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Insect Cell Cultures for Recombinant Protein Production

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재조합 단백질 생산을 위한 곤충세포의 배양

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ABSTRACT

Insect cell cultures were performed in laboratory-scale vessels. The batch growth of insect cells was affected by such parameters as serum content, other nutrients, seeding density, and mechanical agitation. Lactate and ammonium were not likely to be environmental factors that inhibited cell growth at the concentrations observed at the end of batch cultures. In addition, redox potential was found to be a useful index in monitoring low-level dissolved oxygen during the cultivation of insect cells.

Recombinant protein production by cells infected with a genetically-modified baculovirus was also demonstrated. The maximum beta-galactosidase synthesis of 2800 units per reactor volume was achieved at the dilution rate of $0.006hr^{-1}$.

INTRODUCTION

Protein expression systems based on the *Autographa californica* nuclear polyhedrosis virus(AcNPV) have wide applicability as an alternative to prokaryotic or other eukaryotic expression system(1 − 6). However, the application of the baculovirus expression systems has been limited by difficulties in insect cell culture scale-up(7 − 10). Therefore, it is quite natural that the large scale insect cell culture would be a challenging scientific and technological undertaking to ensure efficient production of various significant products for human health and medicine.

In this study, *Spodoptera frugiperda* cells were cultured in static and stirred reactors in order to obtain information for future development of large scale suspension culture. Especially the effect of serum content, other nutrients, seeding density, metabolic wastes, mechanical agitation speed, dissolved oxygen(D.O.), and redox potential on growth kinetics is discussed. This work also investigated the feasibility of recombinant protein production by cells infected with a genetically-modified baculovirus.

MATERIALS AND METHODS

Cell line and culture conditions

The cell line used in this study was *Spodoptera frugiperda* insect cell. The cells were maintained in $25cm^2$ and $75cm^2$ tissue culture flasks(Corning), to provide cells for batch and continuous reactors. The medium used was Grace's insect medium(Gibco), which was supplemented with 50 μ g / ml gentamycin sulfate(Sigma), 2.5 μ g / ml fungizone(Gibco), 0.35g sodium bicarbonate(Sigma) and 10% fetal bovine serum(Sigma), unless otherwise specified. Batch cultures were carried out at the initial pH 6.2 and 28 ℃ in siliconized bottle and spinner flask which were used for static and stirred reactors, respectively.

Continuous culture was conducted in a siliconized spinner flask under the conditions of the initial pH 6.2, 28 ℃, 80rpm agitation and surface aeration.

Analytical methods

The cell number was counted with a hemacytometer under the microscope. The cell viability was determined by the dye exclusion method with 0.4% trypan blue solution. Lactate and ammonium ion assays were performed with enzymatic kits from Sigma. The dissolved oxygen concentration and redox potential value were measured by D.O. meter(Cole-Parmer) and MV meter(Cole-Parmer), respectively. The recombinant protein production by cells infected with genetically-modified baculovirus was estimated by the beta-galactosidase assays that given by Miller(11). One unit of activity is defined as 1.0mM of ONPG cleaved per minute at 37 ℃ and pH 7.3. One mg of pure beta-galactosidase contains approximately 300 units of activity (Sigma, G-06008).

RESULTS AND DISCUSSION

Batch static culture

Fetal bovine serum(FBS) is a costly component in the medium. To determine the extent to which the FBS concentration could be reduced, the effect of serum concentration on the cell growth was studied in a 30ml static reactor. A significant decrease in cell growth rate was observed as the fetal bovine serum concentration was reduced further to 4%(Figure 1). However, the maximum cell density was not dramatically affected by reducing the FBS concentration from 10% to 5%.

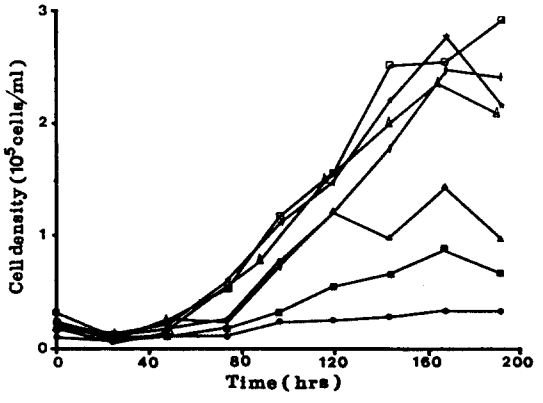

Fig. 1. Effect of serum concentration on the cell growth (●, 0%; ■, 2%; ▲, 4%; △, 5%; ◆, 6%; ★, 8%; □, 10%)

Figure 2 represents the effect of nutrients other than fetal bovine serum in the medium on the cell growth. The cell growth rate is slower in fortified medium(2X) which contained 2 times Grace's insect medium than that in normal medium. The maximum cell density in fortified medium is also lower than that in normal medium. It is implied that ionic strength and osmolarity might be important factors in insect cell growth.

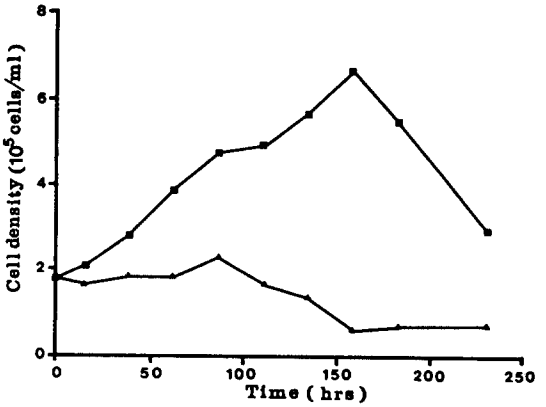

Fig. 2. Cell growth in batch static culture using normal (1x) and fortified medium (2x) (■, 1x; ▲, 2x)

In an attempt to examine the effect of seeding density on the growth, several runs were made in static reactors. As shown in Figure 3, the cultures reach the maximum cell density in a similar way if the culture is seeded at least as high as 2×10^5 cells / ml.

Lactate and ammonium have been reported to be the main factors inhibiting animal cell growth(6, 7). Therefore, we measured the lactate and ammonium concentra-

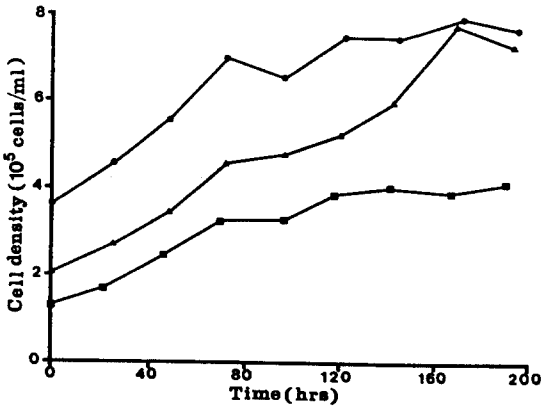

Fig. 3. Effect of seeding density on the maximum cell number

tions during the cell culture. As illustrated in Figures 4 and 5, the final concentration of lactate and ammonium was 8.9mM and 1.2mM. To investigate the effect of lactate and ammonium on the cell growth, the experiments were carried out by exogeneously adding the final amount of the substances to the growth medium. It appears that the levels of lactate and ammonium accumulated at the end of a typical batch culture did not trigger the transition into the death phase(data not shown).

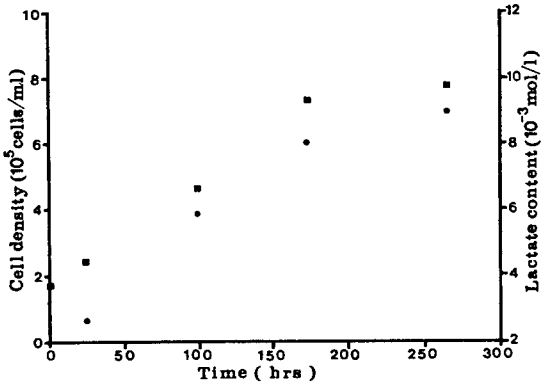

Fig. 4. Lactate formation in batch static culture (■, cell density; ●, lactate content)

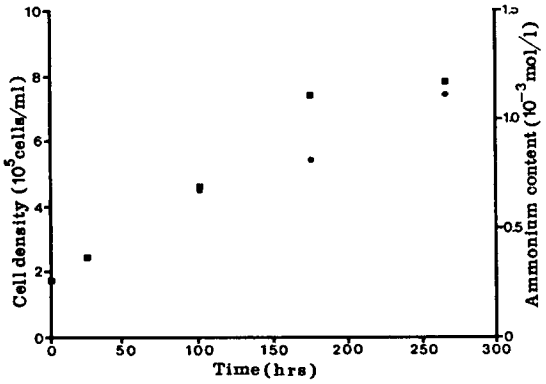

Fig. 5. Ammonium formation in batch static culture (■, cell density; ●, ammonium content)

Batch stirred culture

Figure 6 shows the growth kinetics in suspension culture at different agitation speeds. Up to 80rpm, the maximum cell number increased as agitation speed increased. However, if the agitation speed increases to 100rpm, the value decreases. So an optimum agitation speed exists and would be 80rpm in a spinner reactor. The maximum cell density at this agitation speed is 20 times greater than seeding density.

In the case of 20, 40, and 60rpm, agitation is not enough to maintain homogeneous distribution of cells in the spinner reactors and a clump of cells grows in culture medium. The clump of cells causes internal mass transfer limitation in clumps which results in decreased cell viability and external mass transfer area. As rpm increases, the distribution of cells in the reactor improves and external mass transfer coefficient increases. This results in the enhancement of both the external and internal mass transfer and increase of growth rate. However, as rpm increases the decrease of cell viability due to shear force becomes dominant, so the growth rate starts to decrease again. Therefore an optimum rpm exists in compromising between mass transfer and shear sensitivity.

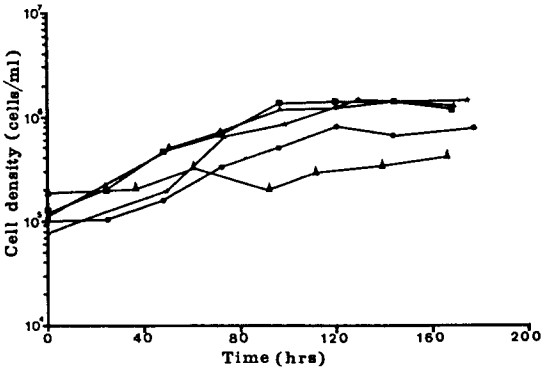

Fig. 6. Effect of mechanical agitation on the cell growth (★, 20 rpm; ●; 40 rpm; ▲, 60 rpm; ■, 80 rpm; ▲, 100 rpm)

The insect cells have a greater oxygen demand than mammalian cells and are sensitive to oxygen limitation(12). The oxygen requirement is such that the dissolved oxygen concentration rapidly decreases to zero during the early phase of the non-aerated culture(Figure 7).

The reactions determining the redox potential in insect cell cultures are quite complex and not fully understood at the present time. Although the characteristics of redox potential can not be generalized in cell cultures, of the particular interest is the following reaction.

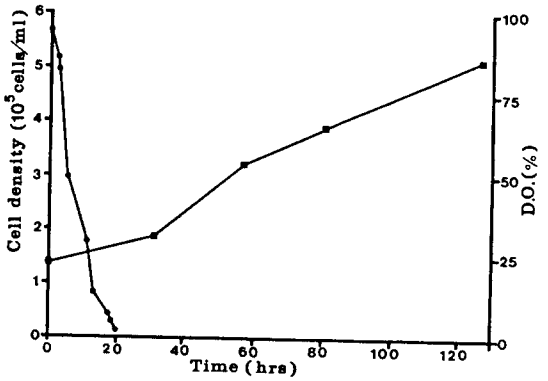

Fig. 7. Time course changes of dissolved oxygen in non-aerated culture (■, cell density; ●, dissolved oxygen)

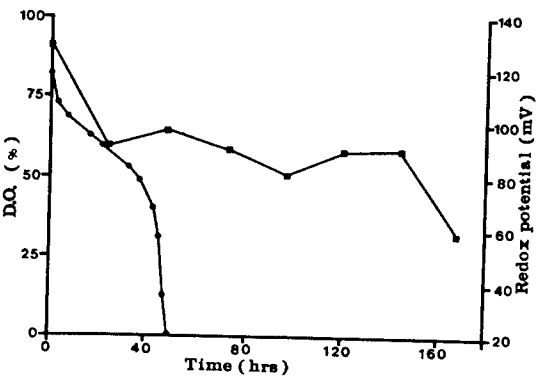

Fig. 8. Time course changes of D.O. and redox potential in surface-aerated culture (●, dissolved oxygen; ■, redox potential)

$$1/2\ O_2 + 2H^+ + 2e^- = H_2O$$

Applying the Nernst equation, the above equation becomes

$$Eh = Eo + \frac{RT}{2F} Ln \frac{(A\ O_2)^{1/2} \times (A\ H^+)^2}{A\ H_2O}$$

This equation can be further simplified to log(D. O.) = a * (Eh) + b in which a and b are specific for each cell culture system. This equation in essense signifies the isolated effect of D. O. and redox potential. An interesting point here is that logarithmic relationship gives an amplifying effect in the detection of low-level dissol-ved oxygen.

The time course of changes in system variables including D. O. and redox potential is shown in Figure 8 for the surface-aerated culture. There is one noteworthy point in this run. The redox potential underwent discernible change throughout the duration of cell culture after D. O. fell to zero level : that is, redox potential might be a useful index in monitoring low-level dissolved oxgen during the oxygen-limited phase of the cell culture.

Continuous culture

The 500ml spinner reactor was inoculated with 150ml of complete medium containing 3×10^5 cells / ml. When the cell concentration reached 1.3×10^6 cells / ml after 5 days of batch growth, the feed containing 5% fetal bovine serum was started at the dilution rate of 0.006hr^{-1} and the reactor volume was maintained at 150ml. The dilution rate was varied from 0.00625hr^{-1} to 0.02433hr^{-1}.

At each dilution rate representative samples were taken from the spinner reactor for viable cell counts and quantification of beta-galactosidase activity. The steady-state cell concentration and recombinant protein (beta-galactosidase) production are shown in Figure 9 as a function of dilution rate. The viable cell content and beta-galactosidase production decrease monotonically as the dilution rate is increased. The amount of beta-galactosidase synthesis per reactor volume was the highest at the dilution rate of 0.006hr^{-1}.

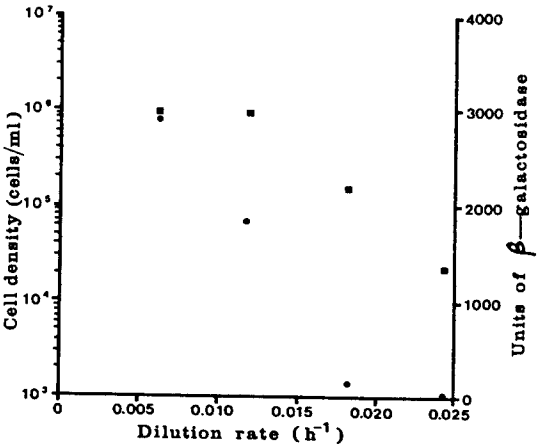

Fig. 9. Cell growth and total beta-galactosidase synthesis in a spinner reactor (■, cell density; ●, units of beta-galactosidase)

요 약

실험실 규모의 배양기에서 곤충세포의 배양을 수행하였다. 회분식 배양에서의 곤충 세포의 성장은 serum의 농도, 다른 영양소, 초기 접종 농도, 기계적인 교반과 같은 변수에 의해 영향을 받는 것으로 나타났다. Lactate와 ammonium은 회분식 배양에서의 말기에 관찰되는 농도에서는 세포의 성장을 저해하는 것은 아닌 것 같다. 또한 redox potential은 곤충세포의 배양 용존산소를 측정할 수 있는 좋은 index임을 알 수 있었다. 아울러 유전공학적으로 재조합된 baculovirus를 곤충세포에 감염시켜 재조합 단백질의 생산을 시도하였으며 dilution rate가 0.006 hr^{-1}일때 반응기당 최대 2800 units의 beta-galactosidase가 생산되었다.

ACKNOWLEDGEMENT

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NOMENCLATURE

A : Activity of redox species
Eo: Redox potential under standard conditions (25 ℃, activities of all the components are unity)
Eh: Redox potential relative to the normal hydrogen electrode
F: Faraday's constant
R: Gas constant
T: Absolute temperature

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