

POSTER PRESENTATION

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

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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, sCR1, 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 CO₂ and O₂, 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 $3 \times 10^5 \text{ ml}^{-1}$ and cultivation was ceased when cell concentration below $1 \times 10^5 \text{ cells mL}^{-1}$. sCR1 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 sCR1, q_s^{CR1} , was obtained by the slope of relationship between sCR1 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 sCR1 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 sCR1 concentration to X_S at 33 °C was the highest in case of more than 33 °C. The final sCR1 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 sCR1 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 sCR1 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 sCR1 concentration to the cell concentration in stationary phase was

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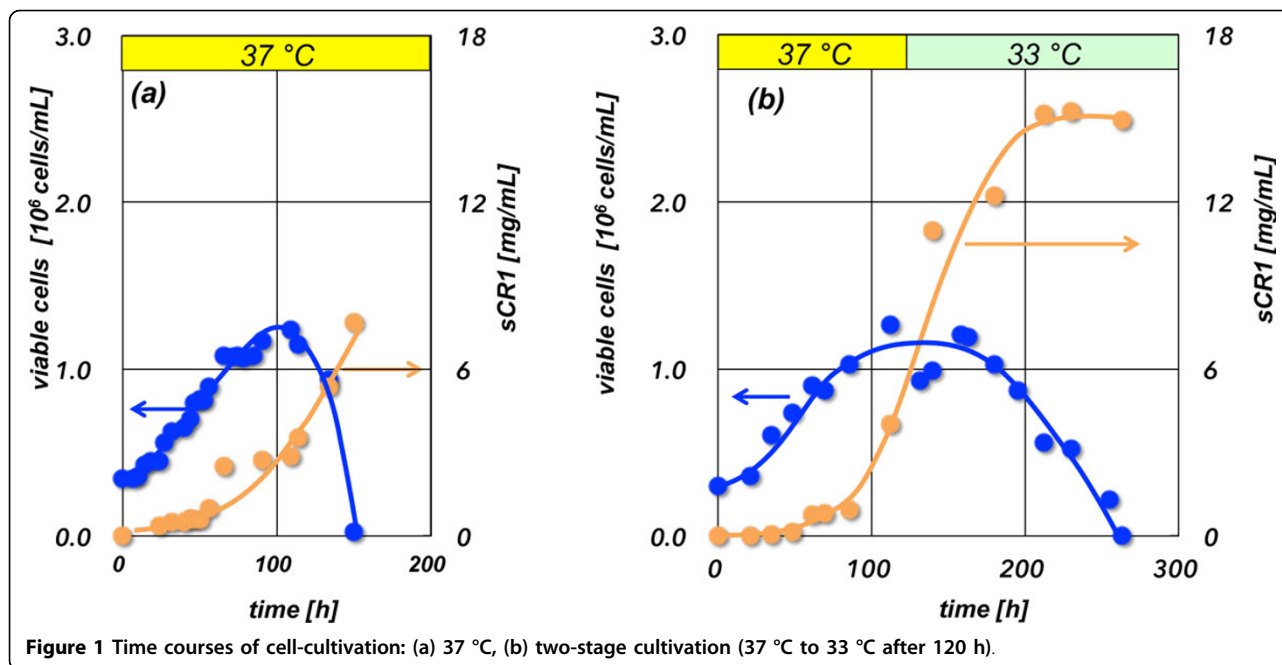


Table 1 Comparison of culture parameters at various temperatures

	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} [$10^9 \text{ g cells}^{-1} \text{ h}^{-1}$]	0.0304	0.0416	0.0407	0.0446	-
final sCR1 [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.

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