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일단 연속 생물반응기의 과도상태 거동을 이용한 이단 연속 생물반응기의 해석

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Analysis of two-stage Continuous Culture System by Transient Response of Single-stage Continuous Culture System

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ABSTRACT

Two-stage continuous culture system has been studied intensively to maximize the productivity of a cloned gene product in unstable recombinant microorganism. As an effort to optimize the two-stage process, transient behavior of the second-stage was studied theoretically as well as experimentally using *Escherichia coli* K12△H1△trp. A mathematical model describing the transient response to a step change in dilution rate was developed based on the assumption that the adaptation rate of cell growth is proportional to the available growth potential, which is defined as the difference in dilution rates between before and after shift-up. The kinetic parameters appearing in the model equations were the dimensionless step increase in growth rate(α) and the adaptation rate constant(k). These parameters were evaluated for various dilution rates and temperatures by washout method. This relatively simple adaptation model could predict the specific growth rate of the second-stage successfully. Advantage and disadvantage of the proposed model are also discussed.

INTRODUCTION

Genetic instability of recombinant microorganism has been considered as one of the most important obstacles on the scale-up of recombinant fermentation processes(1-3). Generally, recombinant microorganisms become more unstable under expressed condition than under repressed condition for the cloned gene. From the bioprocess engineering point of view, therefore, using a two-state culture system to separate the growth stage from the production stage can be a plausible solution to the instability problem(Fig. 1). If the recombinant of interest has a temperature-sensitive genetic switch like P_LO_L site-λcI_{857} repressor, the expression of the cloned gene is controlled very easily by changing the operating temperature. For instance, the first-stage is operated at the temperatures lower than 37℃ for cell growth without cloned gene expression, while the secondstage is maintained at the temperatures higher than 38℃ to inactivate the heat-sensitive λcI_{857} repressor molecules and, hence, to induce the rapid accumulation of

the cloned gene product(4–6).

In actual applications, both stages of a two-stage continuous culture system are operated at steady states for cell density and substrate concentration. However, the operating conditions of dilution rate and temperature in each stage are usually different to each other, for their optimal levels are not the same. As a consequence, upon being transferred from the first-stage to the second-stage, cells experience an abrupt change in their growth environment and each cell goes through a transient period exhibiting different growth rates depending on the mean residence time and the difference in operational conditions between the two stages. This results in a transient, unbalanced growth and brings about the changes in average mass, DNA, RNA and protein contents per cell(7–10). Although the growth rate is considered as the best lumped parameter characterizing the physiology of bacterial cells(11), it is for the cells under a balanced batch growth or the cells growing in a single-stage culture system(including the first-stage of a two-stage culture system) under a steady-state condition. For the second-stage of a two-stage system, the growth rate(μ_2) represents an average value of wide range growth rate of individual cells and its meaning should be interpreted more carefully.

The present study is focused on analyzing transient responses of glucose-limited *Escherichia coli* cells to a step change in dilution rate and temperature in a single-stage as well as in a two-stage culture system. The strain used is *E. coli K12△H1△trp* which has been employed as a host cell for the recombinant plasmid pPLc23*trpA*(4–6). Important parameters considered are dilution rate and temperature. They are directly related to the inactivation of heat-sensitive repressor molecules, thus the product formation in the recombinant *E. coli*(4–6). A mathematical model based on the first-order adaptation kinetics is proposed to describe the change in specific growth rate following a step increase in dilution rate or/and temperature and is compared to experimental results.

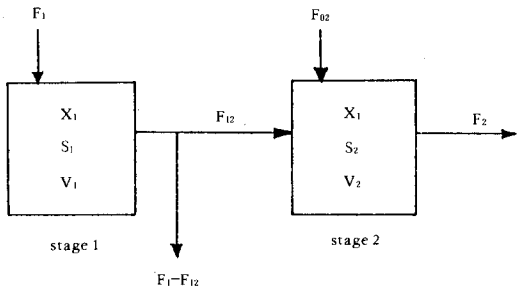

Fig. 1. Schematic diagram of a two-stage continuous culture system.

THEORY

Let's consider a two-stage continuous culture system shown in Fig. 1. The material balances for the cell densities in the first-stage and the second-stage give the following equations(4–6) :

$$\frac{dX_1}{dt} = -D_1X_1 + \mu_1X_1 \quad \cdots\cdots (1)$$

$$\frac{dX_2}{dt} = -D_2X_2 + D_{12}X_1 + \mu_2X_2 \quad \cdots\cdots (2)$$

where, X, D and μ denote cell density, dilution rate, and specific growth rate, respectively; subscripts 1 and 2 denote the first- and second-stage, respectively; D_{12} represents the dilution rate of the second-stage due to the fermentation broth transferred from the first- to the second-stage. Under steady-state conditions, cell density does not change with respect to time, and Eqs. (1) and (2) give :

$$\mu_1 = D_1 \quad \cdots\cdots (3)$$

$$\mu_2 = \frac{D_2X_2 - D_{12}X_1}{X_2} \quad \cdots\cdots (4)$$

Considering the individual cells (X_i) as independent batch bioreactors and denoting their growth rate as μ_i, growth kinetics is expressed as follows(12) :

$$\frac{dX_i}{dt} = \mu_iX_i,\ X_i(0) = X_{i0} \text{ at } t=0 \quad \cdots\cdots (5)$$

$$\text{or, } X_i(t) = X_{i0}\exp(\int \mu_i(t)\,dt) \quad \cdots\cdots (5\text{–}1)$$

If growth condition is kept constant, μ_i is a constant and X_i can be estimated very easily. When a step change in growth condition is introduced (as in

case that cells are transferred from the first- to the second-state), however, μ_i changes to a new level as adaptation proceeds. If we recall that bacterial cells have the tendency to grow with the possible maximal rate under a given set of environmental condition(11), it would be reasonable to assume that the adaptation rate is proportional to the difference between the new growth rate and the pre-shift growth rate before the step change. This would be particulaly true when the new condition allows the highest growth rate(μ_{max}) that the microorganism can inherently exhibit. This "first-order adaptation model" is described as follows :

$$\frac{d\mu_i}{dt}=k(\mu_i^M-\mu_i),\ \mu_i(0)=\mu_{i0}\ \text{at}\ t=0 \quad \cdots\cdots (6)$$

where, μ_i^M is the new growth rate that is determined by new culture condition and k is the adaptation rate constant. Then, the difference between two growth rates, $(\mu_i^M-\mu_i)$, is driving force for adaptation and can be interpreted as available growth potential. The solution of Eq. (6) for the initial condition given is,

$$\mu_i(t)=\mu_i^M-(\mu_i^M-\mu_{i0})\exp(-kt) \quad \cdots\cdots (7)$$

If we assume an ideal stirred tank reactor(CSTR) (12), the cell density of the effluent from the second-stage (X_2) without added fresh medium ($F_{02}=0$) can be expressed as (see Fig. 1) :

$$X_2=\int_0^\infty X_i(t)\,\varepsilon(t)\,dt \quad \cdots\cdots (8)$$

where, $\varepsilon(t)$ is residence time distribution (RTD) function(13). When the cells in the second-stage are growing with varying rates, $\varepsilon(t)$is a complex function of μ_i and D. However, if we assume that one mother cell transferred from the first-stage and its daughter cells derived in the second-stage have the same residence time in the second-stage, $\varepsilon(t)$ has the following form for an ideal CSTR :

$$\varepsilon(t)=D_2\exp(-D_2t) \quad \cdots\cdots (9)$$

This is an approximation which is valid when X_2 is similar to X_1. From Eqs. (5)-(9), X_2 is expressed as follows :

$$X_2=\int_0^\infty X_{i0}\,D_2\left[\mu_i^M t+\frac{(\mu_i^M-\mu_{i0})}{k}\{\exp(-kt)-1\}\right]\exp(-D_2t)\,dt \quad \cdots\cdots (10)$$

If we denote X_2' as the cell density with the added fresh medium into the second-stage (i. e., $F_{02}\neq 0$),

$$X_2'=\frac{F_{02}}{F_{12}+F_{02}}X_2 \quad \cdots\cdots (11)$$

Then, the growth rate of the second-stage(μ_2) can be expressed as follows :

$$\mu_2=D_2-D_{i2}\left(\frac{X_1}{X_2'}\right) \quad \cdots\cdots (12)$$

As an extreme case, when the second-stage is run at high levels of substrate and dilution rate which allow the cells to grow with their fastest rate, μ_{max}, the available growth potential for the cells transferred from the first-stage to the second-stage becomes ($\mu_{max}-\mu_{i0}$). It is this condition that the validity of the proposed first-order adaptation model is tested in the present study, since this extreme case is most interesting for the optimization of the two-stage continuous culture system. We want to run the second-stage at or near the optimal condition, where the gene productivity is not affected by insufficient or limited supply of essential substrate.

MATERIALS AND METHODS

The bacterial strain *E. coli* M72(Sm^R, *lac*Zam, △*bio-uvr*B, △*trp*EA2[*λ*Nam7, *N*am53, *c I* 857, △H1], designated as K12△H1△*trp*, the host cell for the recombinant plasmid pPLc23*trpA*, was used throughout all experiments(4-6). Inoculum cultures were conducted in 500mL flasks and main cultivations in Bioflo units (New Brunswick Scientific). Temperature was maintained at a set point within the range of ±0.1℃ by putting the glass jar into water jacket. The M56 minimal medium(14) supplemented with 10 μg/L biotin, 20mg/L tryptophan and 1g/L glucose(designated as M56) was used for both flask culture and bioreactor experiments. With this medium, glucose was found to be the limiting component determining the final cell yield. In continuous culture

experiments, a steady state was assumed when the absorbance of culture broth at 600nm(A_{600}) were constant in the three consecutive measurements, which occurred at least three mean residence time had passed. Glucose concentration was measured by a glucose analyzer(YSI, model 27). More detailed information on culture conditions and analytical methods can be found elsewhere(4-6).

RESULTS AND DISCUSSION

Measurement of maximum growth rate(μ_{max})

The maximum growth rates(μ_{max}) at different temperatures were measured by a modified washout method(15). At a desired temperature level, bioreactor was run initially under a steady-state condition at a relatively high dilution rate (close to μ_{max}) for more than 6 times the mean residence time, and then a step increase in the fresh medium feed rate was introduced to initiate the washout of the cells. After the cell density was decreased to a level lower than $A_{600}=0.1$, the inflow of fresh medium was stopped and the change in cell density was followed while the bioreactor in a batch mode. Up to the cell density of $A_{600}=0.3$, the plot of (ln A_{600}) vs. time gave a straight line and μ_{max} could be determined accurately from the slope. Based on the Arrhenius plot shown in Fig. 2, activation energy(E_a) was estimated to be 11.1kcal/mol within the temperature range of 27-37 ℃. This value of E_a falls within the range reported by many other researchers(16-17).

Determination of adaptation kinetic parameters

In order to test the validity of the first-order adaptation model proposed and to determine the adaptation rate constant k, a series of washout experiments were carried out(15). One set of results, when dilution rate is shifted from $D=0.45hr^{-1}$ to $1.06hr^{-1}$, are shown in Fig. 3. We find that cell density expressed as A_{600} decreases very fast initially but its rate slows down as time elapses. Since transient growth rate is dependent on dX/dt as shown below in Eq. (13), this indicates that there is a gradual increase in growth rate to be adapted in the new environment. Transient growth rate at each time point is

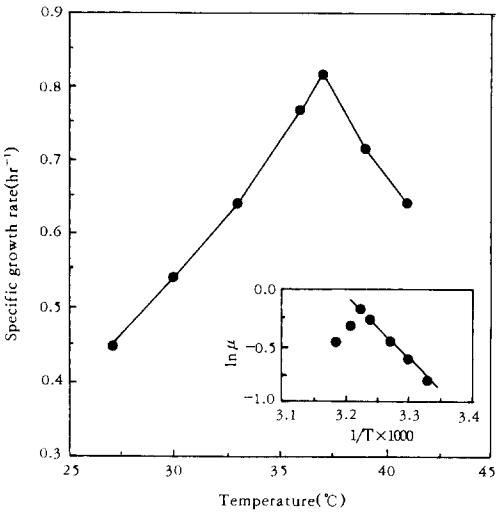

Fig. 2. Effect of temperature on the specific growth rate of *E. coli* K12△H1△trp in M56 minimal medium. Insert: Arrhenius plot for determination of the activation energy.

estimated from the tangential line of the washout curve (after smoothing it) and using the following equation :

$$\mu = D + \frac{1}{X}\frac{dX}{dt} \quad \cdots\cdots (13)$$

The values of μ were also plotted in Fig. 3. Here, it is important to point out that the initial transient growth rate is not the same as the pre-shift, steady-state growth rate. Instead, there is an abrupt increase in the growth rate($\triangle \mu_{in}$) immediately following the step change in dilution rate. The value was estimated to be $0.20hr^{-1}$ in case of Fig. 3.

Once the initial growth rate is obtained, one can calculate k value from the slope of the plot of $-\ln(\mu_i^M - \mu_i)$ vs. time(see Eq. 7), or alternatively, directly from the data of X vs. time. The former method is straightforward but has some difficulty to be applied in the later time period where X changes very slowly with respect to time. The latter one relies on the comparison between the experimentally measured cell densities and the ones calculated from the Eq. (14) shown below. From a material balance equation and the first-order adaptation model given in Eq. (7), cell density during washout is calculated

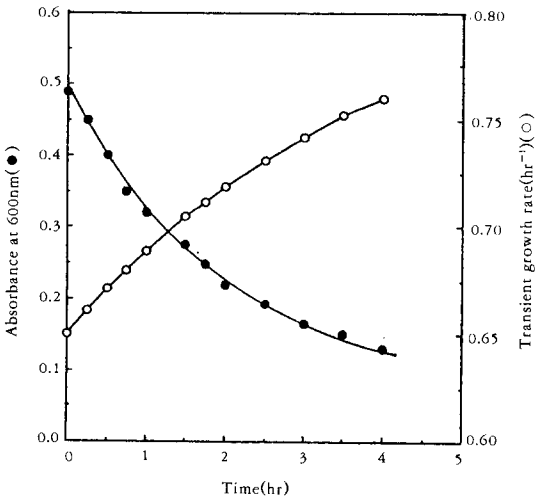

Fig. 3. Transient behavior of a chemostat culture following an increase in dilution rate from 0.45 to 1.06hr^{-1} at 37℃. Solid line for A_{600}(●) shows the model simulation according to Eq. (14).

as follows :

$$X=X_0\exp[(\mu_{max}-D)t+\frac{(\mu_{max}-\mu_{in})}{k}\{\exp(-kt)-1\}] \quad \cdots\cdots (14)$$

where, μ_i^M was substituted by μ_{max} (subscript i was dropped off since the single-stage continuous system was assumed as an ideal CSTR) and the post-shift, initial transient growth rate was denoted as μ_{in} to emphasize that there was some instantaneous increase in growth rate. Since the only unknown in Eq. (14) is k, it is possible to determine its value by regression method from the experimental data like Fig. 3(23). In Fig. 3, the magnitude of k value was about 0.25hr^{-1}.

There are several reports in the literature on the transient responses of bacterial cells to the step changes in dilution rate(18–22). For instance, Beck et. al(18) observed instantaneous increase of the growth rate in a continuous culture as well as in batch culture following the supplementation of medium component. They also reported that the abrupt shift in growth rate was accompanied by a progressive enrichment of the cellular content in stable RNA and a transient decrease in the level of nucleoside triphosphates. Several researchers(19–20) including Beck et. al. have tried to explain this phenomenon based on so-called "RNA-limiting theory" that the overall response of a microbial culture is controlled by the response of the cellular macromolecule and the macromolecule which exerts predominant control over transient response is the RNA. This hypothesis is dependent on the fact that the largest single component of the biomass is protein and protein synthesis is controlled by the cellular complementation of RNA(11). The RNA-limiting theory is generally accepted while there are some other evidences indicating its limitation in explaining the transient responses over a wide range of specific growth rate(22). In the present study, it is not attempted to measure the levels of any intracellular metabolites or to reveal the mechanism by which cells modulate their RNA content with growth rate, since the purpose is the analysis of adaptation kinetics and its application to the optimization of a two-stage continuous culture system.

The washout experiments were performed for several different initial steady-state dilution rates in order to reveal the transient response of the culture in detail. Fig. 4 shows the dimensionless step increase in the specific growth rate(α) and k value, respectively, as a function of initial steady-state dilution rate or growth rate. The dimensionless step increase is defined as follows :

$$\alpha=\frac{(\mu_{in}-\mu_0)}{(\mu_{max}-\mu_0)} \quad \cdots\cdots (15)$$

In Fig. 4, one important finding is that the value of α is practically constant(about 0.5) at the given temperature of 37℃, regardless of the pre-shift steady-state dilution rate. This means that the magnitude of the difference in growth rate which can be accommodated instantaneously following a step change in substrate concentration is almost constant, when it is expressed in terms of the ratio of initial shift-up($\mu_{in}-\mu_0$) to total potential $\triangle\mu_0(=\mu_{max}-\mu_0)$. In other words, *E. coli* cells retain intracellular pool of rate-limiting component(s) (maybe the protein synthesizing system, according to the RNA-limiting theory) 50% in excess of what is required to meet the

given growth rate. Fig. 4 also shows the magnitude of k value. Interestingly, it was almost constant at 0.25±0.05 regardless of the steady-state growth rate, although it appeared to be increasing slightly. This indicates that the cells adapt to the new environment at the rate of about 25% of the total driving force or available growth potential per hour after the initial step change is imposed on them.

From the results of Fig. 4, it is possible to estimate the length of time that is required to reach a new steady-state awhen dilution rate is varied. If we define response time(τ_θ) as the time required for the pre-shift steady-state growth rate(μ_0) to reach (100xθ)% of μ_{max}, it is expressed as follows :

$$\tau_\theta = \frac{1}{k} \ln \frac{(1-\theta)\ \mu_{max}}{(1-\alpha)\ \triangle \mu_0} \quad \cdots\cdots\cdots\cdots\cdots\cdots (16)$$

Fig. 5 shows $\tau_{0.95}$ as a function of the pre-shift growth rate μ_0 when $\mu_{max}=0.82hr^{-1}$(at 37℃ with M56 medium). The response time was as long as 8 hr for the step change of $\triangle \mu_0=0.6hr^{-1}$.

At this moment, it might be appropriate to discuss about the applicability of the first-order adaptation model to two additional transient situations, i. e., the case that the new growth rate after shift-up(μ_i^M) is lower than μ_{max}, and the case that a sudden shift-down (instead of shift-up) in dilution rate is imposed. For the former case, some experiments were conducted (data not shown) with varying μ_i^M. When the changes imposed were less than 0.5 in α value, the bioreactor exhibited almost instantaneous adaptation in terms of specific growth rate. When the changes imposed were more than 0.5 in α value but μ_i^M is lower than μ_{max}, an instantaneous increase in growth rate and a subsequent gradual increase in growth rate were observed. The magnitude of α value was practically the same as the one obtained with $\mu_i^M=\mu_{max}$. This indicates that the first-order adaptation model can properly simulate the dynamic responses of the cells even when $\mu_i^M<\mu_{max}$.

Recently, Balboo et. al. (24) have reported that, when dilution rate is shifted up suddenly in continuous culture of *Klebsiella pneumonia*, there exists an overshoot in cell density and specific growth rate before a new steady state is established. For instance, when dilution rate was shifted up from $0.22hr^{-1}$ to $0.91hr^{-1}$, specific growth rate increased abruptively up to about $0.5hr^{-1}$, increased rather gradually up to $1.05hr^{-1}$, and then decreased to approach the steady-state level of $0.91hr^{-1}$. To simulate this result, they suggested to use the rather complicated "cybernetic model", one of the structured growth model(11). In the present study with μ_i^M less than μ_{max}, no lot some overshoot in the specific growth rate was also

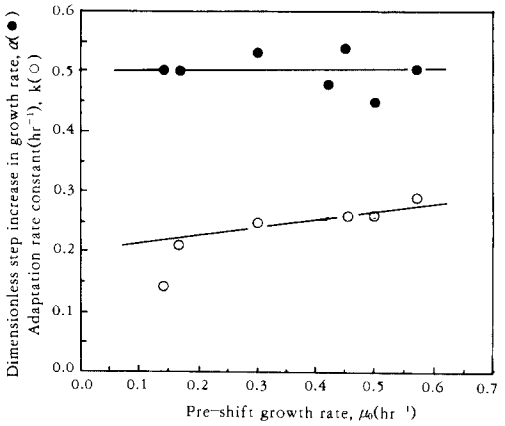

Fig. 4. Effect of the pre-shift specific growth rate on the adaptation rate constant (k) (○) and the dimensionless step increase in growth rate (α) (●). The dilution rate after shift up was $1.05hr^{-1}$ and operating temperature was constant at 37℃.

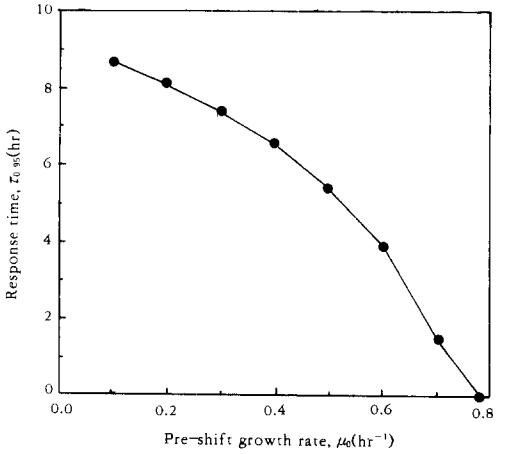

Fig. 5. Effect of the pre-shift specific growth rate on response time, $\tau_{0.95}$, time required for the transient growth rate to reach 95% of the new growth rate.

observed. However, the magnitude of the overshoot was not so significant that the present model can simulate the dynamic responses without a serious error.

For the other case, i.e., when dilution rate is lowered, Balboo et at.(24) have reported that there exist an adrupt decrease in growth rate immediately following a step decrease in dilution rate as well as an undershoot in specific growth rate prior to the new steady state. They claimed that the cybernetic model could also explain this dynamic response properly. in the present study, no experiment has been conducted to test the applicability of the first-order adaptation model to the shift-down case. It is predicted, however, that the present model does not simulate the transient growth rate, since the growth rate during the shift-down is controlled mainly by the supply of external nutrient rather than the internal regulatory mechanism of the cell as in the case of shift-up. This is clearly a drawback of the present model compared to the cybernetic model, but it should be pointed out that the development of a versatile kinetic model is not the purpose of this study. The case of shift-down is not related with the optimization of a two-stage continuous culture system, which the present study ultimately is focused on.

Effect of temperature on adaptation kinetic parametes

The effect of temperature on adaptation kinetics was also studied and the results were shown in Fig. 6. Since α and k were found to be constant regardless $\triangle\mu_0(=\mu_{max}-\mu_0)$, these experiments were conducted for a single $\triangle\mu_0$ only. At each temperature, the selected μ_0 was $0.20\mu_{max}$ and $D_{washout}$ $1.20\mu_{max}$, respectively (see Fig. 2 for μ_{max}).

Fig. 6 shows that both α and k values increase up to 39℃ and decrease as temperature is elevated further. One might conclude that, near the optimal temperature for the cell growth, the response time for the adaptation becomes shorter.

Experments with a two-stage continuous culture system

The two-stage continuous culture experiments were carried out for two cases : first, both stages were operated at the same temperature, and the other, at two different temperatures. The dilution rate of the second-stage was kept constant at $1.0hr^{-1}$ but that of the first-stage was varied to see the effect of the steady-state growth rate of the first stage (μ_1) on that of the second-stage (μ_2). The feed rate of additional fresh medium into the second-stage (F_{02}) and that of the first-stage culture broth (F_{12}) were carefully chosen so that the growth of cells in the sceond-stage was not limited by insufficient supply of glucose. Typically, D_{12} and D_{02} were 0.1 and $0.9hr^{-1}$, respectively.

Experimental results showing the effect of μ_1 on μ_2 at 37℃ are given in Fig. 7, along with those of (theoretical) computer simulation. Here, experimental μ_2 was determined according E_q (4) and theoretical μ_2 according to E_q (12). Although there are some scatterings, experi mental measurements generally well agree with the theoretical calculations. When the α value was modified slightly, the computer simulation gave a better agreement with the experimental results. This observation demonstrate that the first-order adaptation model and the kinetic parameters evaluated from washout experiments in a single-stage culture system can properly describe the transient behavior of the cells growing in the second-stage of a two-stage culture system. Computer simu-

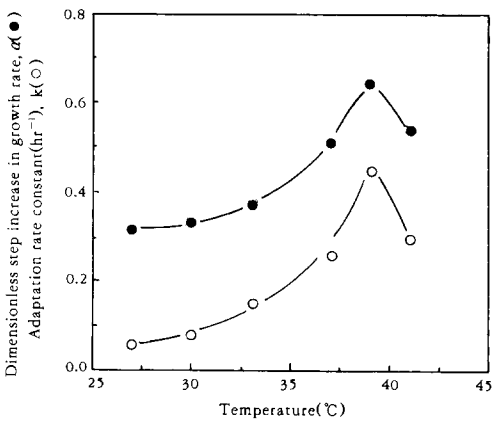

Fig. 6. Effect of temperature on the adaptatiin rate constant (k) (○) and the dimensionless step increase in growth rate(α) (●).

lation in Fig. 7 also shows that, at a constant μ_1, μ_2 is higher when D_2 is low. This means that, the longer the cells stay in the new condition, the more they are adapted.

Fig. 8 shows the effects of both temperature and growth rate in terms of dimensionsless growth rate $U(=\mu/\mu_{max})$. For comparison, the results shown in Fig. 7 were rearranged and also given in Fig. 8. The temperatures chosen for first-stage(T_1) and the second-stage(T_2) were 33℃ and 40.5℃, respectively, since the heat-sensitive λcI_{857} repressor molecules are inactivated above 38℃ and 40.5℃ is close to an optimum for the synthesis of cloned-gene product in the recombinant *E. coli* *K12△H1△trp/pPLc23trp*Al(4-6). Other operating conditions including D_{02} and D_{12} were the same as those of the previous experiment described in Fig. 7. Fig. 8 shows that the operation of the two-stage system at different temperatures for each stage results in a much longer adaptation time than the operation at the same temperature (37℃) for both stages. For example, at $U_1=0.5$, U_2's were 0.76 (when $T_1=33$℃ and $T_2=40.5$℃) and 0.93 (when $T_1=T_2=37$℃), respectively. There are several reports that, within or near the normal growth range (which is defined as range where temperature dependence of growth rate follows Arrhenius relation)(12), cellular composition of *E. coli* cells growing at the same rate is essentially the same. In addition, it was suggested that, when an abrupt change in temperature is exerted to the cultures growing at one of these temperatures, the growth rate assumes the value characteristic of the temperature to which the shift is made without a detectable transient period(17-18). On the contrary, the present study clearly shows that an abrupt change in operating temperature causes some negative effect of adaptation process. This might be due to the high T_2 which slightly departed form the normal growth range. Alternatively, we cannot exclude the posibility that there is some significant difference in cell physiolgy when the cells are growing at different temperature.

For the simulation study with temperature shift-up, the values of α and k should be determined. As shown in Fig. 4, these parameters are functions of

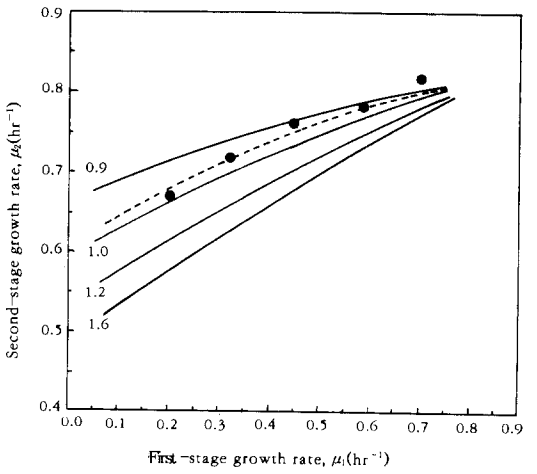

Fig. 7. Effect of the specific growth rate of the first-stage (μ_1) on that of the second-stage (μ_2) in a two-stage continuous culture system. Operating conditions were $D_2=1.0$ and $T_1=T_2=37$℃. Solid lines show the model simulations with $\alpha=0.50$ and $k=0.25$ for varying D_2 (0.9,1.0, 1.2 and 1.6). With the slightly modified α value of 0.55, the computer simulation gives a better agreement with the experimental results (broken line).

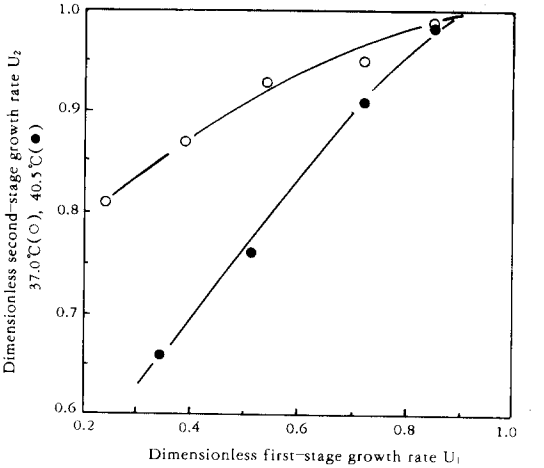

Fig. 8. Two-stage experiment with $T_1=33$℃ and $T_2=40.5$℃(●). Dimensionless growth rate of the second-stage, $U_2(=\mu_2/\mu_{max})$ was plotted against that of the first-stage, $U_1(=\mu_1/\mu_{max})$. The results from Fig. 7 were also given for comparison, where $T_1=T_2=37$℃ (○).

operating temperature. Furthermore, they vary depending on the magnitude of temperature change be-

tween the first-stage and the second-stage. Since T_1 and T_2 can be chosen in many different combinations it seems almost impossible to determine those two parameters for all cases in a systematic way. This is obviously a limitation of the theoretical approach given in the present study. Nevertheless, once optimal T_1 and T_2 for a two-stage continuous culture system have been determined, we can determine the values of α and k exprimentally for those paritcular temperatures. This enables us to analyze the physiological condition of the second-stage more accurately through the prediction of μ_2 at various dilution rates which is one of the key parameters for optimizing a two-stage culture system.

CONCLUSIONS

In the present study, the transient behavior of the second-stage of a two-stage continuous culture system was investigated theoretically as well as experimentally using *E. coli* K12△H1△trp. Important findings can be summarized as follows :

1. Kinetic equations based on first-order adaptation model could describe properly the transient dynamics of cell growth following a step change in dilution rate in a single-stage as well as in a two-stage continuous culture system. Two kinetic parameters, the adaptation rate constan(k) and the dimensionless step increase in growth rate(α) were involved in the kinetic model.
2. There was a step increase in growth rate immediately after the shift-up in dilution rate, but the ratio(α) of the step increase to the total growth potential was constant at a fixed temperature regardless of the pre-shift steady-state dilution rate.
3. The values of k and α showed maximums at around 39℃ and gradually decreased when the temperature changed in either direction.
4. When the temperature of the first-stage was different from that of the second-stage, adaptation took a longer period of time than when both stages were being operated at the same temperature.

국 문 요 약

유전자 재조합 균주의 성질이 불안정할 경우, 2단 연속 배양조를 사용하면 여러가지 이점이 있다. 본 논문은 2단 연속 배양조의 최적화를 목적으로 유전자 재조합 대장균의 과도상태 거동을 1단 및 2단 연속 배양조에서 연구한 결과를 다룬다. 희석율이 갑자기 변할 때 대장균은 새로운 희석율에 적응하기 위해 성장속도를 바꾸는데 적응속도가 성장 잠재력에 비례한다는 가정 아래 수학적 이론식을 도출하였으며, 이때 중요한 동력학적 변수는 무차원의 순간속도 증가(α) 및 적응속도 상수(k)이었다. 이 상수들을 여러 온도와 희석속도에서 실험적으로 측정하였고, 이 측정값을 모델식에 적용한 결과 실제 2단 배양조의 미생물 성장속도의 과도기적인 성질을 잘 묘사하는 것으로 나타났다.

NOMENCLATURE

D dilution rate (hr^{-1})
F fow rate(L/hr)
k first-order adaptation rate constant(hr^{-1})
t time(hr)
U dimensioinless specific growth rate defined as μ/μ_{max}
X cell density(g/L)

Subscript and superscript

0 initial condition at t=0
1 first-stage
2 second-stage
02 flow of fresh medium into the second-stage
12 flow of culture broth form the first- #to the second-stage
i a segregated volume element of bioreactor

Greek

α dimensionless step increase in specific growth rate defined in Eq.(15)
ε residence time distribution #(RTD) function
μ specific growth rate(hr^{-1})
μ_{in} inital transient specific growth rate(hr^{-1}) exhibited after instantaneous step increase in specific growth rate

$\triangle\mu_0$ difference between a new steady-state growth rate and the pre-shift growth rate(hr^{-1})

τ_θ response time required for the pre-shift growth rate to reach 100θ% of μ_{max}

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