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Kinetics of Cultivating Mammalian Cells in Fed-Batch Process for the Production of Erythropoeitin

Yu, Ho-Kuem, Suk-Kyu Choi, Youn-Soo Lee[1], Kwang-Soon Shin[2], Hee-Koo Hwang[2] and Hyeon-Yong Lee*

Department of Food Engineering, Kangweon National University, Chuncheon 200-701, Korea
[1]Lotte Group R & D Center, Seoul 150-104, Korea
[2]Central R & D Center, Green Cross Company, Seoul 137-073, Korea

동물세포의 유가배양 공법에 의한 Erythropoeitin 생산에 관한 동력학적 연구

유호금 · 최석규 · 이윤수[1] · 신광순[2] · 황희구[2] · 이현용*

강원대학교 식품공학과, [1]롯데그룹 중앙연구소, [2]녹십자(주) 중앙연구소

Aabstract — 1.85×10^{-10} (mmole/cell/h) of specific glucose consumption rate was obtained under fed-batch cultivation of recombinant mamalian cells with maintaining 4.7×10^{-7} (μg/cell/h) of average specific erythropoeitin production rate. Higher maximum cell density was also achieved than for both cases of batch and perfusion cultivations. It proves that glutamolysis dominates metabolic pathways at latter period of cultivation where quasi steady state was maintained. Substrate limitation of glucose concentration was estimated as 13 (mmole/*l*) under fed-batch conditions. while specific product production rate was decreased according to cultivation time, erythropoeitin production was increased as glucose concentration in the media increased up to 13.2 (mole/*l*).

Recombinant mammalian cells are still believed to be suitable for the production of pharmaceutically active proteins since they can secrete enough amounts of properly modified products, even though natural products are much positively accepted than recombinant-derived proteins (1-3). There are several disadvantages in scaling-up animal cell culture processes because of stringent requirement of sterility, low concentration of cell density and relatively low productivity with high cost of culture media (4, 5). So far, much improvement has been made to maintain high number of cell density (up to 1×10^{7} cells/m*l*) in bioreactors by employing several types of perfusion systems (6-8). Perfusion process has proved to be more efficient in cultivating mass amounts of cells with high productivity of extracellular proteins than batch cultures (9).

However, this kind of continuous culture system has several difficulties in industrial applications: cell aggregation, clogging of filters by cells and/or debris, mechanical stress on cells by filtration and loss of productivity. Therefore, fed-batch cultivation may be directly applied to scale-up mammalian cell cultures since this process can overcome the above-mentioned problems. In this report kinetics of cell growth and erythropoeitin production from recombinant cells has been investigated under fed-batch conditons.

Key word: Fed-batch cultivation, erythropoeitin, mammalian cells
***Corresponding author**

Materials and Methods

A subline of Chinese hamster ovary (CHO-K) was recombinated with a plasmid (150 kb) containing erythropoeitin (EPO) genes, and named as CHO-SQ.1(10). These cells have been cultivated with Ru-

bbs essential media (GIBCO, USA) enriched by 10% fetal bovine serum (Hyclone, USA) and 20 μM of methylothrexate in a 75 T-flask at 37℃ CO_2 incubator by changing fresh media every 3 days. This medium contains 3 g/*l* of glucose, 0.584g/*l* of glutamine and 40 μg/m*l* of gentamicine. When total cell density was reached to 5×10^6 cells/m*l*, cells were inoculated into a 2*l* air-exchange-type bioreactor (Celligen, USA) for fed-batch cultivation. Cell density was measured by trypan blue dye exclusion method everyday (11).

Cells were cultivated with one liter of fresh media after being inoculated, and then 20 m*l* of fresh was added every 20~24 hours for spike-feeding until cell density was decreased by a peristaltic pump. Total volume of media in the reactor was increased up to about 1.2*l*. Residual concentrations of glucose and glutamine, and produced lactate and ammonia in the reactor were measured by using HPLC (Waters, USA), enzymatic and colorimetric methods every day. EPO concentration in the media was estimated by employing modified immnunoassay technique (12).

Metabolic quotients of glucose to lactate, q_G and glutamine to ammonia, q_g were calculated by the ratio of two components as follows:

$$q=\frac{\Delta C_L}{\Delta C_G} \text{ or } \frac{\Delta C_{NH3}}{\Delta C_g} \quad (1)$$

where ΔC is the consumed and produced concentrations of metabolites (mmole/*l*) in the media and L, G and g is lactate, glucose and glutamine, respectively. Specific EPO production rate, q_P, (μg/cells/h) and specific glucose consumption rate, q_G (mmole/cells/h) in the fed-batch process can be estimated as follows:

$$q_P=(\Delta P/\Delta t+DP_{avg})/X_{avg} \quad (2)$$

$$q_G=(\Delta G/\Delta t+DG_{avg})X_{avg} \quad (3)$$

where P and G are EPO and glucose concentration in the media (μg/m*l*) and (mmole/*l*), respectively, t is the cultivation time (hour), D is the dilution rate (1/h), P_{avg} and G_{avg} are average concentrations of the product and glucose at time t_1 and t_2 (μg/m*l*) and (mmole/*l*), and X_{avg} is average total cell concentration at t_1 and t_2 (cells/m*l*).

Results and Discussions

Fig. 1 is the results of cultivating CHO-SQ.1 cells under fed-batch conditions. 6.7×10^6 (cells/m*l*) of maximum cell density was obtained after 140 hour cultivation when 20 m*l* of fresh medium was supplied every 20~24 hours for spike feeding as shown in solid line of Fig. 1. This fed-batch culture maintained relatively high maxium cell denisty, compared to about 5×10^6 (cells/m*l*) from perfusion process and about 3×10^6 (cells/m*l*) from batch process, respectively (15). Maximum cell density was obtained within 140 hours of cultivation and slowly decreased rather than maintaining stationary state for a period of time. This culture maintained a relatively short period of steady state. It could result from fast feeding rates before reaching the steady state.

Glucose and glutamine comsumption rates were observed along with the production of lactate and ammonia in Figs. 2 and 3, assuming that only glutamine produced ammonia because of low concentrations of ther amino acidss in the media. 1.85×10^{-10} (mmole/cells/h) of specific glucose consumption rate was estimated at 130 hours of cultivation by employing Eq. (3) and its consumption rate was higher than about 0.39×10^{-10} (mmole/cell/h) in ex-

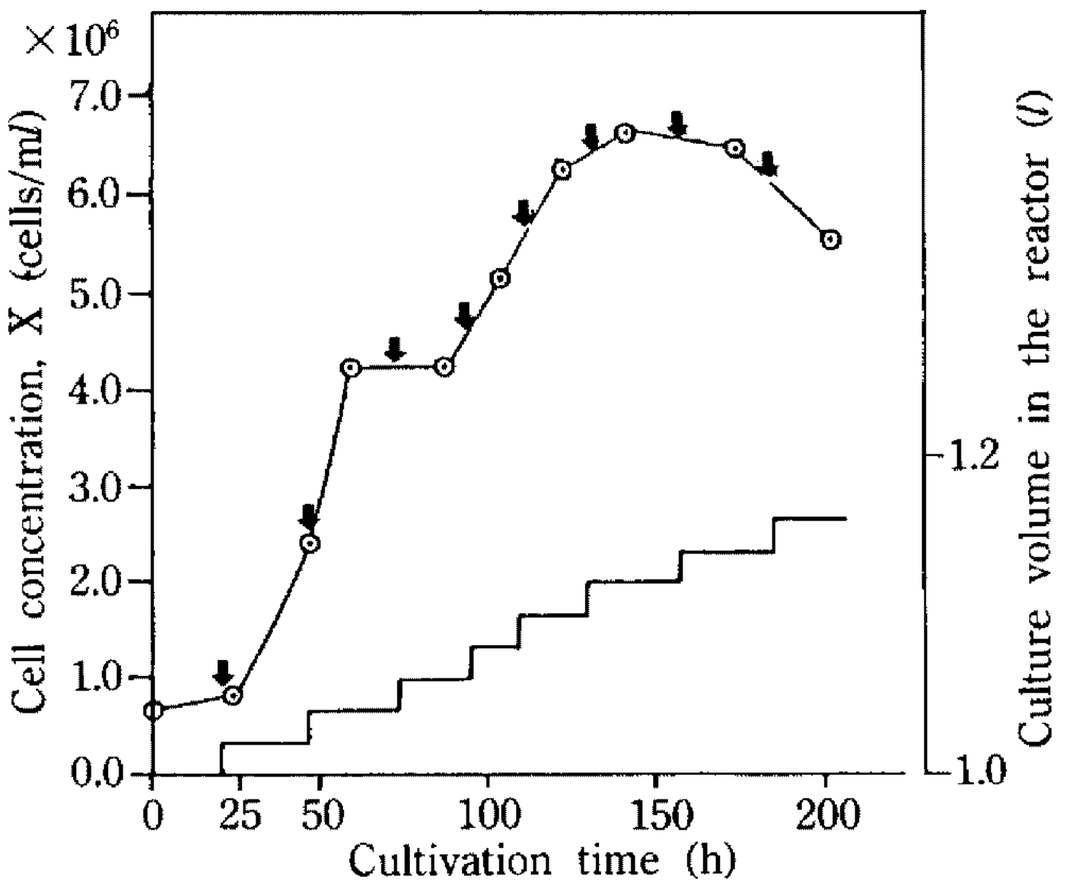

Fig. 1. Growth kinetics of cultivating recombinant CHO-SQ.1 under fed-batch condition.
⊙ and — are cell density (cells/m*l*). Solid line is the increase of culture volume and dark arrows ar the points of spike feeding of fresh media.

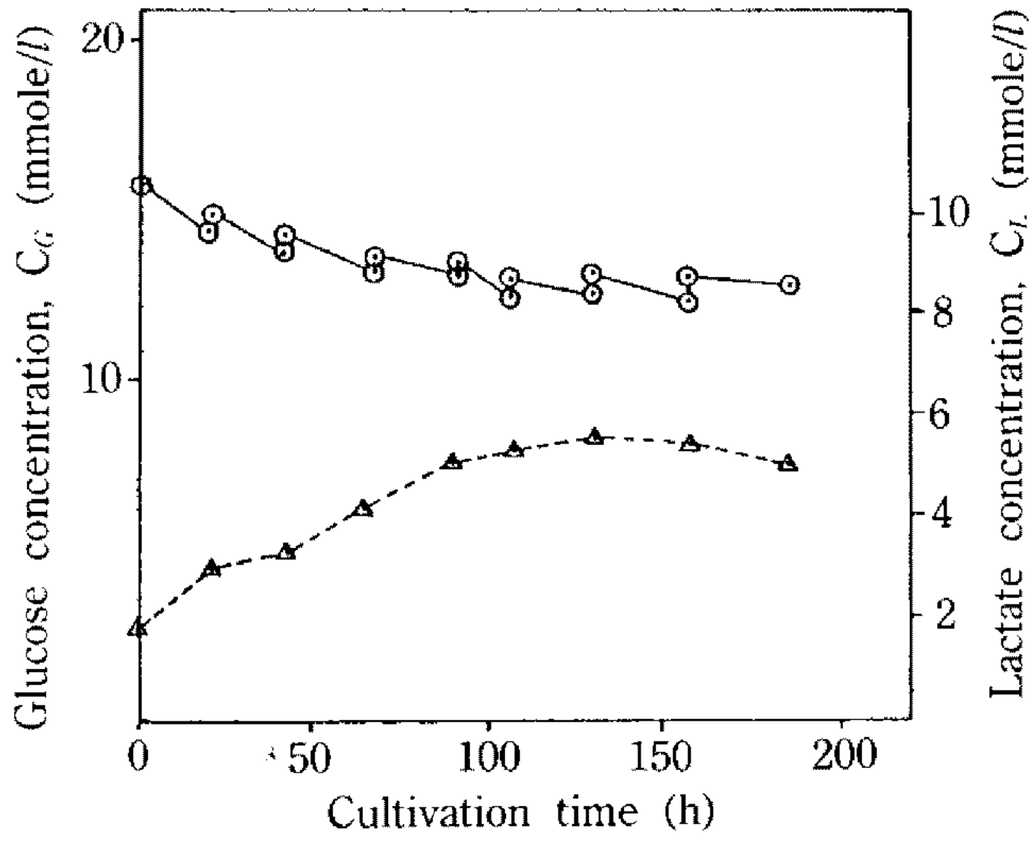

Fig. 2. Consumption of glucose and production of lactate during fed-batch cultivation of recombinant CHO cells.

⊙ and — are the concentrations of glucose in the reactor (mmole/*l*); △ and --- are the concentrations of produced lactate (mmole/*l*).

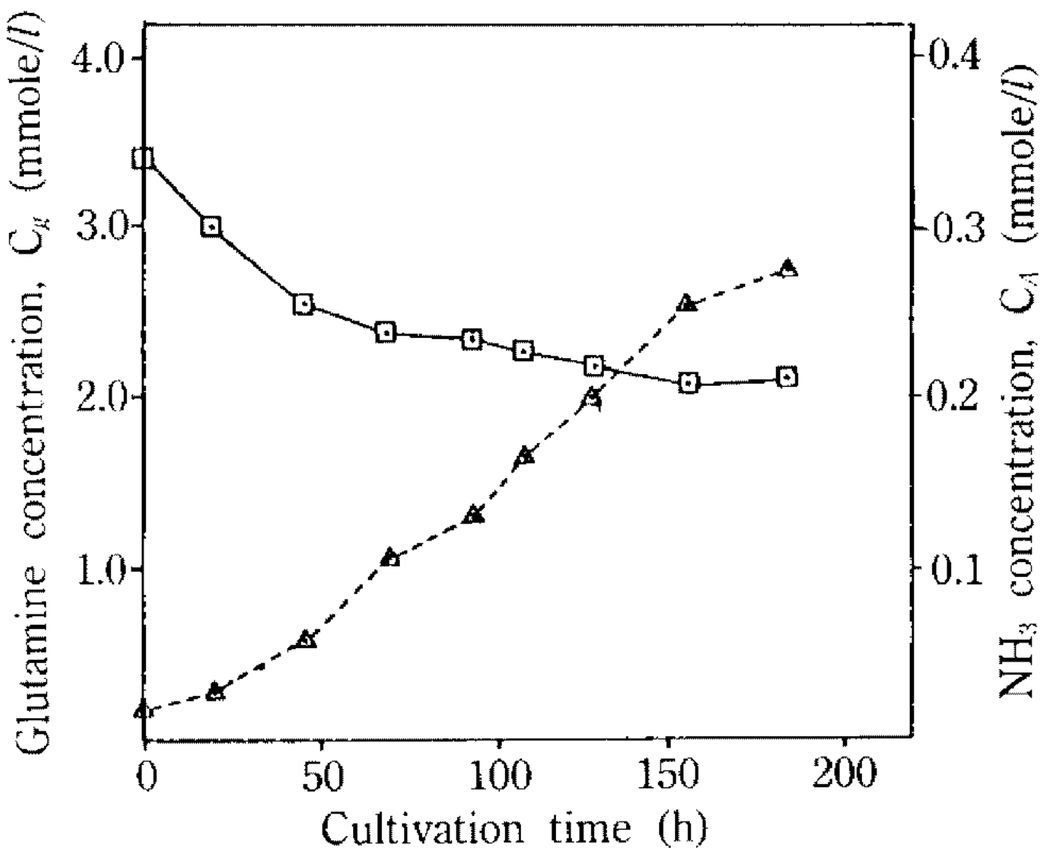

Fig. 3. Concentration of glutamine and NH_3, according to cultivation time under fed-batch condition.

⊙ and — are glutamine concentration (mmole/*l*); △ and --- are ammonia concentration (mmole/*l*).

ponential growth of batch cultivation (data not shown), because of higher glucose concentration. The production of lactate was gradually decreased while glucose concentration remained relatively constant at latter period of cultivation because cells no longer grew in continuously feeding the media. Much higher production of ammonia was observed than the production of lactate at same cultivation time in Fig. 3 even though glutamine concentration was also relatively constant. It implies that glutamolysis can play a more important role in steady state than consumptions of glucose under fed-batch cultivation with low glucose and glutamine concentration (13-15).

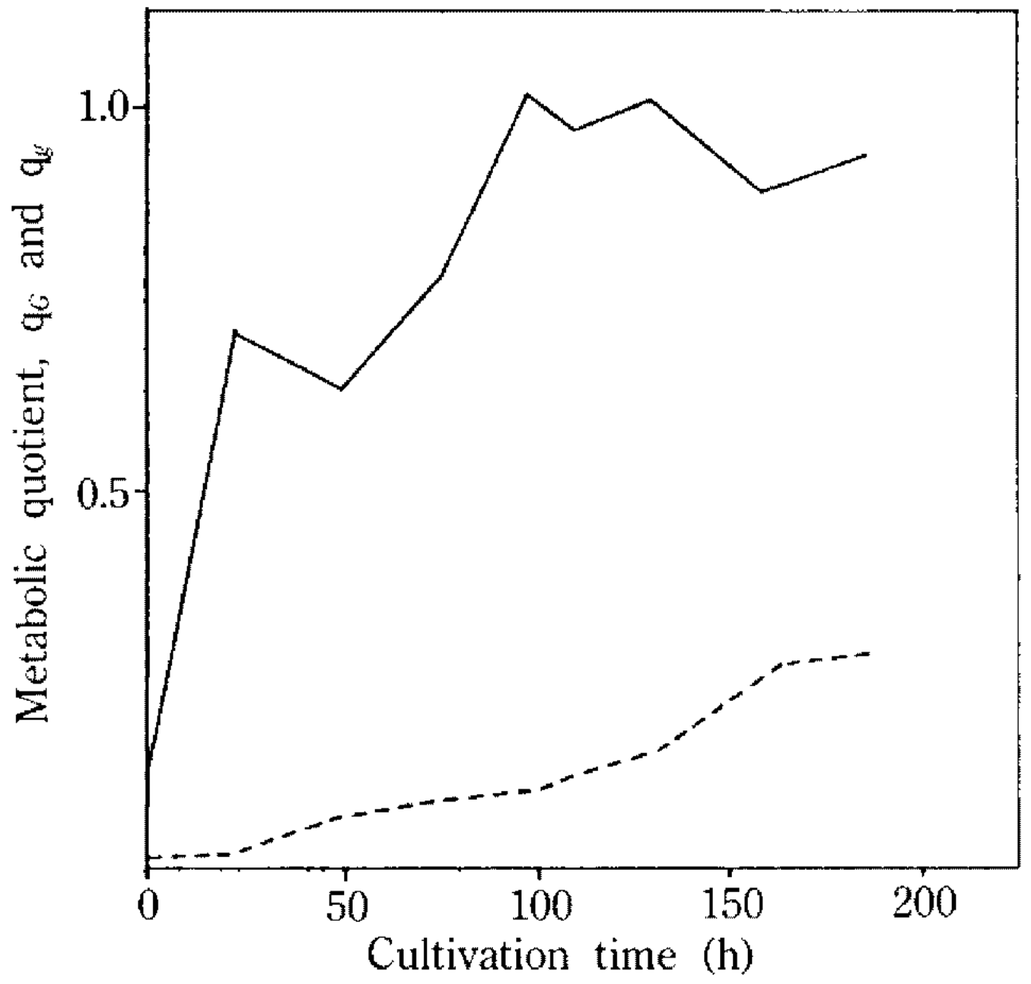

Fig. 4. Conparision of metabolic quotients based on glucose and glutamine consumptions according to cultivation time.

Solid line is the ratio of glucose to lactate from Fig. 2 and dotted line is the ratio of glutamine to ammonia from Fig. 3.

Fig. 4 demonstrates metabolic changes in fed-batch cultivation by showing the ratios of glucose to lactate and glutamine to ammonia. As expected in Fig. 3, while the production of ammonia was continuously increased according to cultivation time, lactate concentration in the media was gradually decreased due to less consuming glucose at latter periods of cultivation than at early portions of cultivation. Fig. 4 also confirms that active metabolic processes occur in the first periods of cultivation with having rapid increase of metabolic quotient. The values of q_G (>1) were due to experimental errors and/or due to incorporation of carbons from other sources besides glucose. It could also infer that glutamine consuming pathway was less efficient process than glycolysis by having low metabolic conversion ratios.

Fig. 5 is the kinetics of secreting EPO from recombinant cells, and the product production seemed to be partially growth related process for this culture because products were continuously produced

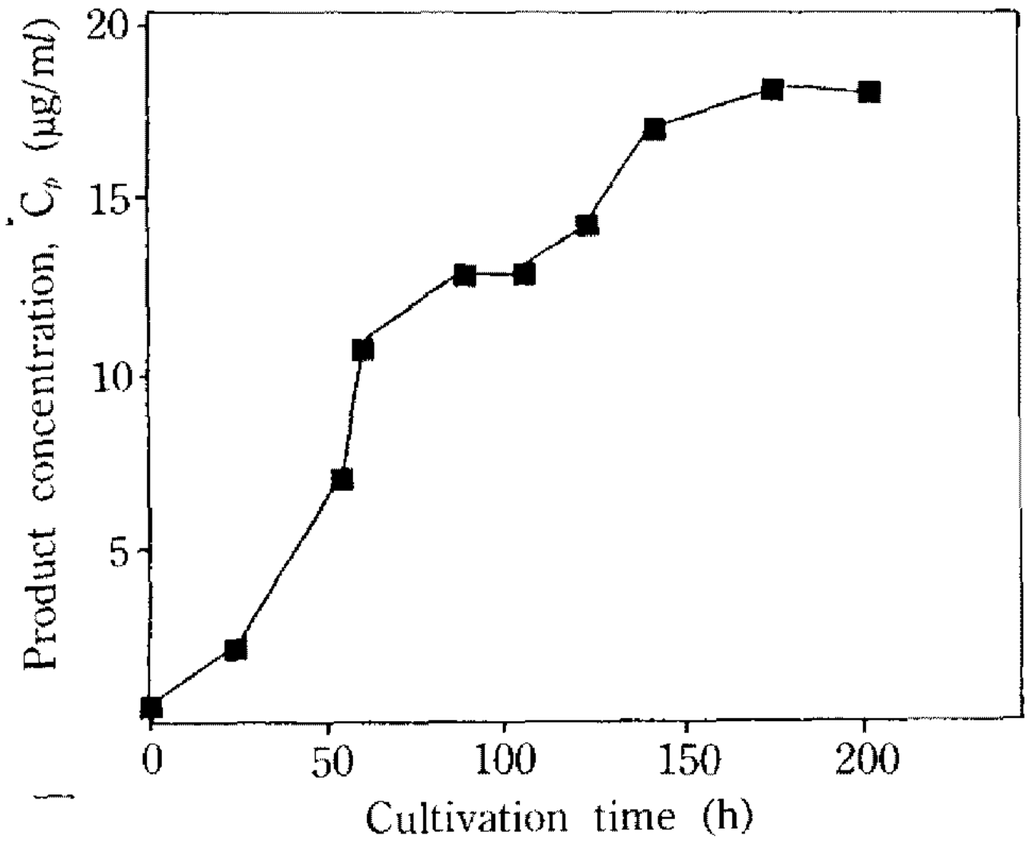

Fig. 5. Kinetics producing EPO in the reactor under fed-batch cultivation of recombinant CHO cells.

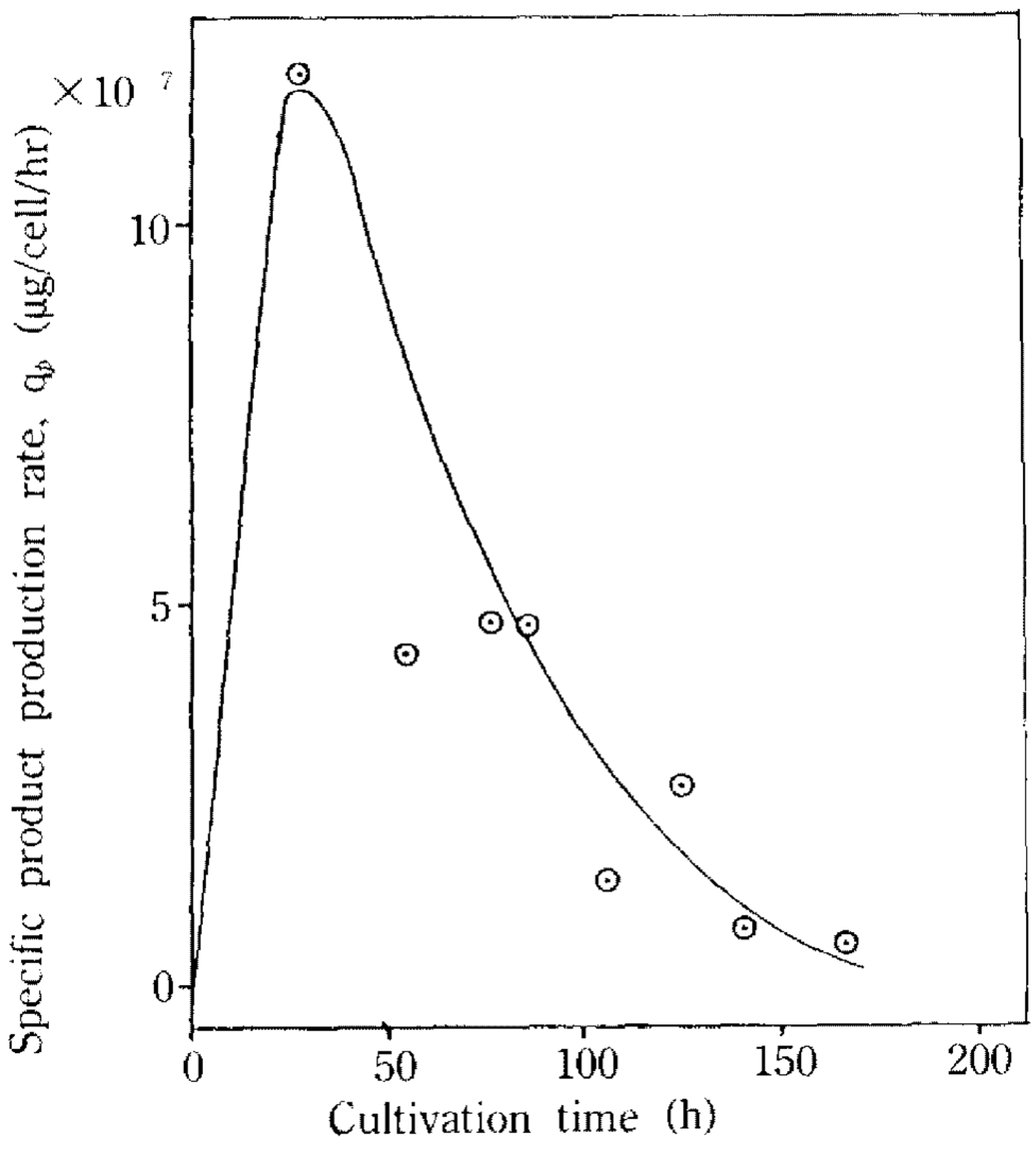

Fig. 6. Specific EPO production rate according to cultivation time by spike feeding of fresh media from the growth of CHO cells.

except when cell density decreased after 160 hours of cultivation. The reason that EPO concentration remained steady in the latter peroid of the cultivation was the release of the product from dead cells. Fig. 6 is the results of estimating specific product production rate by employing Eq. (2) with data from Figs. 1 and 5. Specific product production rate was decreased according to cultivation time with maintaining 4.7×10^{-7} (μg/cell/h) of average productivity

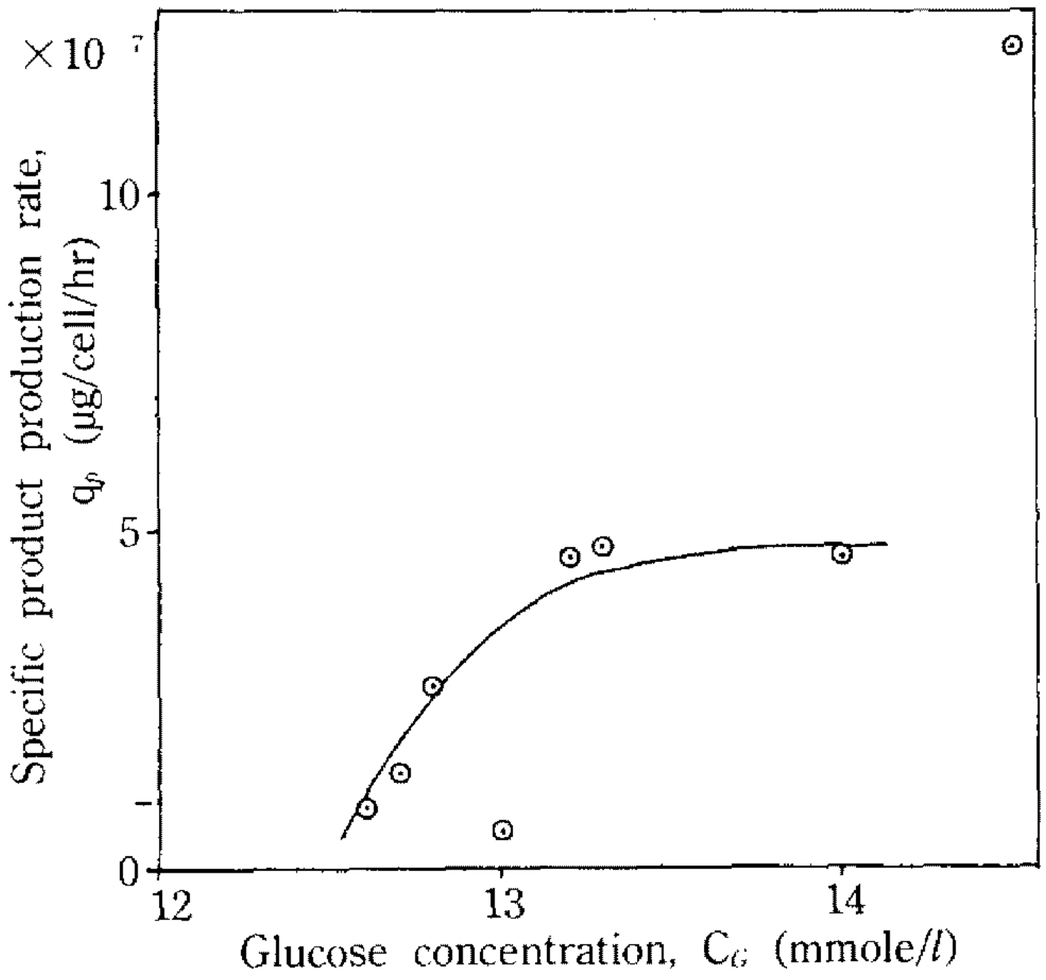

Fig. 7. Correlation between residual glucose concentration and EPO production rate fed-batch cultivation of spike feeding of fresh media.
Solid line is the result of non-linear regression analysis.

because of faster cell growth than product production until the system reached to steady state. Fig. 7 is to compare the productivity with residual glucose concentrations in fed-batch cultivation. Product production rate was linearly related with the increase of glucose concentrations up to 13.34 (mmol/l), then remained constant. It could tell that one of nutrient components in the media (glucose for this case) served as a growth and/or product limiting factor for cell culutures, except for serum, under fed-batch cultivation. One data point at high glucose concentration was deleted as an outlier for non-linear regression analysis in Fig. 7.

Conclusion

Fed-batch cultivation showed the possibility of easy scale-up of mammalian culture systems by maintaining 6.7×10^{6} cells per ml, compared to batch and perfusion cultivations. This culture system can control metabolic pathways of glucose and glutamine along with process parameters such as media feeding rates, D.O. and pH etc. Glutamine played a very important role in growing and producing products by evovling large amount of ammonia, rather than depleting glucose and glutamine at latter

period of cultivations. And at early period of process cells grew actively, then specific product production rate was gradually dereased as well remained relatively constant at high glucose concentrations in the media. Further work on fed-batch cultivation will also be conducted to clearly explain current experimental results and to precisely analyze cell culture systems.

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요 약

유전자 재조합된 동물 세포의 유가 배양시 1.85×10^{-10}(mmole/cell/h)의 비 glucose 소비속도와 4.7×10^{-7}(μg/cell/h)의 erythropoeitin(EPO)비 생산속도를 유지할 수 있었다. 또한 이같은 배양에서 회분 및 연속 배양에서보다 높은 세포수를 얻었으며 전 배양이 유사 안정상태에 도달하는 배양 후기에는 glutamolysis가 생육 공정에 매우 중요한 역할을 하고 있음이 확인됐다. 유가 배양시 13(mmole/*l*)의 glucose 농도에서 생육 제한 현상이 일어났으며, 이같은 농도에 도달할 때까지는 glucose의 농도가 증가함에 따라 배양시간의 경과와 함께 EPO 생산성이 증가했다.

References

1. Zettlmeissl, G., H. Ragg and H. E. Karges: *Bio /Technol.*, **5**, 720 (1987)
2. Fontages, R., Y. Beaudry nd G. Motot: *Biotechnol. Bioeng. Sym.*, **4**, 859 (1974)
3. Pavirani, A., P. Meulien, H. Harrer, F. Schamber and J. Lecocq: *Bio/Technol.*, **5**, 387 (1987)
4. Ratafia, M.: *Pharm. Technol.*, **3**, 48 (1987)
5. Lee, H. Y.: *KSEA*, **10**, 127 (1987)
6. Hu, W. S. and T. C. Dodge: *Biotech. Prog.*, **1**, 209 (1985)
7. Lee, H. Y.: *Kor. J. Biotech. Bioeng.*, **4**, 36 (1988)
8. Murry, M. Y. and Y. Christi: *Bio/Technol.*, **6**, 1291 (1988)
9. Scattergood, E. M., A. J. Schalabach, J. M. McLeer and M. Hilleman: *Annal. N.Y. Acad. Sci.*, **413**, 332 (1983)
10. Lin, F. K., S. Suggs and C. H. Lin: *Proc. Natl. Acad. Sci.* USA., **82**, 7580 (1985)
11. Freshney, R. I. (ed.): *Culture of Animal Cells* (2nd ed.), Alan R. Press, N.Y. (1983)
12. Goldberg, M. A., G. A. Glass, J. M. Cunningham and H. G. Bunn: *Proc. Natl. Acad. Sci.* USA, **84**, 7972 (1987)
13. Miller, W. M.: *A kinetic analysis of hybridoma growth and metabolism*, Ph.D. thesis, Univ. of California at Berkeley (1987)
14. Kang, J. K., S. K. Choi and H. Y. Lee: *Kor. J. Appl. Microbiol. Biotech.*, **18**, 401 (1990)
15. Glacken, M. W., J. K. Linn and S. Y. Lowes: *J. Biotechnol.*, **10**, 39 (1989)

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