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하이브리도마의 고농도 배양과 포도당 농도가 MAb 생산성에 미치는 영향

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High Density Culture of KA112 Hybridoma and Effect of Glucose Concentration on MAb Productivity

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ABSTRACT

Perfusion culture was conducted in Celligen perfusion culture system using a self-constructed hybridoma cell and low serum medium. The culture system employed hollow fiber to separate cells from the culture broth. Maximum cell density of 2.1×10^7cells/ml, 10 times higher than in batch culture, could be achived. Concentration of monoclonal antibody(MAb) was 4 times higher and production rate at maximum feed rate was 9 times higher than in batch culture. Glucose concentration was very important for the cell growth and MAb production. When glucose concentration was below 1g/ℓ, i. e. 0.5~0.9 g/ℓ, specific MAb production rate decreased but cell concentration still increased. As the glucose concentration goes above 1 g/ℓ, specific MAb production rate increased and remained at maximum value at more than 1.5 g glucose/ℓ. The maximum value of the specific Mab production rate was similar to that of batch culture.

INTRODUCTION

The production of bioproducts by the cultivation of animal cell has been limited by low growth rate and low production rate. High density culture was required to increase production rate and total production. In high density animal cell culture, instruments are complex and control is difficult. Separator is required to separate cells from culture broth. In addition, danger of contamination is high because of long operation time(1). However, cell recycled continuous culture is inevitably required to increase cell concentration and productivity. Up to now, several kinds of separators such as hollow fiber(2), stainless steel screen (3, 4), and ceramic filter(5), have been used.

In this paper, high density culture was conducted using hollow fiber as separator and characteristics of MAb productivity during perfusion culture was investigated in terms of glucose concentration.

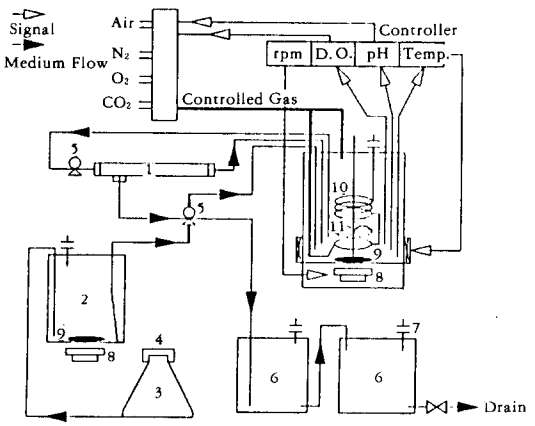

Fig. 1. Schematic diagram of perfusion culture system 1: hollow fiber; 2: media reservoir, 3: sterillization bottle; 4: filter; 5: pump; 6: waste bottle; 7: air vent, 8: magnetic stirrer; 9: magnet; 10: silicon tubing for aeration; 11: marine type impeller.

MATERIALS AND METHODS

Cell line

KA112, a self-constructed hybridoma cell line producing IgM against the surface antigen of *Chlamydia trachoamtis* was used.

Medium

DMEM with high glucose content was used. For the culture of KA112, LSM was used. LSM was composed of insulin, pyruvate, oxaloacetate, pluronic F-68, 1% FBS and DMEM(6).

MAb assay

ELISA(Enzyme Linked Immunosorbent Assay) method was used(6).

Glucose analysis

Glucose was quantified by Somogy-Nelson method(6).

System and operation

The bioreactor used was Celligen(N. B. S., U. S. A.) with marine type impeller. Working volume was 1.0 liter. 4 meters of silicon tubing was wound inside the bioreactor to increase oxygen transfer rate. The separator purchased from A/G Tech. (U. S. A.) was of hollow fiber type with 0. 1 micron pore size and 1mm inner diameter of the fiber. Two peristaltic pumps were used, one for the circulation of culture supernatant and the other for the supply of fresh medium and the removal of waste medium. The ratio of flow rates of the pumps for the circulation and the fresh medium supply was 60:1. The circulation pump was backflushed for 10 minutes ten times a day to prevent cell sedimentation on the inner surface of tubing. Total system was operated in laminar flow bench to reduce contamination. Medium was prepared daily to minimize spontaneous degradation of ingredients and sterilized by passing twice through a 0.2 micron filter. Dissolved oxygen was controlled at 50% of saturation value. rpm was controlled at 40 initially but, later, with the increase of cell density, increased up to 90 to increase oxygen transfer rate. pH was controlled by supplying fresh medium adjusted to very high pH value of about 9 using 3.5~4 g / ℓ $NaHCO_3$ and NaOH.

RESULTS AND DISCUSSION

High density culture

Cells were inoculated into the liquid medium of 100ml volume in spinner flask and cultured for 150 hours in fed-batch operation(Fig. 2). Final volume of liquid medium in spinner flask was 500ml and final cell concentration reached 1.5×10^6 cells/ml. 400ml of this volume was inoculated into Celligen. Initial cell concentration was adjusted to 6.0×10^5 cells/ml and working volume was 1.0 liter. On the 5th day, cell concentration reached 1×10^7 cells/ml and on the 9th day maximum cell density, 2.1×10^7 cells/ml, was obtained with 80% viability(Figs. 3 and 4). Maximum vialbe cell concentration was 1.7×10^7 cells/ml. We did not observe clogging in hollow fiber, and flow rate across the hollow fiber membrane was constant and sufficient for 3 Vol./day feed rate. Above 3×10^6 cells/ml, D. O. could not be con-

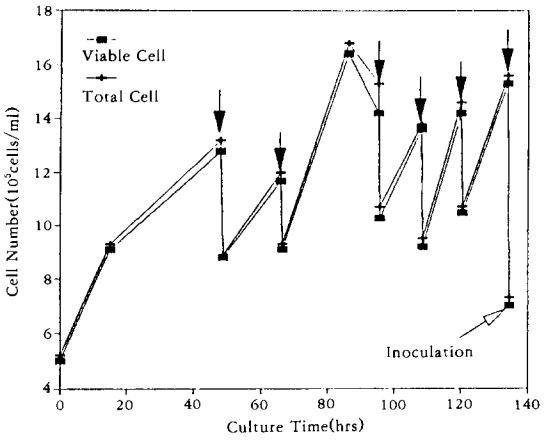

Fig. 2. Cell growth profile in spinner flask in fed-batch operation mode.
→ : Media Feeding Point

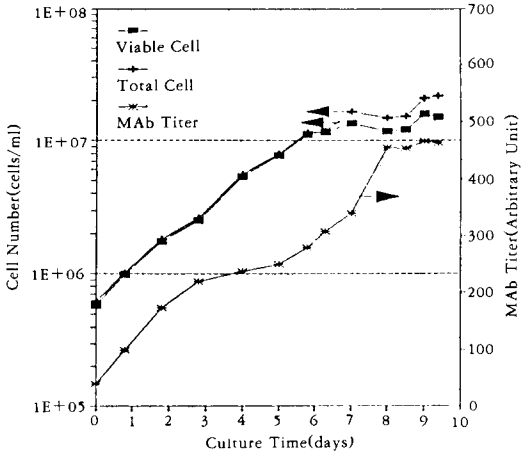

Fig. 3. Cell growth and antibody titer profiles during perfusion culture.

trolled by the supply of plain air and the air supply line was switched to pure O^2. With continuous increase of cell concentration, oxygen limitation occurred and above 1×10^7 cells/ml, D. O. was maintained below 30%. Beyond 7th day, the decrease of viability was caused by oxygen limitation(Fig. 4). Some researchers showed the fact that the concentration of viable cells could be considerably increased with the increase of the feed rate of fresh medium(3). In current study, despite the considerable increase in feed rate on

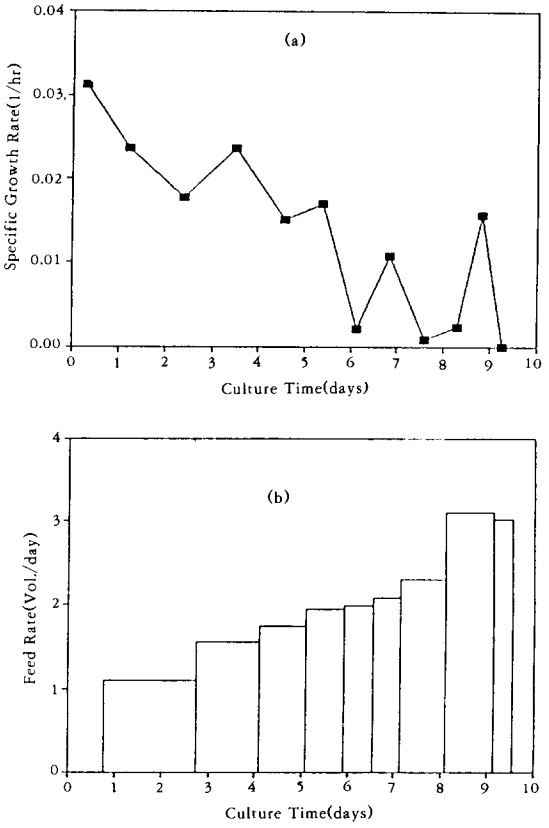

Fig. 4. Specific growth rate and feed rate profiles during perfusion dulture.
(a) Specific growth rate profile.
(b) Media feed rate profile.

the 8th day to 3 Vol./day, the oxygen limitation was not eliminated and held viable cell concentration constant with a gradual decrease in viability (Fig. 5). For this reason specific growth rate was decreased nearly to zero on the 6th day.

Effect of glucose concentration on specific MAb production rate

Our intention was to retain glucose concentration above 1 g / ℓ but, between day 3 and day 7 glucose concentration was about 0.80 g / ℓ. During this period, cell growth was not repressed but specific MAb production rate was reduced(Figs. 5, 6). Beyond the 7th day, with the increase of feed rate, glucose concentration could be main-

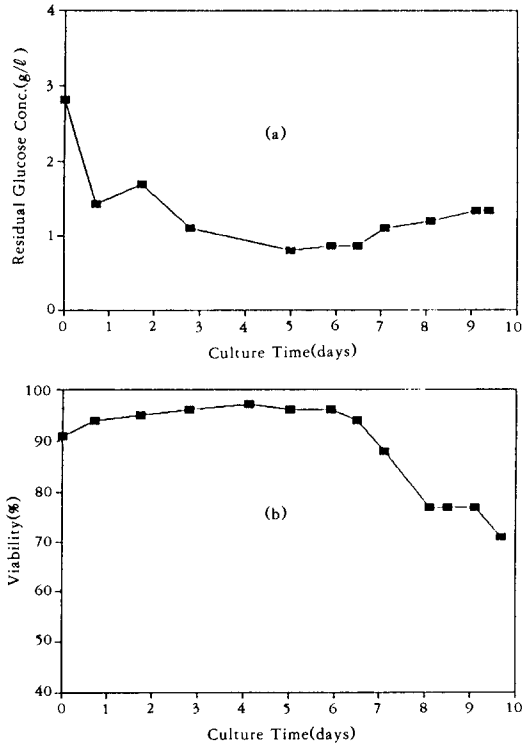

Fig. 5. Residual glucose concentration and viability profiles during perfusion culture.
(a) Residual glucose concentration profile
(b) Viability profile.

tained at 1.2~1.3 g / ℓ and specific productivity increased again. Specific glucose consumption rate profile was similar to that of specific productivity(data not shown). Form these observations, one can ascertain that at glucose concentration slightly below 1 g / ℓ, the limiting glucose concentrations for cell growth and MAb production, respectively, are such that cell growth is not affected but MAb production does decrease. Probably below the glucose concentration level of 0.5 g / ℓ, both cell growth and MAb productivity may be limited. For the production of MAb and the growth of cells, glucose concentration should be controlled above 1.5 g / ℓ. This value will be sufficient for the two objectives. For the production of MAb without growth of cell, some component may have to be added or deleted to limit cell

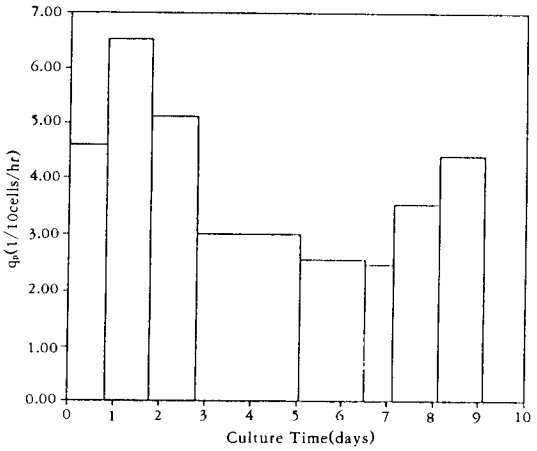

Fig. 6. Specific MAb productivity change during perfusion culture. unit of MAb titer is arbitrary.

growth.

In the current study of perfusion culture with Celligen bioreactor, the concentration of MAb reached at maximum cell concentration, 4 times higher than in bath culture, and a specific productivity, 9 times higher than in batch culture, could be achieved. Cell concentration was 10 times higher as compared with batch culture.

Once glucose concentration can be kept above 1.5 g / ℓ and D. O. can be controlled well, very high MAb productivity and also very high cell concentration with excellent viability can be achieved. Difficulties were encountered in the correct measurement of cell concentration and in securing sufficient level of oxygen transfer rate at high cell density. Indirect measurement of cell concentration(7) and better ideas to increase oxygen transfer rate at high cell density(8-10) may well deserve further studies.

요 약

LSM을 이용하여 KA112 균주의 고농도 배양을 시도하였다. Separator로는 hollow fiber를 사용하였고 reactor로는 Celligen을 이용하였다. Wroking volume 1리터로 10일간 배양하여 최고 세포농도가 회분식 배양에 비하여 10배 이상 증가한 2.1×10^{7}

cells/ml이었고, 항체의 농도는 4.5배 정도 높았다. 최고 feed rate에서 항체생산속도는 회분식 배양보다 9배 높았으며 배양 중 glucose농도가 1 g/ℓ 이상일 때 specific productivity가 증가하였고, 1 g/ℓ 이하일 때 세포성장은 영향을 받지 않으나 specific prodictivity는 감소하였다.

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