Performance of HyCell CHO and Cell Boost 2, 5, and 6 supplements in fed-batch CHO cultures

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Performance of HyCell™ CHO and Cell Boost™ 2, 5, and 6 supplements in fed-batch CHO cultures

Chinese hamster ovary (CHO) cells are extensively used for production of recombinant proteins. However, different cell clones exhibit a large degree of variation in their nutritional requirements. For high cell growth and productivity, culture conditions need to be optimized to meet the specific requirement of the cell clone used. This application note shows how cell growth and productivity of an IgG-producing CHO cell line could be significantly improved by optimizing fed-batch culture conditions using HyClone™ HyCell CHO base medium and Cell Boost supplements.

Introduction

HyCell CHO is a chemically defined and animal-derived component-free culture medium optimized for CHO cells. The medium supports high peak cell densities and enhanced product yield and quality.

To further optimize protein yields with HyCell CHO medium, a fed-batch process utilizing Cell Boost supplements can be employed. The Cell Boost supplements are chemically defined and animal-derived component-free. Cell Boost formulations are designed to provide a selection of nutrients such as amino acids, vitamins, lipids, cholesterol, glucose, and growth factors in concentrations optimized for various mammalian cell types.

The Cell Boost supplements used in this study were selected through a series of design of experiments studies in which the concentration, timing, and combination of feeds were optimized. Feed optimization resulted in a greater than five-fold increase in yield compared with the batch control culture.

Materials and methods

A proprietary CHO cell line producing recombinant human IgG was used in this study.

Medium and feed supplements used were HyCell CHO medium and Cell Boost 2 (hydrated at 100 g/L in water for injection [WFI]-grade water and pH adjusted to 7.0), Cell Boost 5 (hydrated at 100 g/L in WFI water and pH adjusted to 7.0), and Cell Boost 6 (hydrated at 70 g/L in WFI water with 15 g/L sodium bicarbonate added and pH adjusted to 7.0) supplements.

The study was performed in 125 mL cell culture flasks with a starting volume of 30 mL and a seed density of 2.5×10^5 viable cells/mL. Cells were incubated at 37°C in 5% CO_2 and rotated at 130 rpm. For fed-batch cultures, cells were fed on days 2, 4, 6, and 8. The following conditions were investigated in duplicate:

1. Non-fed control culture
2. Cell Boost 2 + Cell Boost 5, both fed at 5% (v/v)
3. Cell Boost 2 fed at 10% (v/v) + Cell Boost 5 fed at 5% (v/v)
4. Cell Boost 2 + Cell Boost 6, both fed at 10% (v/v)

Cell count was performed daily. Biochemical analyses were conducted every second day (same day as feeding). Based on analysis results, the cultures were fed L-glutamine (200 mM) and glucose (200 mM) as needed. The cultures were sampled for IgG content every second day (day between feedings).

Results and discussion

Growth and productivity

For the fed-batch cultures, the results show slightly longer doubling times, yet overall longer plateau phases. Peak cell densities for the non-fed control culture reached just above 17×10^6 viable cells/mL, with the cultures maintaining a viability of > 50% up to day 10. The fed-batch conditions reached densities ranging from 12.0 to 18.7×10^6 viable cells/mL. Cells of the most productive fed-batch cultures did not grow quite as rapidly at the beginning, yet reached acceptable cell densities. These cultures also maintained a plateau phase for a few days before declining.

In terms of IgG production, the non-fed control culture reached 80 mg/L on day 9, while the fed-batch cultures produced from 384 to 430 mg/L by the end of the study. This result equates to an average five-fold increase in overall productivity for fed-batch cultures compared with the control. The best performing culture was fed Cell Boost 2 and Cell Boost 5 at 5% each. This culture reached a peak cell density of 18.68 × 10^6 viable cells/mL, a peak IgG concentration of 430 mg/L, and remained productive for a total of 13 days. The second highest performing culture was fed Cell Boost 2 at 10% and Cell Boost 5 at 5%. This culture reached a peak cell density of 12.2 × 10^6 viable cells/mL, a peak IgG concentration of 399.7 mg/L, and remained productive for a total of 15 days. Finally, the lowest performing fed-batch culture was fed Cell Boost 2 and Cell Boost 6, both at 10%. This culture reached a peak cell density of 16.44 × 10^6 viable cells/mL, a peak IgG concentration of 384.9 mg/L, and remained productive for a total of 14 days.

Growth and production data are displayed in Figure 1. All cultures were terminated when the viability dropped below 50%.

Ammonium, glutamine, and glutamate levels

In all fed-batch cultures investigated, the ammonium levels became a concern. Peak ammonium concentrations of 20 to 35 mM were observed. Figure 2 illustrates the ammonium levels along with the levels of glutamine and glutamate throughout the study. Note that the glutamate levels rose higher than the range of the biochemical analyzer, causing an error for glutamine readings (glutamine is dependent of glutamate in this analysis). Thus, only the non-fed control culture had glutamine concentration readings throughout the entire study. The fed-batch cultures were fed L-glutamine at 4 mM every other day beginning on day 5. This likely contributed to the increased levels of ammonium in those cultures.

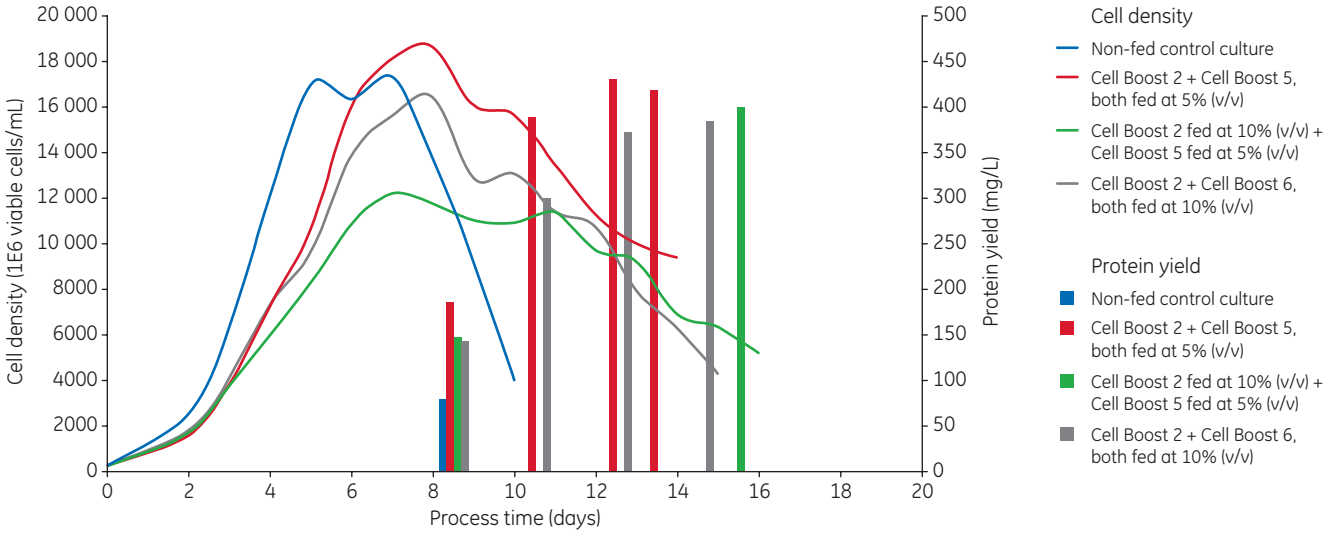

Fig 1. Cell growth (line charts) and productivity (bars) over the duration of the study.

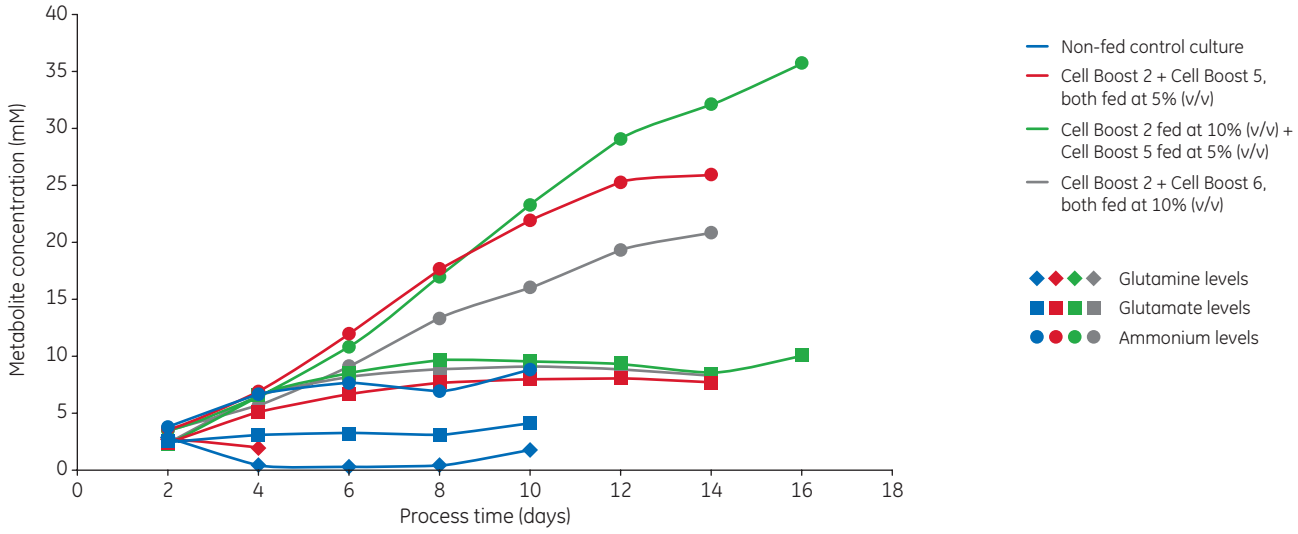

Fig 2. Ammonium, glutamine, and glutamate levels over the duration of the study.

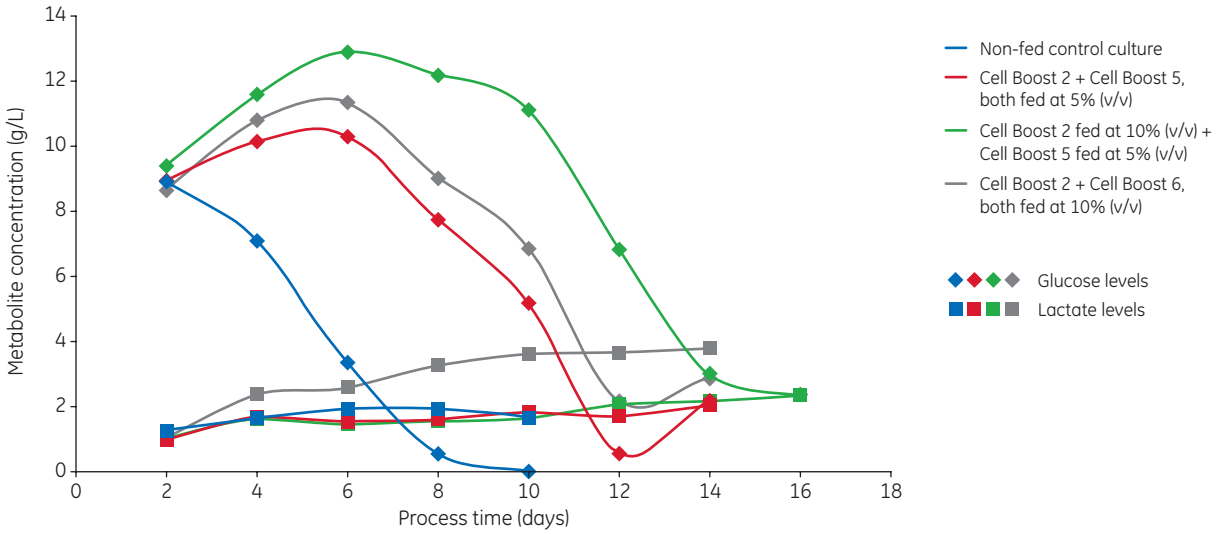

Fig 3. Glucose and lactate levels over the duration of the study.

Glucose and lactate levels

In addition to feeding with glutamine, the fed-batch cultures were also fed glucose as needed. Figure 3 shows the glucose and lactate concentrations over the time of the study.

Conclusions

By optimizing the feed strategy for HyCell CHO medium, it was demonstrated that IgG productivity could be increased more than five-fold compared with the non-fed control culture. HyCell CHO medium promotes excellent growth and quick population doubling times. When strategically combined with the Cell Boost feed supplements, a longer plateau phase and an extensive increase in productivity could be achieved.

Ordering information

Product	Product code
HyCell CHO	SH30934
Cell Boost 2	SH30596
Cell Boost 5	SH30865
Cell Boost 6	SH30866
L-glutamine, 200 mM	SH30034

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