Matsuoka et al. BMC Proceedings 2013, 7(Suppl 6):P50
http://www.biomedcentral.com/1753-6561/7/S6/P50

BMC Proceedings

POSTER PRESENTATION Open Access

Improvement of production rate on recombinant CHO cells in two-stage culture

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From 23rd European Society for Animal Cell Technology (ESACT) Meeting: Better Cells for Better Health
Lille, France. 23-26 June 2013

Background

Cultivation temperature is a key environmental parameter that influences cell growth and recombinant protein production. Recombinant CHO (rCHO) cells are usually cultivated at 37 °C. Although lowering culture temperature below 37 °C decrease specific growth rate, in many cases, the specific production rate, q, of CHO cells was not enhanced by lowering the culture temperature. Unlike the specific growth rate, effects of low temperature cultivation on specific productivity rate are not so clear [1]. In the present study, we investigated the effect of low temperature cultivation on rCHO cell growth and production rate. We proposed a two-stage culture that the cultivation was carried out at 37 °C and then a culture temperature become lower. We report that the final production concentration by the two-stage culture is higher than that in case of a flat temperature at 37 °C.

Materials and methods

CRL-10052 was used as the cell line of rCHO, which is the CR1 plasmid was transfected to CHO cells. Target product is the soluble CR1, *s*CR1, which is a soluble form of a human complement receptor type1, could be expressed and secreted by rCHO [2]. Although an original rCHO was an adherent cell, we changed it to be a floating one and used in this experiment. Batch cultivations were carried out in a 1 L-fermentor with a 400 mL working volume at various temperatures. pH and DO were maintained at 7.2 and 40% of air saturation by CO_2 and O_2, respectively. Agitation speed was 100 rpm. A serum-free medium on the basis of IMDM with 1% penicillin-streptomycin-neomycin antibiotics mixture was used. An initial cell concentration was 3×10^5 ml^{-1} and cultivation was ceased when cell concentration below 1×10^5 cells mL^{-1}. *s*CR1 concentration was determined by using HPLC gel filtration column chromatography (TSK gel G3000SWXL, TOSOH), in which the Tris buffer (pH = 7.4) containing 0.05% CHAPS was used as elution buffer.

Results

All batch cultivations were carried out until viable cells become equal to zero. Cells grew well at more than 33 °C, however cells didn't grow at 30 °C. Compared to 37 °C-cultivation, lower specific growth rates were observed in the lower temperature cultivations. The specific production rate of *s*CR1, q_s^{CR1}, was obtained by the slope of relationship between *s*CR1 concentration and time integrated cell concentration within a linear range. The q_s^{CR1} at each temperature were the almost same except at 30 °C.

The final *s*CR1 concentrations at 33 °C was rather higher than those at 37 and 35 °C. The cell concentration in stationary phase, X_S, at 33 °C was lower than those at 37 and 35 °C. Thus the ratio of the final *s*CR1 concentration to X_S at 33 °C was the highest in case of more than 33 °C. The final *s*CR1 concentration to X_S at 30 °C is rather higher than that at 33 °C, however it makes no sense because of the extremely low specific growth rate at 30 °C.

In order to increase the final *s*CR1 concentration, we proposed a two-stage culture that at first cultivation temperature was set to 37 °C and then a culture temperature became lower at late logarithm phase. Thus the final *s*CR1 concentration by using a two-stage culture, in which the temperature was 37 °C initially and changed to 33 °C after 120 h-cultivation, increased by 1.75 and 1.99, compared as a flat temperature culture at 33 °C and 37 °C, respectively (Figure 1, Table 1).

Conclusions

The conclusions are as follows:

1. It was shown that the ratio of the final *s*CR1 concentration to the cell concentration in stationary phase was

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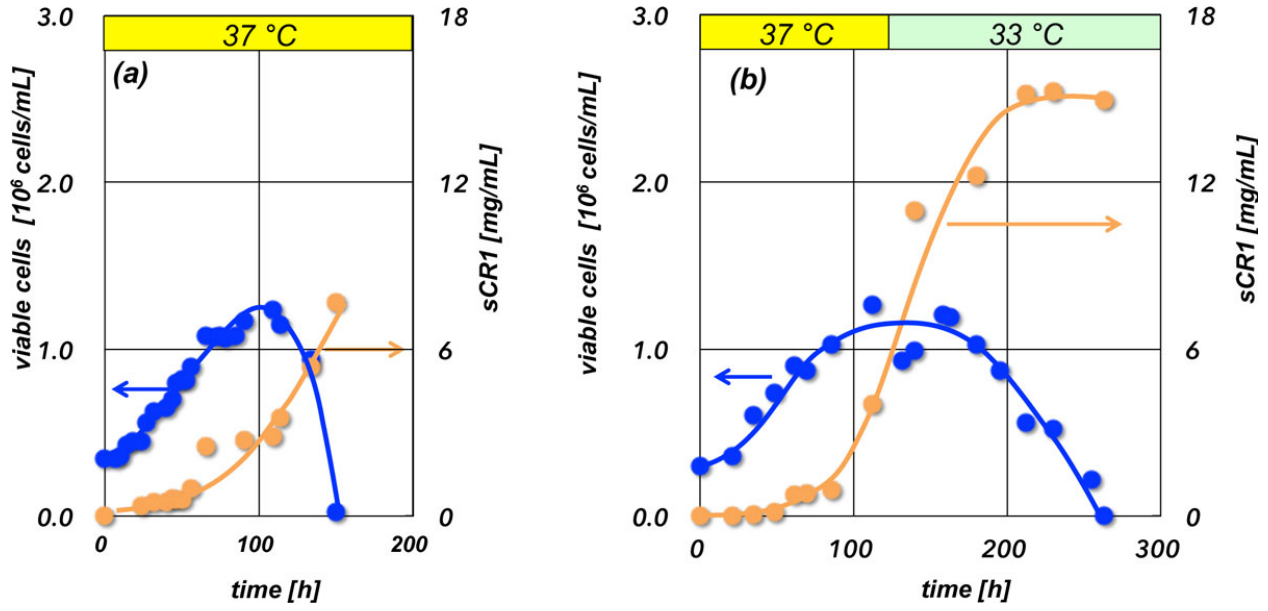

Figure 1 Time courses of cell-cultivation: (a) 37 °C, (b) two-stage cultivation (37 °C to 33 °C after 120 h).

Table 1 Comparison of culture parameters at various temperatures

	30 °C	33 °C	35 °C	37 °C	37 °C→33 °C
specific growth rate [h^{-1}]	>0.0002	0.0072	0.0107	0.0136	-
q_s^{CR1} [10^9 g $cells^{-1}$ h^{-1}]	0.0304	0.0416	0.0407	0.0446	-
final *s*CR1 [mg/mL] (a)	3.04	8.68	8.11	7.67	15.2
X_S [10^6 cells/mL] (b)	0.223	0.788	1.09	1.15	1.20
(a)/(b)	13.6	11.0	7.43	6.68	12.7

rather higher at lower temperature than that in 37 °C-cultivation.

2. A two-stage cultivation with temperature change from 37 °C to lower temperature was proposed and it was shown that the final product concentration was considerably improved.

Published: 4 December 2013

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doi:10.1186/1753-6561-7-S6-P50
Cite this article as: Matsuoka *et al.*: Improvement of production rate on recombinant CHO cells in two-stage culture. *BMC Proceedings* 2013 7(Suppl 6):P50.