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Kinetics of continuous GM-CSF production by recombinant *Saccharomyces cerevisiae* in an airlift bioreactor

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Abstract

Continuous production of murine GM-CSF by recombinant *Saccharomyces cerevisiae* in an airlift bioreactor was studied at three different dilution rates. The reactor was initially fed with a selective medium to increase cell concentration, and then was fed with a rich, nonselective medium for GM-CSF production. Ethanol was used as the main carbon source to provoke GM-CSF expression. In continuous culture, GM-CSF production was maintained for over 150 h, even though the fraction of plasmid-carrying cells continuously dropped to lower than 20%. The stable GM-CSF production during the later phase of the continuous culture was attributed to increased specific cell productivity possibly resulting from an increase in the plasmid copy number in plasmid-carrying cells. This also indicated the possibility of natural selection of high-copy number cells in continuous culture. Plasmid stability was found to be growth rate (dilution rate) dependent; it increased with the dilution rate. Reactor productivity and specific productivity also increased with the dilution rate. A two-parameter kinetic equation was used to model the plasmid stability kinetics. The growth rate ratio between plasmid-carrying and plasmid-free cells increased from 0.996 to 0.998 while the segregational instability or the probability of plasmid loss from each cell division increased from 1.1% to 16% as the dilution rate decreased from 0.11 h^{-1} to 0.05 h^{-1}. Oscillation of the dilution rate between 0.05 h^{-1} and 0.11 h^{-1} stabilized the plasmids and gave a higher productivity than that achieved without oscillation.

Keywords: Saccharomyces cerevisiae; GM-CSF; Kinetics; Air-lift bioreactor

1. Introduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein that stimulates a population of committed granulocyte-macrophage progenitor cells to generate the granulocytes and macrophages (Metcalf, 1985).

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GM-CSF has been cloned and expressed in bacterial, yeast, and mammalian cells and is now mass-produced for clinical uses (Metcalf, 1991). The yeast, *Saccharomyces cerevisiae*, grows rapidly in a relatively simple medium to high cell densities with high levels of product secretion and, thus, has been used as a host organism for production of several recombinant proteins (Heslot and Gaillardin, 1991). Recently, Price et al. (1987) developed a recombinant *S. cerevisiae* strain XV2181 (pαADH2) containing murine GM-CSF genes; however, information regarding the production of recombinant GM-CSF by this yeast is scarce in the available literature (Shu, 1992).

Plasmid instability is a major concern in industrial production of heterologous protein products using recombinant cells (Caunt et al., 1988). Poor plasmid stability and low growth rate of recombinant cells during the course of a production process could significantly reduce the production level of the desired protein product. Although employing a selective pressure to prevent the growth of plasmid-free cells during the culturing process may effectively maintain plasmid stability (Schultz et al., 1987), it is generally cost-ineffective and undesirable for large-scale processes. Regulatory promoters for gene expression control are commonly used to minimize plasmid loss in the growth phase, but not in the production phase (Lee et al., 1988; Hortacsu and Ryu, 1991). More recently, several operating strategies were developed to overcome the plasmid instability problem, including cell immobilization (De Taxis du Poet et al., 1987; Kumar and Schügerl, 1990), recycle of plasmid-carrying cells by sedimentation (Henry et al., 1990), and oscillation of culturing conditions (Impoolsup et al., 1989; Weber and San, 1989; Stephens et al., 1992).

In this work, the kinetics of GM-CSF production by *S. cerevisiae* strain XV2181 (pαADH2) in continuous culture in an air-lift bioreactor was studied. A two-parameter kinetic equation previously developed by Ryu et al. (1991) was used to model the plasmid instability kinetics in the continuous culture. The effects of dilution rate and oscillation in dilution rate on plasmid stability and GM-CSF production were also studied and reported here.

2. Materials and methods

2.1. Culture

Saccharomyces cerevisiae XV2181 (pαADH2) was obtained from Immunex (Seattle, Washington). The vector pαADH2 consisted of pBR322, the yeast TRP1 gene, 2μ origin of replication, and cDNA for murine GM-CSF. Gene expression of the GM-CSF protein was controlled by the glucose-repressible ADH2 promoter. The selective marker for this tryptophan auxotroph is TRP1 gene. Detailed description of the vector can be found elsewhere (Price et al., 1987). The recombinant yeast culture was maintained on agar plates with bi-weekly transfers.

2.2. Media

Two different media were used. The selective medium contained 6.7 g l^{-1} yeast nitrogen base without amino acids (Difco), 5 g l^{-1} casamino acid (Difco), 20 g l^{-1} glucose, 10 mg l^{-1} adenine, and 20 mg l^{-1} uracil. This selective medium was used in preparing inocula for batch cultures and during reactor start-up for continuous cultures. A rich (non-selective) medium containing 10 g l^{-1} yeast extract, 20 g l^{-1} peptone, 80 mg l^{-1} adenine, 80 mg l^{-1} uracil, and 20 g l^{-1} ethanol was used in continuous culture study. Ethanol was used, instead of glucose, in the rich medium to ensure that the ADH2 promoter was not repressed by glucose and to attain high product expression level. Without any adjustment, the pH of these media was about 6.5. For agar plates, the media also contained 2% (w/v) Bacto-agar.

2.3. Batch culture

Batch cultures were carried out in 5-l reactors (Marubishi MD-300) at 30°C, containing 3 l media. They were inoculated with 100 ml flask culture grown overnight in the selective medium. The agitation rate was 500 rev./min and the aeration rate was 3 l min^{-1}. Plasmid stability and the concentrations of substrate, cell, and GM-CSF were monitored throughout the batch culture.

2.4. Continuous culture

A draft tube air-lift bioreactor with a working (liquid) volume of ~350 ml was used for continuous culture. As shown in Fig. 1, the bioreactor was made of a glass column (30 cm long, 5.0 cm in diameter), a draft tube (20 cm long, 2.6 cm in diameter), and a filter disc (3.0 cm in diameter). To study the effect of dilution rate, the continuous cultures were operated at three different dilution rates, 0.05, 0.08, and 0.11 h^{-1}. A 5-ml, 24-h old culture grown in the selective medium was used to inoculate the bioreactor filled with the selective (glucose) medium. The culture was allowed to grow for 18 h in the bioreactor. The bioreactor was then shifted to GM-CSF production phase by continuously feeding the rich (ethanol) medium at a selected dilution rate. Unless otherwise noted, the bioreactor was maintained at 30°C and aerated at a volumetric flow rate of 0.50 l min^{-1} through a filter (60 μm pore size). The pH of the bioreactor was not controlled and changed from 5.5 to 7.5, depending on the dilution rate. Effluent samples from the bioreactor were taken at regular time intervals and assayed for pH, cell density, substrate and product concentrations, and plasmid stability.

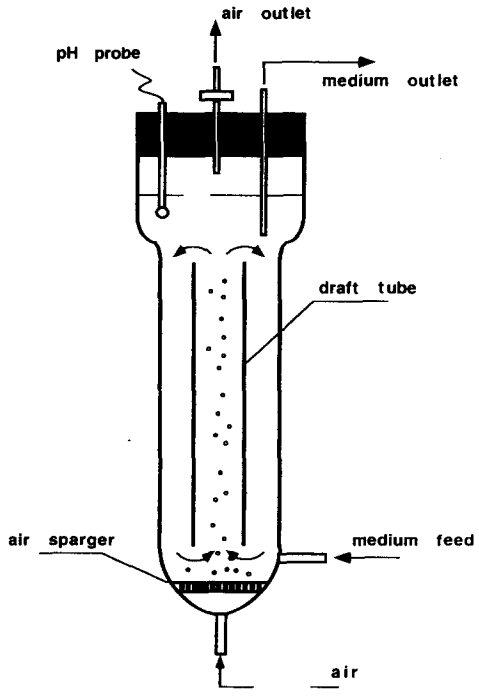

Fig. 1. Schematic diagram of the air-lift bioreactor.

2.5. Assay methods

2.5.1. Cell concentration

Cell concentration was determined by measuring the optical density of the cell suspension at 660 nm (OD_{660}) in a 1.5-ml polystyrene cuvette (with a light path of 10 mm) and comparing the measured value to a standard curve. One unit of OD was equivalent to 0.64 g l^{-1} cell when OD was less than 0.45. Samples were diluted with distilled water if the OD was greater than 0.45.

2.5.2. Glucose and ethanol concentrations

Concentrations of ethanol and glucose in the sample solution were analyzed using high performance liquid chromatography (HPLC) as previously described by Yang et al. (1987).

2.5.3. GM-CSF concentration

The concentration of GM-CSF was determined using a reverse-phase high performance liquid chromatography (Shu, 1992). Twenty-five μl of cell-free sample solution were injected into a reverse-phase column (Vydac C4, 5 mm reverse-phase silica). A binary solvent system (Solvent A: 0.1% trifluoroacetic acid (TFA) in water; Solvent B: 0.1% TFA in acetonitrile) was used as the eluent at a flow rate of 1.0 ml min^{-1} to separate the GM-CSF from other proteins in the sample. A gradient controller (Isco model 2360 gradient programmer) was used to provide linear gradients of 0–100% Solvent B in the eluent at the following increasing rates: 3% min^{-1} for 10 min, 0.67% min^{-1} for 30 min, and 5% min^{-1} for 10 min. Absorbance at 280 nm was measured using a UV spectrophotometer (Waters Lambda-Max model 481 LC). A standard containmg 100 mg l^{-1} murine GM-CSF (Mochizuki et al., 1986) provided by Immunex Corp. was used to determine the concentration of GM-CSF in process samples.

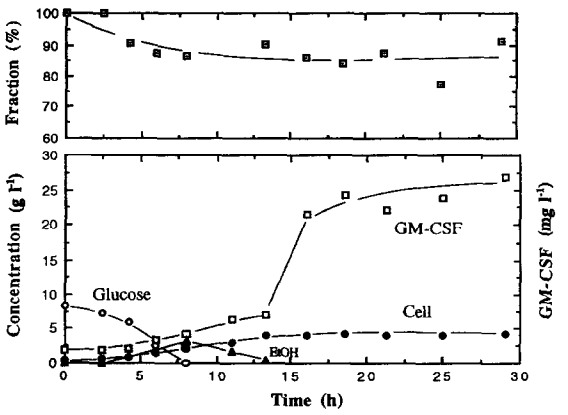

Fig. 2. Kinetics of GM-CSF production in batch culture grown in a selective medium containing glucose as the carbon source. Fraction, the fraction of plasmid-carrying cells in the total cell population; EtOH, ethanol concentration.

2.5.4. Fraction of plasmid-carrying cells

The fraction of plasmid-carrying cells in the total cell population was determined from the ratio of the colony counts on the selective medium agar plate and the rich (non-selective) medium agar plate. Cell samples were diluted to get the colony counts within the range of 30–300. All plate counts were determined from the average of at least three replicates.

3. Results and discussion

3.1. Kinetics of batch culture

3.1.1. Growth on glucose

The kinetics of GM-CSF production from yeast grown on glucose in the selective medium at pH 6.0 is shown in Fig. 2. Glucose was first consumed for cell growth and ethanol production. During this period, only a small amount of GM-CSF was produced because the ADH2 promoter was repressed by glucose. After glucose was depleted, ethanol was used as the carbon source for continued cell growth, and large amounts of GM-CSF were produced in this period. The plasmid stability was maintained at over 85% for the fraction of plasmid-carrying cells. The cell yield resulting from growth on glucose was only 0.2 g g^{-1}, but was 0.49 g g^{-1} based on total carbon consumption during the entire batch culture period. The specific growth rate, yields, and productivity for the culture grown on glucose are summarized in Table 1.

3.1.2. Growth on ethanol

The kinetics of GM-CSF production from yeast grown on ethanol in the rich medium at pH 6.0 is shown in Fig. 3. GM-CSF production in this batch culture was growth-associated; the GM-CSF concentration increased during the exponential growth phase but remained nearly unchanged in the stationary phase. The fraction of plasmid-carrying cells continuously dropped to about 70%. The batch culture reached a maximum GM-CSF concentration of 70.8 mg l^{-1} at pH 6.0. Batch culture grown at pH 4.0 was also studied. The results showed that lower GM-CSF production and poorer plasmid stability were obtained at pH 4.0 than at pH 6.0, although pH did not significantly affect cell growth.

The comparisons of specific growth rate (μ), cell yield ($Y_{x/s}$), product yield ($Y_{p/s}$), specific GM-CSF production ($Y_{p/x}$), volumetric productivity (P), and specific productivity (P/X^+) are given in Table 1. The specific productivity was determined from the volumetric productivity divided by the plasmid-carrying cell concentration, X^+. As can be seen in Table 1, faster growth was obtained with glucose than with ethanol, but higher GM-CSF production was obtained with ethanol than with glucose as the major carbon source in the culture. Thus, for the subsequent continuous culture study, glucose was used for reactor startup and ethanol was used for GM-CSF production.

3.2. Kinetics of continuous culture

The time-course data of continuous cultures at three different dilution rates (0.05, 0.08, and 0.11 h^{-1}) are shown in Figs. 4–6, respectively. In general, all reactors reached relatively stable levels of cell density, pH, and ethanol concentration within 50 h, and as expected, the fraction of plasmid-carrying cells continuously dropped to $\sim$ 20% throughout the culturing period of $\sim$ 150 h. In response to the change from the glucose

Table 1
Kinetics of GM-CSF producton in batch cultures

Substrate	pH	μ (h^{-1})	$Y_{x/s}$ (g g^{-1})	$Y_{p/s}$ (mg g^{-1})	$Y_{p/x}$ (mg g^{-1})	P (mg l^{-1} h^{-1})	P/X^+ (mg h-1 g-1)
Glucose	6.0	0.168	0.49	3.2	6.5	0.92	0.25
Ethanol	6.0	0.130	0.44	4.3	9.8	1.77	0.28
Glucose	4.0	0.126	0.44	2.8	6.4	1.06	0.18

μ: specific growth rate; $Y_{x/s}$: product yield; $Y_{p/x}$: specific GM-CSF production; P: productivity; P/X^+: specific productivity.

medium to ethanol medium, GM-CSF production initially increased to a higher level. It then decreased gradually, following the occurrence of plasmid loss in the culture. However, the decrease in GM-CSF production in the bioreactor was generally slower than the plasmid loss rate. Also, the decline in GM-CSF production slowed down or leveled off after 50–100 h of culturing time, depending on the dilution rate and reactor pH. The high reactor pH in Reactor 1 also helped to stabilize GM-CSF production, as was observed in batch fermentation.

As also shown in Figs. 4–6, the specific productivity increased significantly during the last ~50 h of the culturing period. The recombinant yeast used in this work had a reported plasmid copy number of ~30 (Price et al., 1987). Since specific productivity is usually proportional to the copy number, the stabilized GM-CSF production could be attributed to the increase in the plasmid copy number. It is well known that plasmid copy number may vary with the culturing conditions and usually increases for cells grown at low growth (dilution) rate (De Taxis du Poet et al., 1987). This explains why product concentration decreased at a slower rate than that of the fraction of plasmid-carrying cells. The increase in copy number might have resulted from the high generation numbers during continuous culture, since plasmid-carrying cells with fewer plasmid copy numbers should lose plasmids at a faster rate than those with high copy numbers. Specific productivity was calculated as total product expression averaged for all plasmid-carrying cells with different copy numbers. After a certain number of generations, plasmid-carrying cells with fewer copy number were eliminated and cells with high copy numbers were left in the culture, resulting in higher specific productivities. However, this hypothesis remains to be verified experimentally.

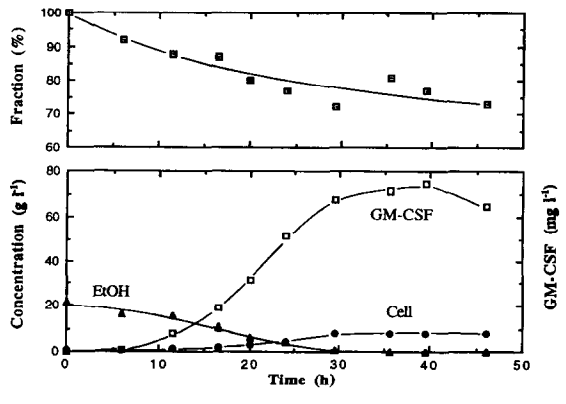

Fig. 3. Kinetics of GM-CSF production in batch culture grown in a rich, non-selective medium containing ethanol as the carbon source.

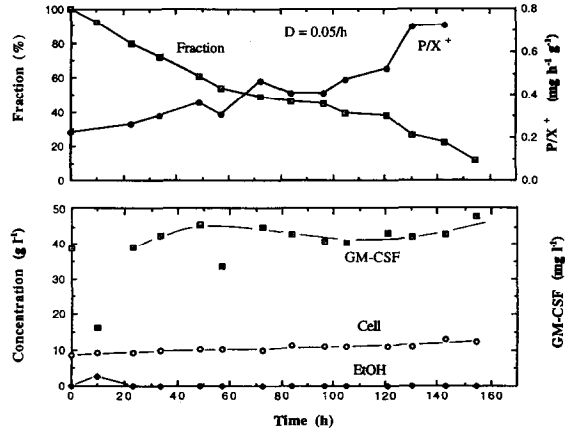

Fig. 4. GM-CSF production in continuous air-lift bioreactor at 0.05 h^{-1} dilution rate (Reactor # 1).

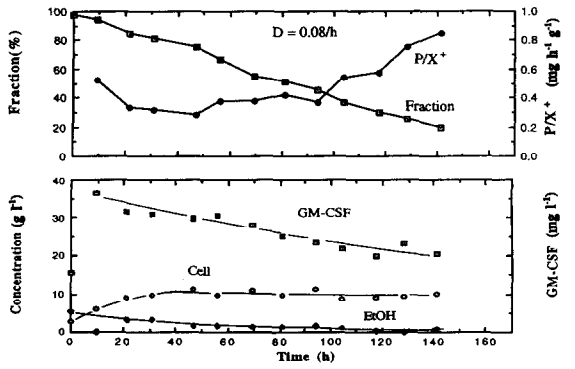

Fig. 5. GM-CSF production in continuous air-lift bioreactor at 0.08 h^{-1} dilution rate (Reactor # 2).

3.3. Effects of dilution rate

As the dilution rate was increased, the cell density decreased from 11.5 g l^{-1} at 0.05 h^{-1} to 8.5 g l^{-1} at 0.11 h^{-1}, the average GM-CSF concentration decreased from 42.5 mg l^{-1} at 0.05 h^{-1} to 24.1 mg l^{-1} at 0.11 h^{-1}, and the residual ethanol concentration increased from 0 g l^{-1} at 0.05 h^{-1} to 3.2 g l^{-1} at 0.11 h^{-1}. The average reactor pH value was 6.9 at 0.05 h^{-1} and ~ 5.5 at both 0.08 and 0.11 h^{-1}. The effects of dilution rate on cell yield, product yield, specific GM-CSF production, volumetric productivity, and specific productivity are shown in Table 2.

The effect of dilution rate on plasmid stability is seen in a plot of the fraction of plasmid-carrying cells versus generation number (Fig. 7). As shown in this figure, plasmid stability increased as the dilution rate increased. One possible explanation proposed by Kleinman et al. (1986) is that yeast divides with larger buds at faster growth rates, and the large bud increases the possibility of an even distribution of plasmids between the mother cell and the daughter cell. Plasmid stability was also affected by pH, as previously found in batch cultures. Cells grown at a higher pH showed better plasmid stability. Since the reactor pH at 0.05 h^{-1} dilution rate was higher than those at 0.08 and 0.11 h^{-1}, it is clear that the higher dilution rate gives better plasmid stability.

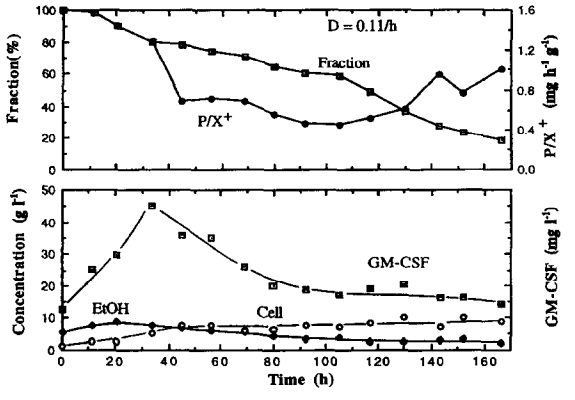

Fig. 6. GM-CSF production in continuous air-lift bioreactor at 0.11 h^{-1} dilution rate (Reactor # 3).

3.4. Comparison of cell yield and product yield

In general, cell yield from the continuous culture was higher than that from the batch culture, but product yield and specific GM-CSF production were much lower for the continuous culture. The lower product yield and specific GM-CSF production in continuous culture were attributed to the lower fraction of plasmid-carrying cells resulting from plasmid loss over the long operation period. The higher cell yield in continuous culture could be attributed to the presence in the bioreactor of a large fraction of plasmid-free cells, which usually grew faster and had higher cell yields than plasmid-carrying cells. For the continuous cultures, cell yield decreased from 0.55 to 0.46 g g^{-1} and product yield decreased from 2.11 to 1.35 mg g^{-1} as the dilution rate increased. The higher product yield at 0.05 h^{-1} also might result from the higher reactor pH.

3.5. Comparison of productivity and specific productivity

In general, average reactor productivity from continuous culture was higher than the productivity from batch culture. Also for continuous culture, productivity increased with increasing dilution rate; from ~ 2.1 mg l^{-1} h^{-1} at 0.05 h^{-1} to ~ 2.7 mg l^{-1} h^{-1} at 0.11 h^{-1}. The productivity at 0.05 h^{-1} was slightly higher than that at 0.08 h^{-1}, probably because pH was higher at 0.05 h^{-1}.

Table 2
Kinetics of GM-CSF producton in continuous cultures

Reactor #	pH	D (h^{-1})	$Y_{x/s}$ (g g^{-1})	$Y_{p/s}$ (mg g^{-1})	$Y_{p/x}$ (mg g^{-1})	P (mg l^{-1} h^{-1})	P/X^+ (mg h^{-1} g^{-1})
1	6.9	0.05	0.55	2.11	3.84	2.10	0.26~1.5
2	5.4	0.08	0.53	1.40	2.63	2.07	0.28~0.85
3	5.5	0.11	0.46	1.35	2.93	2.65	0.45~1.5
3c	5.5	0.05/0.11	-	-	-	2.52	-

D: dilution rate; $Y_{x/s}$: cell yield; $Y_{p/s}$: product yield; $Y_{p/x}$: specfic GM-CSF production; P: productivity; P/X^+: specific productivity.

The specific productivity based on plasmid-carrying cells present in the continuous culture initially was about the same as that in batch culture, but became several fold higher than that in batch culture toward the end of the culturing period studied. Comparing the two runs with similar medium pH, the continuous culture at the higher dilution rate also had a higher specific productivity. A higher specific productivity at a higher dilution rate has also been reported for several other recombinant cell cultures (Seo and Bailey, 1986; Lee et al., 1988).

3.6. Continuous culture with oscillation in dilution rate

It has been previously shown by other researchers that oscillation of the dilution rate may improve plasmid stability and reactor productivity of a recombinant cell culture (Impoolsup et al., 1989; Weber and San, 1989; Stephens et al., 1992). To study the oscillation effect on the performance of the reactor shown in Fig. 6, at 170 h the dilution rate of the reactor was oscillated between 0.05 h^{-1} and 0.11 h^{-1} every 36 h. The average dilution rate was thus 0.08 h^{-1}. The reactor performance is shown in Fig. 8. The average productivity over the 150-h period studied was 2.5 mg l^{-1} h^{-1}. Compared to the reactor performance at a constant 0.08 h^{-1} dilution rate, both plasmid stability and GM-CSF production were improved with the oscillation.

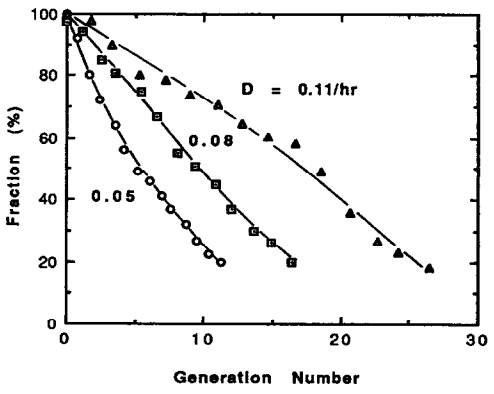

Fig. 7. Effect of dilution rate on plasmid stability.

3.7. Kinetics and modeling of plasmid stability

Estimation of major genetic parameters and understanding their effects on productivity of genetically engineered recombinant cells are essential to the design, control, and optimization of large-scale recombinant cell culture processes. A mathematical model previously proposed by Ryu et al. (1991) was used to evaluate the kinetics of plasmid instability in the continuous culture. In this model, two important parameters (θ and α)

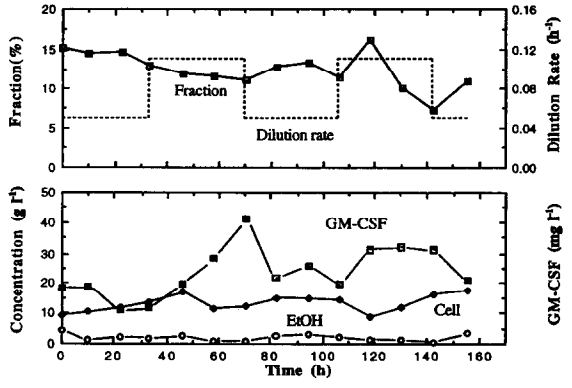

Fig. 8. GM-CSF production in continuous air-lift bioreactor at dilution rate oscillating between 0.05 h^{-1} and 0.11 h^{-1} every 36 h (Reactor # 3 continue).

are defined. The relative plasmid loss rate or probability of plasmid loss during each cell division, θ, is equal to the ratio of the specific production rate of plasmid-free cells, Θ, to the specific growth rate of plasmid-carrying cells, μ^+. θ is indicative of segregational instability of the recombinant cell. The growth rate ratio between plasmid-carrying cells and plasmid-free cells, α, is equal to the ratio of the two specific growth rates, μ^+/μ^-, and is indicative of competitive stability of the culture.

For the continuous culture in the air-lift bioreactor, changes of the heterogeneous cell populations, X^+ and X^-, with culturing time, t, can be described by the following two equations:

$$\frac{dX^+}{dt} = -DX^+ + \mu^+(1 - \theta)X^+ \quad (1)$$

$$\frac{dX^-}{dt} = -DX^- + \mu^+\theta X^+ + \mu^- X^- \quad (2)$$

However, it is difficult to estimate μ^+ and μ^- separately in a continuous culture since the cell population is continuously changing in the heterogeneous populations and the limiting substrate concentration varies with the dilution rate. Thus, the ratio of plasmid-free cell concentration to plasmid-carrying cell concentration, $\Omega = X^-/X^+$, and the growth rate ratio of these two cell populations, $\alpha = \mu^+/\mu^-$, are used in the model.

$$\frac{d\Omega}{dt} = \frac{1}{X^+}\left(\frac{dX^-}{dt} - \frac{X^- dX^+}{X^+ dt}\right)$$

$$= (\mu^- - \mu^+ + \mu^+\theta)\Omega + \mu^+\theta \quad (3)$$

$$= \mu^-[(1 - \alpha + \alpha\theta)\Omega + \alpha\theta]$$

Now, define an apparent specific growth rate as follows:

$$\mu_{app} = \frac{\mu^+X^+ + \mu^-X^-}{X^+ + X^-} = \mu^-\frac{\alpha + \Omega}{1 + \Omega} \quad (4)$$

At pseudo-steady state, $\mu_{app} = D$. Combining Eq. (4) with Eq. (3) gives the following equation:

$$\frac{d\Omega}{dt} = D\frac{1 + \Omega}{\alpha + \Omega}[(1 - \alpha + \alpha\theta)\Omega + \alpha\theta] \quad (5)$$

Eq. (5) can be solved by using the method of separation of variables with a proper initial condition, provided that α and θ are practically constant during a certain period of cultivation under well-defined experimental conditions. With initial condition of $\Omega = \Omega_o$ at $t = 0$, the solution of Eq. (5) is found as follows (Ryu et al., 1991):

$$\ln(\Omega + A) = BDt - B\ln\left(\frac{1 + \Omega}{1 + \Omega_o}\right) + \ln(\Omega_o + A) \quad (6)$$

where $A = \alpha\theta/(1 - \alpha + \alpha\theta)$ and $B = (1 - \alpha + \alpha\theta)/(\alpha - \alpha\theta)$. The values of A and B in Eq. (6) can be determined by fitting the experimental data, $\Omega(t)$, with the model using non-linear regression method. The values of α and θ can then be calculated as: $\alpha(1 + AB)/(1 + B)$ and $\theta = AB/(1 + AB)$.

Table 3
Effects of dilution rate on α and ϑ in the kinetic model, eq. (6)

D(h^{-1})	α	ϑ
0.05	0.998	16.0%
0.08	0.997	5.6%
0.11	0.996	1.1%

By using a non-linear optimization method, the parameters α and θ in the kinetic model were estimated, and the results are given in Table 3. As shown in Table 3, the growth rate ratio α at various dilution rates was almost identical, but the relative plasmid loss rate θ decreased significantly with increasing the dilution rate. The model thus suggested that plasmid instability in the recombinant yeast culture studied was mainly caused by the segregational instability instead of competition instability. The results from the model were consistent with the experimental results shown in Fig. 7 that plasmid stability in continuous culture increased with the dilution rate. As shown in Fig. 9, the model simulates the experimental data well. Also shown in Fig. 9 is the comparison between data from oscillation experiment and model simulation without considering any possible oscillation effects on cell physiology. It is clear that oscillation of the dilution rate stabilized the plasmids of the recombinant cells in the continuous culture.

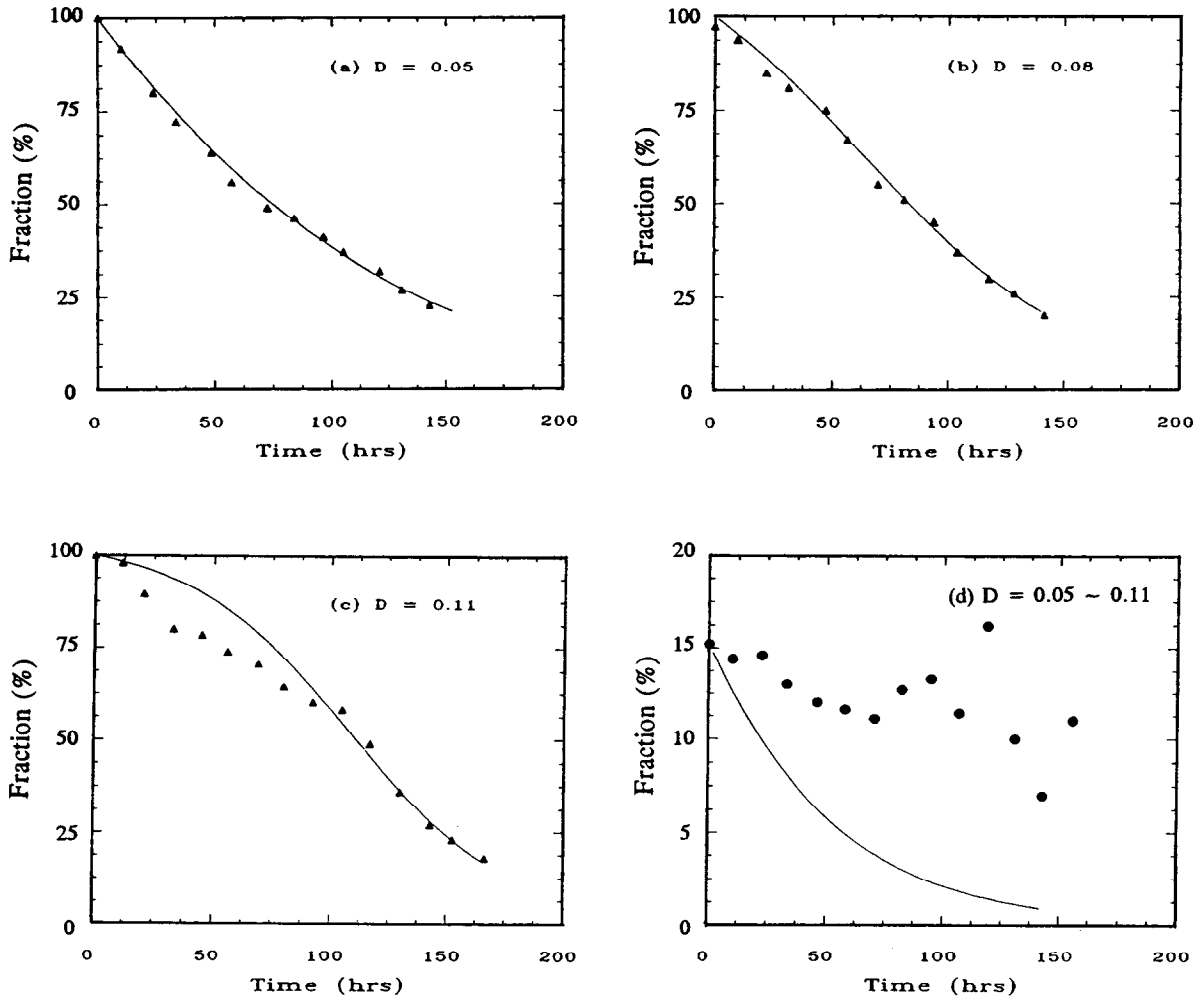

Fig. 9. Comparison of model simulations and data on the fraction of plasmid-carrying cell population in continuous cultures at various dilution rates: (a) 0.05 h^{-1}; (b) 0.08 h^{-1}; (c) 0.01 h^{-1}; and (d) oscillation between 0.05 h^{-1} and 0.11 h^{-1}. (Curves show model simulation and symbols show experimental data).

4. Conclusion

Compared to batch culture, continuous culture improved the productivity of recombinant GM-CSF in the air-lift bioreactor. The specific productivity of plasmid-carrying cells increased with culturing time in the continuous culture, possibly because of natural selection of high-copynumber cells over the long operation period. Both the plasmid stability and GM-CSF production were higher at higher dilution rates. Oscillation in the dilution rate also improved plasmid stability and productivity.

5. Nomenclature

A	Coefficient in Eq. (6)
B	Coefficient in Eq. (6)
D	Dilution rate; h^{-1}
X^+	Cell density of plasmid-carrying cells; g l^{-1}
X^-	Cell density of plasmid-free cells; g l^{-1}
t	Time; h or min

Greek

α	Growth rate ratio; $\alpha = \mu^+/\mu^-$
μ^+	Growth rate of plasmid-carrying cells; h^{-1}
μ^-	Growth rate of plasmid-free cells; h^{-1}
μ_{app}	Apparent growth rate in suspension cells; h^{-1}
θ	Probability of plasmid loss during each cell division; $\theta = \Theta/\mu^+$
Θ	Specific plasmid loss rate; $\Theta = \mu^+\theta$; h^{-1}
Ω	Ratio of X^- to X^+
Ω_o	Initial value of Ω at time = 0

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