Improving Protein Quality in CHO cells with a Next-Generation Medium and Feed System Developed Using Multi-Omics and DOE Bioinformatic Analysis

Megan Pajak-Lee, Yoana Hammer, Fatima Zara, Mary Reynolds, Anna-Barbara Hachmann, Paul Gulde, Chengjian Tu, Christopher Brau, Rich Hassett, Ryan Boniface, James W. Brooks
Thermo Fisher Scientific, Research and Development, Grand Island, NY, USA

Thermo Fisher SCIENTIFIC

Bioprocessing

ABSTRACT

Advances in Chinese Hamster Ovary (CHO) cell line development continue to push traditional cell density and titer boundaries. Fed-batch cultures that exceed 6x10^7 cells/mL and 5-10 g/L protein titers are not uncommon. Media and feed systems are expected to support these demanding cell line requirements. To address the evolving bioproduction landscape, we have taken a multi-omics and bioinformatics modeling approach in developing the Gibco™ Efficient-Pro™ Medium and Feeds. We have employed proteomics and metabolomics techniques to characterize the needs of the cells during cell growth and protein production and combined these with traditional approaches of spent media analysis and Design of Experiment (DOE) to develop a next-generation CHO system. For both CHO-S and CHO-K1 cell lines, we were able to improve the glycan profile compared to a competitive fed-batch bioreactor process. Protein aggregation profiles are comparable, and charge variant profiles are improved compared to a benchmark medium and feeds. Both the innovative medium and feeds are available in liquid and easy-to-use Advanced Granulation Technology (AGT™) format and can maximize process efficiency, simplify preparation, and streamline CHO workflows.

INTRODUCTION

CHO cells are the most common mammalian cell line used in the biopharmaceutical industry for the production of therapeutic proteins. Traditional medium and feed development for CHO cells has relied on spent medium analysis to determine the depletion rates of medium components, often limited to basic components (e.g., amino acids and vitamins), which are then added back to the basal medium or incorporated into a feed.

To incorporate novel medium components, lengthy screening experiments are performed to assess toxicity and suitable concentration ranges, followed by several rounds of DOE experiments. These methodologies, although successful, only give basic insights into the metabolic needs of a particular cell line, can be labor intensive, and require significant time to reach a suitable formulation.

Our goal was to develop a next generation medium and feeds for CHO cells. To aid in the development, we have employed a novel and proprietary multi omics-based approach leveraging informatics to design a medium and feeds to target cell-specific metabolic needs. Combining this novel approach with traditional DOE, we optimized the medium and two feeds for CHO-S and CHO-K1 cells. Here, we describe protein quality characteristics of the Efficient-Pro Medium with the Efficient-Pro Feed 1 for CHO-K1 cells and the Efficient-Pro Feed 2 for CHO-S cells.

MATERIALS AND METHODS

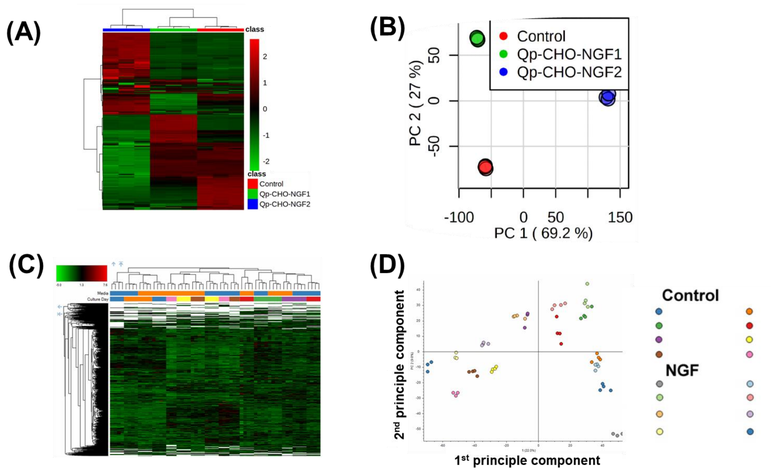
Cell culture: CHO-S and CHO-K1 cells expressing IgG molecules were grown in Efficient-Pro medium and a commercially available benchmark medium supplemented with 6mM L-glutamine (Gibco, 25030) and 1:100 Anti-Clumping Agent (Gibco, 0010057). Culture conditions were maintained at 37˚C, 8% CO_2, 125 rpm. Cell densities and viabilities were measured using a Vi-CELL® counter (Beckman Coulter). Metabolites (glucose, ammonia, lactate) and IgG were measured using a Cedex ® BioHT Analyzer (Roche). Glycan profiles were analyzed with Genetic Analyzer 3500xL (Applied Biosystems ™) and HPLC UltiMate ™ 3000 (Dionex).

Bioreactors: Ambr® 15 bioreactors (Sartorius AG) were seeded at 0.3x10^6 viable cells/mL in triplicate in Efficient-Pro medium and a benchmark medium. Efficient-Pro Feed 1 (3%) was fed on days 3-13, Efficient-Pro Feed 2 (2%) was fed on days 3-13, and the manufacturer recommended feeding protocol was followed for the benchmark feeds on days 3-13. Culture conditions were maintained as follows; pH 7.05 +/- 0.05, 50% DO, 37˚C, and 1200 rpm. Glucose was fed to 6g/L when measured glucose dropped below 3.5g/L.

Multi-Omics: For metabolomic and proteomic analysis, cells were pelleted from cellular suspensions containing approximately 1x10^6 cells via centrifugation at 700xg at 4˚C for 5 minutes. Quenched samples were kept and stored at -80˚C for further analysis. Cells were processed for proteomics using the EasyPep™ Kit (Thermo Scientific™). Metabolomic analysis was performed on a Vanquish™ UHPLC (Thermo Scientific) system coupled with Orbitrap ID-X™ Tribrid™ MS (Thermo Scientific) or Orbitrap Fusion™ Lumos™ (Thermo Scientific). Generated MS raw files were analyzed by Compound Discoverer™ 3.0 (Thermo Scientific) or Proteome Discoverer™ 3.0 (Thermo Scientific). Data were interpreted for feed development using a proprietary bioinformatics process.

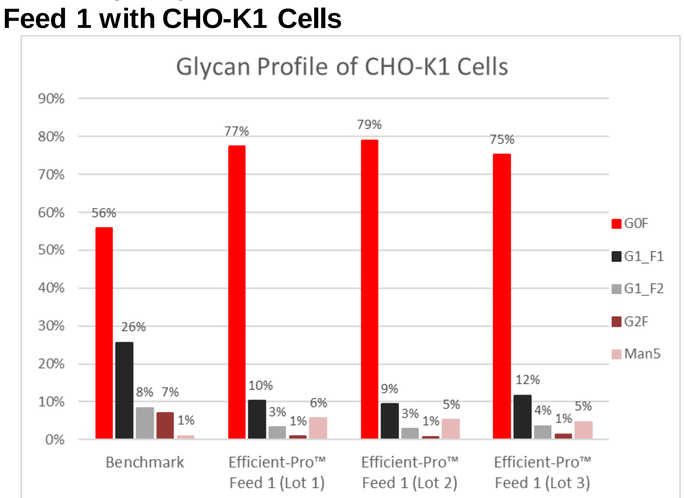
RESULTS

Figure 1. Multi-Omics Analysis



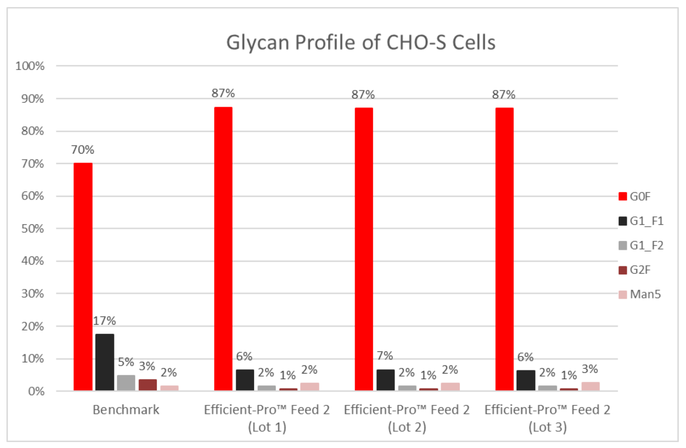
(A,C) Heat maps demonstrating the distribution of metabolites **(A)** and proteins **(C),** that are statistically different among different feeds; their relative abundances were normalized using z-scores and color-coded; clustering analysis was also performed, with distances measured using Euclidean method and clustered using Ward algorithm. **(B,D)** Two-dimensional principal component analysis (PCA) score plot depicting clear differences in the metabolite **(B)** and protein **(D)** profiles among different feeds and across time scales.

Figure 2. Glycosylation Profile with Efficient-Pro Medium and Feed 1 with CHO-K1 Cells



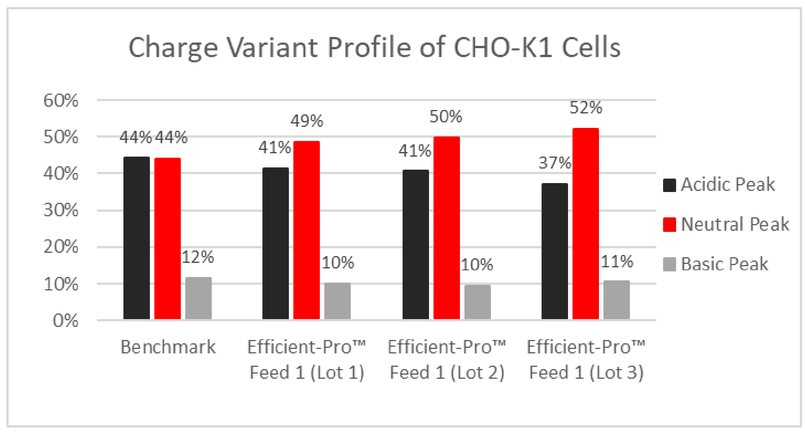
CHO-K1 cells were evaluated in Ambr™ 15 bioreactors. Triplicate reactors were set up to test the Efficient-Pro Medium and Feed 1 with IgG producing CHO-K1 cells. Day 14 glycosylation profile with Efficient-Pro Medium and Feed 1 was compared to a benchmark medium and feed process. Comparisons show the Efficient-Pro Medium and Feed 1 has a 20% increase in less differentiated glycans over the benchmark process.

Figure 3. Glycosylation Profile with Efficient-Pro Medium and Feed 2 with CHO-S Cells



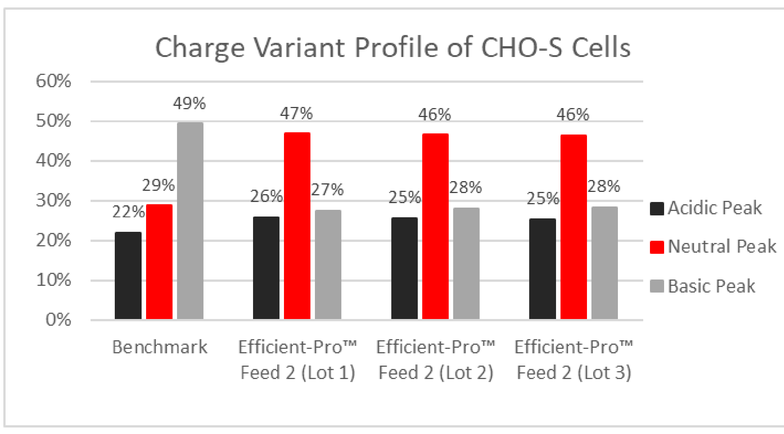
In triplicate, IgG producing CHO-S cells were tested with the Efficient-Pro Medium and Feed 2 using Ambr 15 bioreactors. Day 14 glycosylation profile with Efficient-Pro Medium and Feed 2 was compared to a benchmark medium and feed process. Comparisons show the Efficient-Pro Medium and Feed 2 has a 16% increase in less differentiated glycans over the benchmark process.

Figure 4. Charge Variant Profile with Efficient-Pro Medium and Feed 1 with CHO-K1 Cells



In triplicate, IgG producing CHO-K1 cells were tested with the Efficient-Pro Medium and Feed 1 using Ambr 15 bioreactors. Day 14 charge variant profile with Efficient-Pro Medium and Feed 1 was compared to a benchmark medium and feed process. Comparisons show the Efficient-Pro Medium and Feed 1 has an improved charge variant profile with a larger neutral peak over the benchmark process.

Figure 5. Charge Variant Profile with Efficient-Pro Medium and Feed 2 with CHO-S Cells



CHO-S cells were tested in Ambr 15 bioreactors. Triplicate reactors were set up to test the Efficient-Pro Medium and Feed 2 with IgG producing CHO-S cells. Day 14 charge variant profile with Efficient-Pro Medium Feed 2 was compared to a benchmark medium and feed process. Comparisons show the Efficient-Pro Medium and Feed 2 has an improved charge variant profile with a larger neutral peak over the benchmark process.

Table 1. Aggregation Profile with Efficient-Pro Medium and Feeds

Cell Line	Condition	HMW (High Molecular Weight)	MAIN PEAK	LMW (Low Molecular Weight)
CHO-K1	Benchmark	2.1%	94.7%	3.1%
	Efficient-Pro Feed 1 (Lot 1)	1.3%	96.4%	2.3%
	Efficient-Pro Feed 1 (Lot 2)	1.2%	96.5%	2.3%
	Efficient-Pro Feed 1 (Lot 3)	1.7%	95.9%	2.4%
CHO-S	Benchmark	3.6%	94.5%	1.9%
	Efficient-Pro Feed 2 (Lot 1)	2.5%	95.1%	2.3%
	Efficient-Pro Feed 2 (Lot 2)	2.5%	95.1%	2.4%
	Efficient-Pro Feed 2 (Lot 3)	1.9%	95.2%	2.9%

Both CHO-K1 and CHO-S cells were tested in Ambr 15 bioreactors. Triplicate reactors were set up to test the Efficient-Pro Medium and Feeds with IgG producing CHO cells. Day 14 aggregate profiles with both Efficient-Pro Medium and Feeds were compared to a benchmark medium and feed process. Comparisons show that the Efficient-Pro Medium and Feeds have a comparable aggregate profile to the benchmark process.

CONCLUSIONS

Development of the Efficient-Pro Medium and Feeds for CHO cells utilized traditional medium development approaches (component screenings and DOE), combined with novel proprietary multi-omics analysis, enabling more rapid optimization. The differences between CHO-S and CHO-K1 cells presented the need to develop cell line specific feeds. Thus, two next generation feed formulations were developed, Efficient-Pro Feed 1 designed for use with CHO-K1 cells and Efficient-Pro Feed 2 designed for use with CHO-S cells.

The Efficient-Pro Medium and Feed 1, when used with CHO-K1 cells, shifted the overall IgG glycan profile to less mature glycans compared to the benchmark process, allowing for more customized differentiation. Similarly, the Efficient-Pro Medium and Feed 2, when used with CHO-S cells, also shifted the overall IgG glycan profile to less differentiated glycans compared to the benchmark process. Further, the IgG charge variant profile is improved with both Efficient-Pro Feeds, indicating a more stable molecule.

REFERENCES

Lakshmanan M *et al.* Multi-omics profiling of CHO parental hosts reveals cell line-specific variations in bioprocessing traits. Biotechnol bioeng. 2019 Sep; 116(9):2117-2129. doi: 10.1002/bit.27014. Epub 2019 May 20. PMID: 31066037.

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TRADEMARKS

megan.pajak-lee@thermofisher.com

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