Fed-batch supplements to boost cell culture performance

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CY13648-21May20-AN

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To increase the yield of a target protein, fed-batch culture is often employed in biomanufacturing processes. This application note demonstrates the enhanced performance of commonly used cell lines for MAb production when supplemented with HyClone™ cell culture supplements. Cultures of Chinese hamster ovary (CHO) cells and the cholesterol-sensitive NS0 murine myeloma cell line were fed with HyClone Cell Boost™ nutrient supplements and HyClone LS1000 cholesterol supplement, respectively. Cell growth and MAb production in the fed-batch cultures were compared with batch cultures. The results demonstrate increased viable cell yields and productivity in the fed-batch cultures.

Introduction

The fed-batch version of stirred-tank cell culture is commonly employed at large process scales. A primary driver of this mid-run addition of nutrients to a batch culture is to increase the quantity of product harvested. The prevalence of fed-batch over other modes is due to many practical factors such as reliability, scalability, and application breadth. Increases in the integral of viable cell concentration and volumetric productivity are examples of significant improvements commonly obtained.

Primary factors determining optimum feeding of cultures include:

- Maintaining desired metabolic pathways in high-density cultures
- Prolonging cell viability and preventing induction of apoptosis
- Promoting longevity of cultures over short-lived peak densities
- Promoting high-quality product accumulation rather than mere quantity
- Encouraging product accumulation rather than simple culture biomass

Today's experienced fed-batch professionals can expect more than 10 times the efficiencies possible from a few years ago. For some secreted recombinant protein biologicals, yields are now expected to be 1 to 3 g/L of culture, with 5 to 10 g/L potentially achievable. As the latest research is applied to production scale, yields exceeding 10 g/L are anticipated.

The Cell Boost supplements each provide an exclusive selection of nutrients such as amino acids, vitamins, lipids, cholesterol, glucose, and growth factors in complements optimized for multiple mammalian cell types Table 1). All Cell Boost supplements are chemically defined and animal-derived component-free (ADCF). In addition to the Cell Boost products, other supplement products provide identified nutrients for specific applications, such as concentrated lipids and ADCF cholesterol for such cell lines as NS0, or selected metabolites for cells using the glutamine synthetase (GS) system. This application note compares cell growth and productivity of CHO and NS0 cells when cultured in batch processes or in fed-batch processes supplemented with Cell Boost nutrient supplements and LS1000 cholesterol supplement, respectively.

Materials and methods

Cell cultures

CHO cells were cultured in HyClone HyCell™ CHO medium using single-use, stirred-tank bioreactors, employing both batch and fed-batch culture formats. Fed-batch cultures were supplemented with 10% v/v feed of Cell Boosts 2 and Cell Boost 5 supplements at a ratio of 60:40 (hydrated to a total concentration of 50 g/L) fed every other day beginning on Day 4.

HyClone CDM4NS0 medium was used for culturing of NS0 cells in single-use, stirred-tank bioreactors. Both batch and fed-batch culture formats were employed. Fed-batch cultures were supplemented with LS1000 feedings on day 1, 3, and 6.

Table 1. Cell Boost supplement matrix

	Amino acids	Vitamins	Glucose	Trace elements	Growth factors	Hypoxanthine/ thymidine	ADCF* lipids	ADCF* cholesterol	Suitable for	Product code
Cell Boost 1 Supplement (R05.2)	●	●	●						HEK293 CHO	SH30584
Cell Boost 2 Supplement (R15.4)	●		●						PER.C6™ CHO	SH30596
Cell Boost 3 Supplement (JM3.5)	●	●	●	●		●			Hybridoma Myeloma	SH30825
Cell Boost 4 Supplement (PS307)	●	●	●	●	●		●	●	CHO	SH30857
Cell Boost 5 Supplement (CN-F)	●	●	●	●	●	●	●	●	Hybridoma NS0 HEK293 CHO	SH30865
Cell Boost 6 Supplement (CN-T)	●	●	●	●	●	●	●	●	T-Cells Hybridoma NS0 HEK293 CHO	SH30866

* Animal-derived component-free

Yields of viable cells and produced MAb were monitored over the culture period. Cell counts and viabilities were determined with a Vi-CELL™ automated cell counter (Beckman Coulter) using standard trypan blue exclusion technique. IgG product titers were determined via biolayer interferometry on a ForteBio™ Octet™ QK 384 system (Pall Corporation) using protein A-based probes (1).

Results and discussion

Fed-batch CHO and NS0 formats, particularly in the expression of MAb, have demonstrated significant production gains through the application of the Cell Boost supplements. Figure 1 shows the result of Cell Boost 2 and Cell Boost 5 feeding of a suspension CHO cell line cultured in HyCell CHO medium. An up to three-fold increase in MAb expression levels in this CHO fed-batch culture was observed compared with batch cultures.

Additionally, fed-batch supplements can be used to overcome process-specific challenges. NS0 cells are known for being cholesterol auxotrophs requiring substantial levels of cholesterol to be present in the cell culture medium. The industry move toward single-use cell culture systems using low density polyethylene (LDPE) culture contact surfaces has created an additional challenge in NS0 (or other cholesterol-sensitive cell lines) processes as LDPE film readily adsorbs cholesterol. This competitive uptake of cholesterol between the NS0 cells and the LDPE film is compensated for by feeding with the ADCF LS1000 cholesterol supplement. Figure 2 demonstrates the rapid decline of an NS0 cell culture in a single-use bioreactor compared with the same cell culture system fed with LS1000.

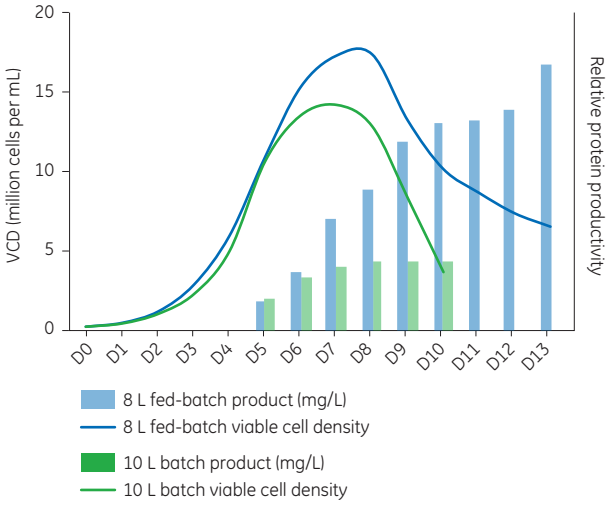

Fig 1. Growth and productivity of CHO-derived cell lines in HyCell CHO medium, comparing batch to fed-batch culture modes in a 10 L bioreactor. Optimized feed was delivered at times indicated.

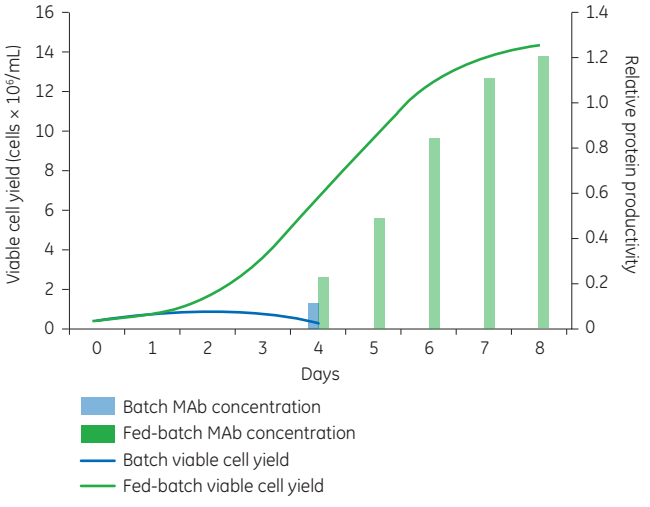

Fig 2. Suspension NS0 cultures in CDM4NS0 medium. Fed-batch culture employed LS1000 feedings on days 1, 3, and 6.

Conclusion

Advantages of the application of Cell Boost supplements in fed-batch cultures shown here represent defined protocols. However, the inherent variability between both individual clones and production platforms often determines the need for specific development and optimization. To support the potential diversity of this work, six Cell Boost supplements are available in powder format, which can be hydrated at a variety of concentrations to support diverse feeding component complements, amounts, and frequencies. The HyClone Cell Boost kit contains 100 g of each of the six Cell Boost products to support individual culture demands and responses to the various formulations.

Reference

Elwood, A., Prince, S., Garner, E., Larsen, J., Manwaring, J., and Wight, M. Determining the optimal feed and feed strategies to enhance protein production of various CHO clones. *BioProcess International Conference, Providence,* RI; Oct. 8-12 (2012).

Ordering information

Product	Product code
Cell Boost 2	SH30596
Cell Boost 5	SH30865
LS1000	SH30554
Cell Boost kit	SH30890
HyCell CHO powder medium	17548911
without L-Glutamine	SH30933.02
HyCell CHO liquid medium without L-Glutamine	SH30934.01
CDM4NS0 powder medium without L-Glutamine	SH30578.02
CDM4NS0 liquid medium without L-Glutamine	SH30579.02

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29142169 AA 08/2015