled to 6.0 with 2 *N* H_2SO_4 and 2 *N* NaOH. The seed culture for obtaining inoculum was conducted in a 500-mL shake flask. Inoculum of 1% (v/v) was used for the main culture.

Fed-Batch Cultures Using Exponential Feeding of Methanol

Fed-batch cultures were performed in the described 2.5-L bioreactor. *H. polymorpha* was grown in batch phase until depletion of methanol (20 g/L). Following the batch phase, a methanol feeding phase was started using a computer-controlled peristaltic pump (Masterflex L/S computerized drive; Cole-Parmer, Vernon Hills, IL). The feed solution contained 200 g/L of methanol, 40 g/L of yeast extract, and 80 g/L of bactopeptone. The yeast extract and bactopeptone solution were autoclaved and allowed to cool before the methanol was added. The methanol feed rate was increased exponentially according to the calculation results of feeding rate. The calculation method of the rate is described in the following section. During culture, agitation speed was gradually increased to 1500 rpm to keep the DO level above 20% of saturation. The culture was continued until the agitation speed reached 1500 rpm. The culture pH was controlled to 6.0 with 2 *N* H_2SO_4 and 2 *N* NaOH, and the temperature was controlled to 37°C. Antifoam 204 (Sigma, St. Louis, MO) was added prior to inoculation, and as needed thereafter, to prevent excessive foaming.

Fed-Batch Cultures Using Independent Feeding of Two Substrates

Fed-batch cultures were performed in the same bioreactor used in the previous section. On depletion of methanol in the batch phase, constant feeding of glycerol or glucose medium was initiated for 1 h to prevent accumulation of the substrates in the early part of the fed-batch phase of the mixed-feed experiments. Following this adaptation period, a methanol/glucose or methanol/glycerol exponential feeding was started. The supplementary carbon source, glycerol or glucose, was fed separately from methanol. The respective methanol, glycerol, and glucose feed contained 200 g/L of the carbon source, 50 g/L of yeast extract, and 100 g/L of bactopeptone. During culture, agitation speed was gradually increased to 1500 rpm to keep the DO level above 20% of saturation. When the agitation speed reached 1500 rpm, pure oxygen was supplied to meet the oxygen consumption. The feeding lasted for 40 h. Note that referred culture conditions were the same as those used in the culture using methanol feed.

Assay

Cell mass was determined by measuring optical density (OD) at 600 nm and calculated using a cell standard curve (1 OD = 0.32 g of cell/L). Methanol concentrations in the samples were measured by gas chromatography (YoungLin M600D, Anyang, Korea) with a flame ionization detector and a Porapak Q80/100 column using 1% (v/v) isopropanol as an internal standard. Glucose and glycerol were determined enzymatically using a glucose kit (Glucose-E kit; Yeongdong Pharm., Seoul, Korea) and a diagnostic kit for glycerol (Sigma), respectively. The hEGF in the broth was assayed by indirect enzyme-linked immunosorbent assay as described by Voller et al. *(23)*. A standard curve was obtained by using purified recombinant hEGF

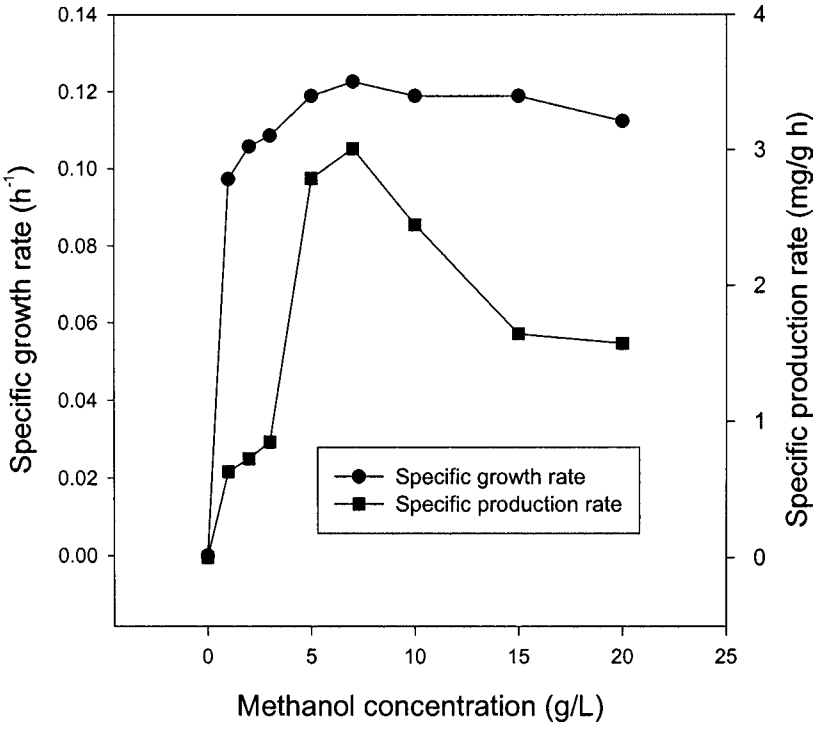

Fig. 1. Influence of initial methanol concentration on specific growth rate and specific production rate in batch cultures of recombinant *H. polymorpha* DL-1.

supplied by the manufacturer (Upstate Biotechnology, Waltham, MA). The monoclonal anti-hEGF was purchased from Serotec (Oxford, United Kingdom), and peroxidase-labeled antimouse IgG was purchased from Bio-Rad (Hercules, CA). The peroxidase substrate used was TMB (Pierce, Rockford, IL). The minimum concentration of hEGF that could be detected was 1.25 ng/mL, and the standard deviation was obtained from quadruplicate for each sample.

Results and Discussion

Pattern of Specific Growth Rate and Specific Production Rate in Batch Culture

The relationship between specific growth rate and specific production rate plays a crucial role in optimizing a fed-batch culture *(24)*. Batch cultures were carried out for different methanol concentrations to obtain the relationship. The production pattern of hEGF followed that of growth-associated products (data not shown). Data collected in the early part of the culture after 5 h of the start when methanol was used in the cell growth were used to determine specific growth rate and specific production rate. The results showed a nonmonotonic pattern in both specific growth rate and specific production rate, as presented in Fig. 1. The optimum methanol

concentration for maximizing the specific production rate was 7.0 g/L, and at this methanol concentration, a maximum specific growth rate of 0.12 h^{-1} was obtained. Because it was reported that the exponential feeding of a growth-limiting substrate in a fed-batch culture resulted in a constant substrate concentration and control of the specific growth rate *(25)*, the exponential feeding strategy was adopted in this study. Although the kinetics of fed-batch culture to keep methanol level constant is not in accordance with that of batch culture because the physical and biochemical environments of the fed-batch culture differ from those of batch culture, it is possible to obtain a required guideline to set up an optimal production strategy.

Calculation of Substrate Feeding Profile

In the case of *H. polymorpha*, it was reported that their regulation follows a repression/derepression. Methanol is considered the most derepressing substrate, and glycerol is a derepressing carbon source of *MOX* promoter. Methanol must be present in quantities sufficient to fully induce heterologous gene expression without inhibiting growth or product formation *(10)*. Glycerol must be supplemented in quantities to enhance cell growth while not repressing both the gene expression and metabolic pathway of methanol driven by *MOX* promoter. Therefore, in the present study, an individual exponential feeding strategy was introduced to satisfy the requirement that methanol and glycerol concentrations be kept constant at a certain level. In our feeding strategy, not only methanol but also glycerol was fed exponentially based on a theoretical model describing the mixed-feed fed-batch culture. To our knowledge, there has been no report on the exponential feeding of glycerol in a mixed-feed fed-batch culture. So far, as feeding strategies of a supplementary carbon source, a feeding protocol as a mixture form with methanol *(6–11)* or a constant feeding *(12,13)* has been used. These methods have limitations in optimizing the feeding rate or the concentration of supplementary carbon source. The exponential feeding strategy of supplementary carbon source will give an alternative method to control the concentration of the carbon source if a reliable sensor for the carbon source is not available.

Mass balance equations to describe the fed-batch culture assuming that during mixed-substrate feeding the cell growth rates from glycerol and methanol are additive are as follows *(26)*:

Cell mass balance

$$\frac{d(XV)}{dt} = (\mu_M + \mu_G)XV \tag{1}$$

Glycerol mass balance

$$\frac{d(GV)}{dt} = F_G G_0 - q_G XV \tag{2}$$

Methanol mass balance

$$\frac{d(MV)}{dt} = F_M M_0 - q_M XV \tag{3}$$

hEGF mass balance

$$\frac{d(PV)}{dt} = q_p XV \tag{4}$$

Working volume

$$\frac{dV}{dt} = F_M + F_G \tag{5}$$

In the case in which the specific growth rates on methanol and glycerol are controlled at a constant value, μ_M and μ_G will be constant. Given at t = 0 that $V = V_0$ and $X = X_0$, the cell mass balance equation can be integrated and written as follows:

$$XV = X_0 V_0 \exp[(\mu_M + \mu_G)t] \tag{6}$$

Assuming that quasi–steady states exist for the glycerol and methanol concentrations, the left terms in Eqs. 2 and 3 are zero. If we also assume that $Y_{X/G}$ and $Y_{X/M}$ are constant, by substituting Eq. 6 into the glycerol and the methanol mass balance, we can obtain glycerol and methanol feeding profiles, respectively:

$$F_G = \frac{q_G XV}{G_0} = \frac{\mu_G X_0 V_0 \exp[(\mu_M + \mu_G)t]}{Y_{X/G} G_0} \tag{7}$$

$$F_M = \frac{q_M XV}{M_0} = \frac{\mu_M X_0 V_0 \exp[(\mu_M + \mu_G)t]}{Y_{X/M} M_0} \tag{8}$$

Table 1 provides the parameters used to calculate F_G and F_M.

Exponential Feeding of Methanol Feed

To examine the reliability of the calculation of the feeding rate and the effect of methanol feeding rate on hEGF production in the fed-batch culture, exponential feeding experiments of methanol feed were conducted (Fig. 2). The batch phase of the fed-batch culture was carried out using methanol in order to minimize the effect of cell history by eliminating a methanol adaptation phase required in the case of the fed-batch culture using glycerol as a batch-phase substrate. In all experiments, the fed-batch phase was started after methanol exhaustion of the batch phase. When the agitation speed reached 1500 rpm, the fed-batch culture was intentionally stopped to examine hEGF production under the same limited oxygen

Table 1
Parameters Used
in Calculation of Feeding Profile[a]

Parameter	Value	Source
M_0 (g/L)	200	Measured
D_0 (g/L)	200	Measured
G_0 (g/L)	200	Measured
X_o (g/L)	6.2	Measured
V_0 (L)	0.8	Measured
$Y_{X/M}$ (g/g)	0.27	Batch data
$Y_{X/D}$ (g/g)	0.35	Batch data
$Y_{X/G}$ (g/g)	0.64	Batch data

[a]M_0, feed methanol concentration; D_0, feed glucose concentration; G_0, feed glycerol concentration; X_0, cell concentration at the beginning of fed-batch culture; V_0, volume at the beginning of fed-batch culture; $Y_{X/M}$, cell yield on methanol; $Y_{X/D}$, cell yield on glucose; $Y_{X/G}$, cell yield on glycerol.

Table 2
Results of Exponential Feeding of Methanol[a]

μ_{sp} (h^{-1})	X_{max} (g/L)	P_{max} (mg/L)	μ_{act} (1/h)	$Y_{P/X}$ (mg/g)	π (mg/[g·h])	Π (mg/[L·h])	t_c (h)
0.040	16.7	130.0	0.030	5.28	0.16	2.22	58.5
0.060	28.2	290.5	0.063	11.43	0.72	6.05	48.0
0.080	26.0	249.0	0.082	11.14	0.91	6.22	40.0
0.100	25.3	230.5	0.102	12.10	1.22	6.40	36.0
0.120	20.4	123.0	0.127	6.16	0.782	3.96	31.0

[a]μ_{sp}, set point of specific growth rate; X_{max}, cell concentration at the end of culture; P_{max}, hEGF concentration at the end of culture; μ_{act}, actual specific growth rate; $Y_{P/X}$, product yield on cell mass; π, specific production rate; Π, volumetric productivity; t_f, final culture time.

supply. It was observed that the actual values of specific growth rate obtained in the fed-batch phase were close to set points of specific growth rates except the set point of specific growth rate, 0.04 h^{-1}. The results of exponential methanol feeding are summarized in Table 2.

The highest hEGF production was obtained at μ_{sp} = 0.06 h^{-1}. This was presumably owing to the extended culture time by the lower oxygen consumption than those of μ_{sp}, ranging from 0.08 to 0.12 h^{-1}, while the

Fig. 2. *(opposite page)* Influence of set point of specific growth rate on **(A)** cell growth, **(B)** hEGF production, and **(C)** methanol concentration in methanol exponential feeding fed-batch cultures of recombinant *H. polymorpha* DL-1. DO level was controlled at 20% of saturation. When agitation speed reached 1500 rpm, operation of the bioreactor was stopped.

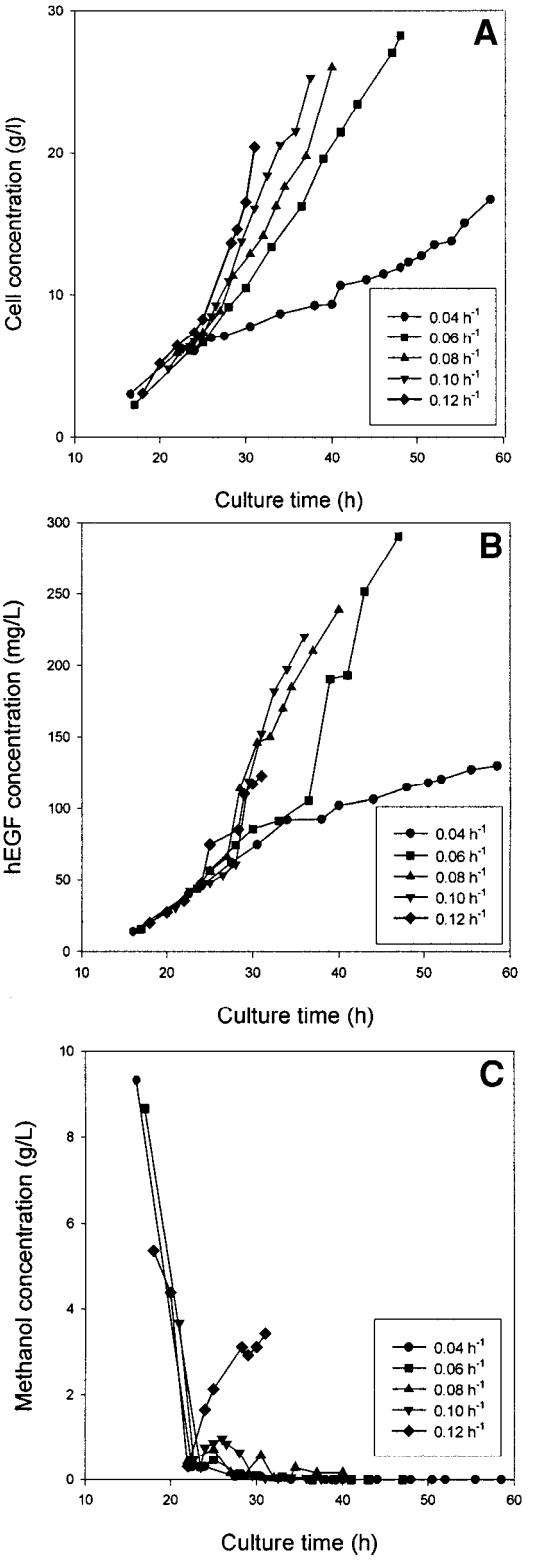
A
Cell concentration (g/l)
Culture time (h)
0.04 h-1
0.06 h-1
0.08 h-1
0.10 h-1
0.12 h-1
B
hEGF concentration (mg/L)
Culture time (h)
C
Methanol concentration (g/L)
Culture time (h)

Fig. 2.

product yield on cell mass ($Y_{P/X}$) was similar in the range of specific growth rate between 0.06 and 0.10 h^{-1}. It is known that unlimited feeding of methanol leads to excessive oxygen consumption and heat dissipation that finally can exceed the capacities of production-scale bioreactors *(27)*. Our results indicate that if the culture system has limited oxygen transfer capabilities, the maximum protein concentration is obtained in low feeding.

The highest specific production rate and volumetric productivity, which was calculated by dividing the maximum production by the culture time when the production was observed, was obtained at $\mu_{sp} = 0.10\ h^{-1}$. The set point of specific growth rate on methanol of 0.10 h^{-1} was used for the independent exponential feeding of two substrates. It has been reported that when the feeding rate in the exponential fed-batch culture is not feedback controlled around a set point, the system becomes unstable even if methanol accidentally fluctuates slightly *(28)*. Methanol serves as a growth nutrient up to a certain level, and it is a growth inhibitor at high concentration. A transient exposure to toxic methanol levels leads to an immediate and complete breakdown of productivity of the culture, although growth can be fully restored during prolonged fermentation. Therefore, the set point of specific growth rate on methanol of 0.10 h^{-1}, slightly lower than the maximum specific growth rate, was chosen for the independent exponential feeding fed-batch culture considering both the volumetric productivity and stable operation.

Independent Exponential Feeding of Glycerol and Methanol Feed

Two different set points of specific growth rate on glycerol ($\mu_{G,sp}$), 0.01 and 0.02 h^{-1}, were used with $\mu_{M,sp} = 0.10\ h^{-1}$ as the optimum set point of specific growth rate on methanol in order to evaluate the validity of the calculation method of the feeding rates and to investigate the effect of supplementary glycerol feeding (Fig. 3A,B). In addition to the glycerol feeding, glucose/methanol as another combination of carbon sources was tried in the dual-feed fed-batch culture (Fig. 3C,D). It is well known that derepression of the methanol pathway is observed when glucose is present in limiting concentrations. The respective glucose and methanol feed were supplied using the same calculation method used in the glycerol and methanol feed as mentioned earlier. In both cases, glycerol and glucose were kept at very low concentration, not detected by the enzymatic method. The minimum detection levels of glucose and glycerol based on the method were about 20 and 40 mg/L, respectively. When the fed-batch cultures were conducted with the independent feeding protocol of glycerol/methanol and glucose/methanol, the actual specific growth rates (μ_{act}) were slightly lower than the corresponding set points of specific growth rate, as shown in Table 3. The deviation increased as the set point of specific growth rate on glycerol and glucose increased from 0.01 to 0.02 h^{-1}. This result was thought to be caused by the lower expression of the methanol oxidase itself in the presence of glycerol or glucose. This explanation can be further supported by the elevated remaining methanol concentration, as shown in

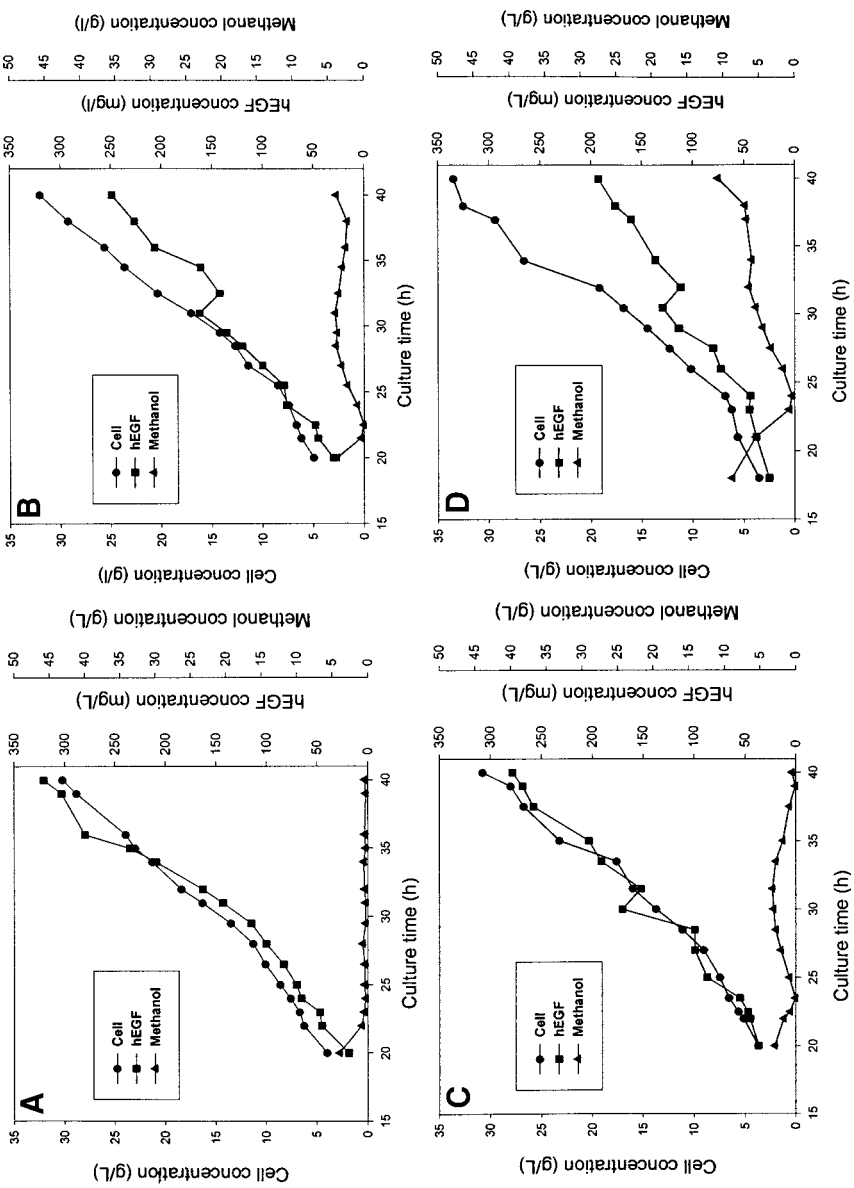

Fig. 3. Time-course variations of cell concentration, hEGF concentration, and methanol concentration in mixed-feed exponential feeding fed-batch cultures at various combinations of carbon sources and set point of specific growth rates: **(A)** $\mu_{G,sp}$ = 0.01 h^{-1}, **(B)** $\mu_{G,sp}$ = 0.02 h^{-1}, **(C)** $\mu_{Glu,sp}$ = 0.01 h^{-1}, **(D)** $\mu_{Glu,sp}$ = 0.02 h^{-1}. In all conditions, $\mu_{M,sp}$ = 0.10 h^{-1}.

Table 3
Results of Exponential Feeding of Dual Substrates[a]

Carbon sources	$\mu_{M,sp}$ (h^{-1})	$\mu_{G,sp}$ (h^{-1})	$\mu_{Glu,sp}$ (h^{-1})	X_{max} (g/L)	P_{max} (mg/L)	μ_{act} (h^{-1})	$Y_{P/X}$ (mg/g)	π (mg/[g·h])	Π (mg/[L·h])
Methanol/glycerol	0.10	—	0.01	30.2	320.5	0.106	11.94	1.26	8.01
Methanol/glycerol	0.10	—	0.02	32.0	248.7	0.112	6.97	0.78	6.21
Methanol/glucose	0.10	0.01	—	30.7	278.4	0.108	9.19	0.99	6.96
Methanol/glucose	0.10	0.02	—	33.4	192.4	0.112	4.96	0.55	4.81

[a]$\mu_{M,sp}$, set point of specific growth rate on methanol; $\mu_{G,sp}$, set point of specific growth rate on glucose; $\mu_{Glu,sp}$, set point of specific growth rate on glycerol; X_{max}, cell concentration at the end of culture; P_{max}, hEGF concentration at the end of culture; μ_{act}, actual specific growth rate; $Y_{P/X}$, product yield on cell mass; π, specific production rate; Π, volumetric productivity.

Fig. 3B,D. It has been observed that the presence of even a small amount of glycerol is inhibitory in achieving the full induction from the *MOX* promoter in *H. polymorpha* DL-1 *(29)*. The characteristic of promoter control is distinguishable from the CBS strain of *H. polymorpha* and similar to that of *AOX1* promoter of *P. pastoris*. When the set point of specific growth rate on supplemented glycerol and glucose was 0.01 h^{-1}, the volumetric productivities of hEGF were higher compared with the methanol-only feeding, while the product yield on cell mass ($Y_{P/X}$) was slightly lower than that of methanol-only feeding in both cases. The volumetric productivities obtained from the high set point of specific growth rate on glycerol and glucose, 0.02 h^{-1}, were lower than those obtained from the methanol-only feeding. These results suggest that the critical glycerol concentration at which suppression of *MOX*-driven expression occurs may be extremely low.

In conclusion, independent feeding of glycerol and methanol by the exponential feeding strategy increased the volumetric productivity of hEGF from 6.4 to 8.01 mg/(L·h) compared with methanol-only feeding. The feeding strategy can be used to investigate systematically the effects of supplementary carbon sources on the heterologous gene expression and cell growth without the available sensors detecting the carbon sources. The results that we obtained can be used to optimize the feeding rate of the supplementary carbon source and ultimately to enhance the volumetric productivity of recombinant proteins in the culture of recombinant methylotrophic yeast cells.

Nomenclature

D_0 = feed glucose concentration (g/L)
F_G = feeding rate of glycerol (L/h)
F_M = feeding rate of methanol (L/h)
G = glycerol concentration (g/L)
G_0 = feed glycerol concentration (g/L)
M = methanol concentration (g/L)
M_0 = feed methanol concentration (g/L)
P = hEGF concentration (mg/L)
P_{max} = hEGF concentration at end of culture (mg/L)
q_G = specific glycerol consumption rate (g/[g·h])
q_M = specific methanol consumption rate (g/[g·h])
t = time (h)
t_f = final culture time (h)
V = culture volume (L)
V_0 = culture volume at beginning of fed-batch phase (L)
X = cell concentration (g/L)
X_0 = cell concentration at beginning of fed-batch phase (g/L)
X_{max} = cell concentration at end of culture (g/L)
$Y_{P/X}$ = product yield on cell mass (mg/g)

$Y_{X/D}$ = cell yield on glucose (g/g)
$Y_{X/G}$ = cell yield on glycerol (g/g)
$Y_{X/M}$ = cell yield on methanol (g/g)
μ_{act} = actual specific growth rate (h^{-1})
μ_{G} = specific growth rate on glycerol (h^{-1})
$\mu_{Glu,sp}$ = set point of specific growth rate on glucose (h^{-1})
$\mu_{G,sp}$ = set point of specific growth rate on glycerol (h^{-1})
μ_{M} = specific growth rate on methanol (h^{-1})
$\mu_{M,sp}$ = set point of specific growth rate on methanol (h^{-1})
π = specific production rate (mg/[g·h])
Π = volumetric productivity (mg/[L·h])

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