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 decreases specific growth rate, in many cases, the specific production rate, \( q_s \), 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 became 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, \( s\text{CR1} \), 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 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 × 10^5 ml^-1 and cultivation was ceased when cell concentration below 1 × 10^5 cells mL^-1. \( s\text{CR1} \) 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 \( s\text{CR1}, \ q_s^{\text{CR1}} \), was obtained by the slope of relationship between \( s\text{CR1} \) concentration and time integrated cell concentration within a linear range. The \( q_s^{\text{CR1}} \) at each temperature were the almost same except at 30 ºC. The final \( s\text{CR1} \) 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 \( s\text{CR1} \) concentration to \( X_S \) at 33 °C was the highest in case of more than 33 °C. The final \( s\text{CR1} \) 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 \( s\text{CR1} \) 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 \( s\text{CR1} \) 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 \( s\text{CR1} \) concentration to the cell concentration in stationary phase was
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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