Effects of feeding strategy on CHO cell performance in fed-batch cultures using HyClone™ ActiPro™ medium and Cell Boost™ 7a and 7b supplements

ActiPro cell culture production medium is intended to be used in combination with Cell Boost 7a and 7b supplements to enhance recombinant protein production in fed-batch or perfusion processes. This work demonstrates the development of a fed-batch process for a Chinese hamster ovary (CHO) cell line producing a monoclonal antibody (mAb). The results emphasize the importance of an appropriate feeding strategy to achieve optimal cell growth and productivity.

Introduction

ActiPro medium and supplements were developed to support high productivity of CHO cells in biomanufacturing of recombinant proteins, and the products have delivered excellent results with various CHO cell lines (1). ActiPro production medium and Cell Boost feed supplements are intended to be used together, following an easy-to-use starting protocol. The feed supplements are designed to complement each other and the recommended ratio of Cell Boost 7a to 7b is 10/1 (v/v). As a start, recommended feed volumes of Cell Boost 7a and 7b are 3% and 0.3%, respectively, of the total culture volume. In previous experiments, this feeding strategy was also shown to be optimal for a high-titer mAb-producing CHO DG44 cell line (internal data). However, the feed volumes should be adjusted with respect to the nutritional requirements of the specific cell clone used. In this work, a feeding strategy was developed in shake flasks for a CHO-S cell line producing a monoclonal antibody in lower titers. The productivity was further optimized in bioreactor cultures using ReadyToProcess WAVE™ 25 bioreactor system.

Materials and methods

Cell lines

Studies were performed with CHO-S cells (licensed from Cobra Biologics Ltd.). For shake flask cultures, the cells were passaged four times after thawing and cultured for 72 h prior to inoculation. For bioreactor cultures, the cells were passaged six times after thawing and cultured for 96 h prior to inoculation.

Batch cultures in shake flasks

Batch cultures were performed in 500 mL shake flasks filled with 100 mL ActiPro medium. Cells were inoculated at 0.3×10^6 viable cells /mL. Cultures were maintained in 37°C and 7.5% CO_2 at a rocking speed of 105 rpm. All experiments were performed in duplicate. Samples were taken daily and measured for cell density, viability, and productivity as well as for metabolite content.

Fed-batch cultures in shake flasks

Fed-batch culturing in shake flasks were conducted to obtain an optimal feeding strategy. Studies were performed in 1000 mL shake flasks with a starting working volume of 250 mL ActiPro medium. Cells were inoculated at 0.3×10^6 viable cells/mL. Starting on day 3, Cell Boost feed supplements were added once daily at volumes listed in Table 1. Glucose was added to the cultures when glucose concentration dropped below 2 g/L. Cultures were maintained in 37°C and 7.5% CO_2 at a rocking speed of 105 rpm. All experiments were performed in duplicate. Samples were taken daily and measured for cell density, viability, and productivity as well as for metabolite content.

Table 1. Setup of shake flask experiment

Shake flask volume (mL)	ActiPro starting working volume (mL)	Cell Boost 7a/7b (% of working volume)	Cell Boost 7a (mL)	Cell Boost 7b (mL)
1000 mL	250 mL	4.0/0.4	11	1.1
1000 mL	250 mL	3.0/0.3	7.6	0.76
1000 mL	250 mL	2.0/0.2	5.4	0.54

Fed-batch bioreactor cultures

Bioreactor cultures were performed in the ReadyToProcess WAVE 25 system operated in dual mode. Two 10 L Cellbag™ bioreactor bags for the duplicate cultures were place on the same rocker. Culture parameters are listed in Table 2. Cultures were controlled using the UNICORN™ system control software.

Cellbag bioreactor bags were filled with 3 L ActiPro medium and inoculated to a cell density of approximately 0.3 × 10^6 viable cells/mL. Starting on day 3, cultures were fed with 2% Cell Boost 7a/ 0.2% Cell Boost 7b once daily, as was shown to be optimal in shake flask cultures. Starting on day 5, glucose concentration was maintained at 2 g/L in the cultures. The cultures were harvested on day 14. Samples were taken daily and measured for cell density, viability, and productivity as well as for metabolite content.

Table 2. Culture parameters for 10 L fed-batch bioreactor cultures

Starting volume	3 L
Cellbag bioreactor	10 L
Temperature	37°C
Dissolved oxygen	40% air saturation
pH	7.1
Rocking speed	22 to 29 rpm
Supplementation (from day 3)	2.0% Cell Boost 7a and 0.2% Cell Boost 7b
Glucose	Added on demand to maintain a concentration of > 2 g/L.
Harvest criteria	9 days of feeding or when the cell viability dropped below 70%.

Analytical methods

Cell density and viability, glucose, lactate, ammonium, glutamine, glutamate levels were measured in cell suspension using a Bioprofile FLEX™ analyzer (Nova Biomedical Corp.) The amount of produced mAb was measured in clarified culture feed with a CEDEX™ Bio analyzer (Roche). Glycoanalysis was performed by Cobra Biologics Ltd., Staffordshire, UK. Charge variant distribution was determined by cation exchange chromatography on a ProPac WCX-10 2 × 250 mm protein column (Dionex).

Results

Shake flask cultures

Cell growth and productivity for shake flask batch cultures grown in ActiPro medium are displayed in Figure 1. To determine optimal feeding regimen, shake flask cultures were supplemented with Cell Boost 7a and 7b in ratios of either 4%/0.4%, 3%/0.3%, or 2%/0.2% of total culture volume. Cell growth and productivity for the tested feed volumes are shown in Figure 2. Results indicate optimal feeding at 2% Cell Boost 7a/0.2% Cell Boost 7b, giving the highest cell density and productivity for the selected cell line.

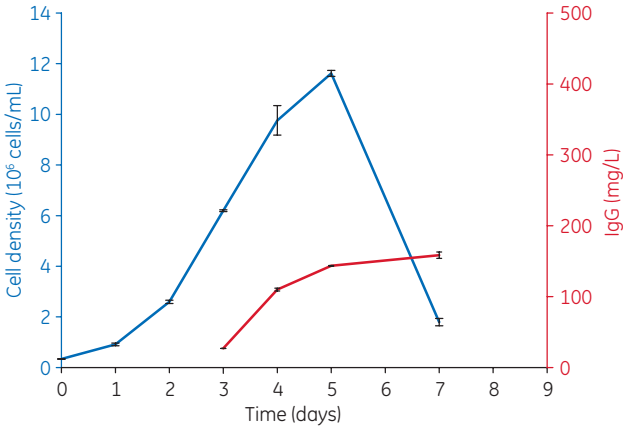

Fig 1. Viable cell density and productivity in shake flask batch cultures.

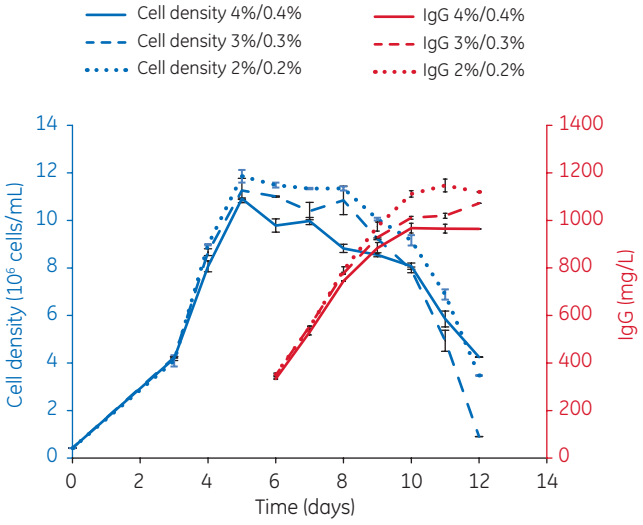

Fig 2. Viable cell densities and productivity in shake flask cultures supplemented with Cell Boost 7a and 7b to varying volumes.

Fed-batch bioreactor cultures

Viable cell density (VCD) and productivity of the bioreactor cultures are shown in Figure 3. A peak VCD of 15 × 10^6 cells/mL and productivity of 1.6 g IgG/L were achieved, as compared with the VCD of 12 × 10^6 cells/mL and productivity of approximately 1.2 g IgG/L achieved in shake flasks. Metabolite concentrations are displayed in Figure 4, showing the concentration profiles for glutamine, glutamate, ammonium, glucose, and lactate during the culture. Product quality attributes, that is, charge variant distribution and glycoprofiles for day 12, 13, and 14 of the culture period, are depicted in Figures 5 and 6, respectively. In general, only subtle changes in the quality attributes were observed during the last three days of the culture.

Shake flask culturing is a good tool for screening of certain culture conditions. However, in many cases, bioreactor systems offer improved growth conditions over culturing in shake flasks, as the culture parameters can be more accurately controlled. Hence, as expected, both cell growth and productivity were further improved in the bioreactor fed-batch cultures.

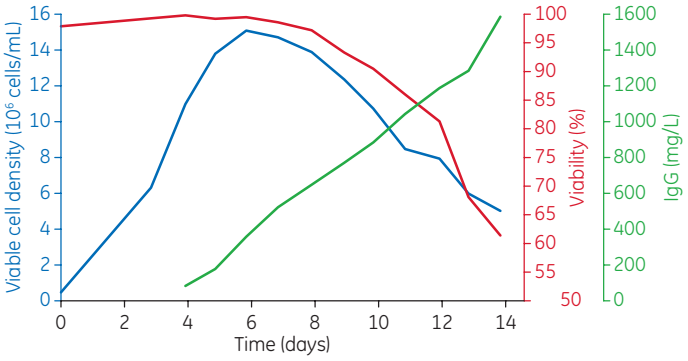

Fig 3. Cell growth and productivity in the fed-batch bioreactor culture.

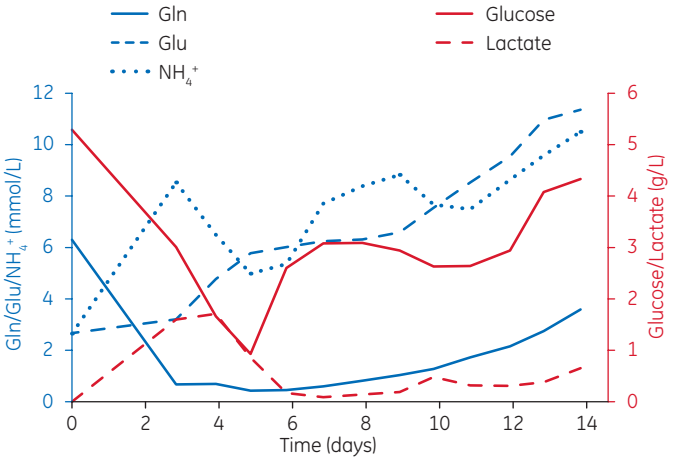

Fig 4. Metabolite concentrations in the fed-batch bioreactor culture.

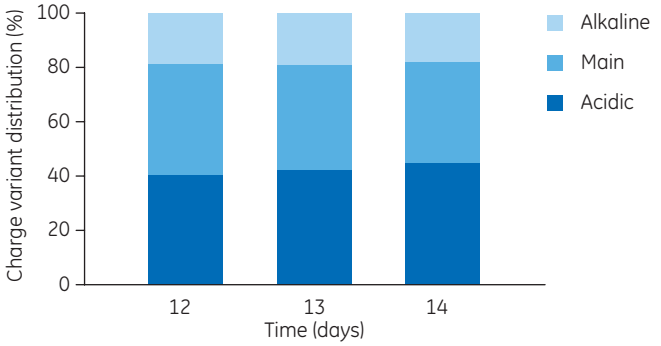

Fig 5. Charge variant distribution in the fed-batch bioreactor culture.

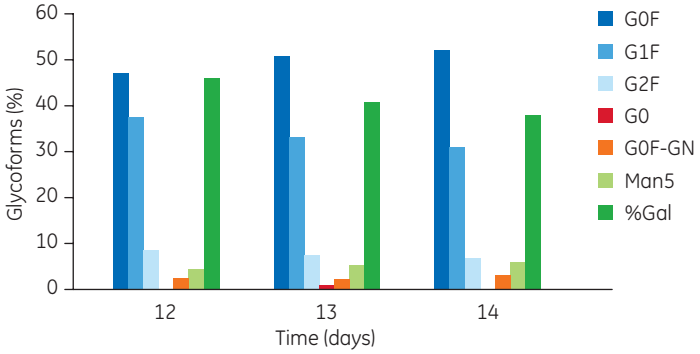

Fig 6. Glycoprofile of the fed-batch bioreactor culture.

Conclusions

This work shows the development of a fed-batch process for a low-producing CHO cell line using ActiPro culture medium and supplements. Feed ratios of 2.0%/0.2%, 3.0%/0.3%, and 4.0%/0.4% Cell Boost 7a to 7b were assessed. For this cell line, optimal cell growth and productivity were obtained at 2% Cell Boost 7a and 0.2% Cell Boost 7b. The results reflect the importance of determining appropriate feeding regimen based on the nutritional requirements of a specific cell line to avoid over or under feeding of the culture. As charge variants and glycoform distribution can vary significantly between cultures depending on selected feeding strategy, as well as during the culture, these important product quality parameters should also be considered when selecting feeding strategy and scaling up the process.

Reference

1. Application note: Scale-up of CHO cell fed-batch cultures in HyClone ActiPro medium supplemented with Cell Boost 7a and 7b. GE Healthcare, 29175257, Edition AB (2016).

Ordering information

Product	Product code
ActiPro basal medium, powder	SH31037
ActiPro basal medium, liquid	SH31039
Cell Boost 7a supplement	SH31026
Cell Boost 7b supplement	SH31027
ReadyToProcess WAVE 25, rocker	28988000
ReadyToProcess™ CBCU Full	29044081
ReadyToProcess Pump 25	29032003
Tray 50	29044474
Lid 50	29044477
10 L Cellbag bioreactor	CB0022/10-31

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

gelifesciences.com/bioprocess

29216535 AB 04/2017