# Improving Protein Titers in CHO Cells with a Next Generation Medium and Feed System

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

Thermo Fisher SCIENTIFIC

Bioprocessing

## ABSTRACT

Feed supplementation is essential for efficient therapeutic protein production with Chinese Hamster Ovary (CHO) cells. Advances in cell line engineering have resulted in higher producing clones that demand higher performing feeds. With more molecules being used for commercial development, clonal diversity creates an additional challenge. Here, we have employed a multi-omics approach to develop a medium and feed for high performing CHO cell lines. Using proteomics and metabolomics analyses, combined with traditional approaches of spent media analysis and Design of Experiment (DOE), we have developed a CHO medium as well as feeds that are designed for CHO-S and CHO-K1 cell lines. The next generation CHO medium and feed supports higher specific productivity compared to a commercially available benchmark medium and feed process. Compared to a competitive fed-batch bioreactor process, the next generation CHO medium and feed system improved protein titers up to 210% with CHO-K1, CHO-K1 GS, DG44, and CHO-S cell lines. The higher specific productivity is expected to improve protein quality and downstream purification. The medium and feed system is chemically defined and available in a ready to use liquid format and easy to reconstitute Advanced Granulation Technology (AGT™) format for large scale bioproduction applications.

## INTRODUCTION

CHO cells are the predominant cell line used in the biopharmaceutical industry for the production of therapeutic proteins. Traditional medium and feed development for CHO cells has relied heavily 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 modified in the basal medium or the feed.

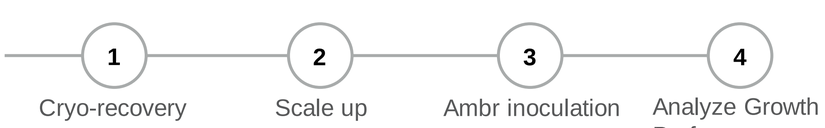
To incorporate novel medium components, screening experiments are performed to assess toxicity and suitable concentration ranges. Then, novel components can be incorporated into several rounds of DOE experiments. These methodologies, although successful, only give basic insights into the metabolic needs 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, Efficient-Pro™ Feed 1 for CHO-K1 cells and Efficient-Pro™ Feed 2 for CHO-S cells. Here, we describe the protein titer and metabolite profiles of the Efficient-Pro™ Medium with the Efficient-Pro Feeds.

## MATERIALS AND METHODS

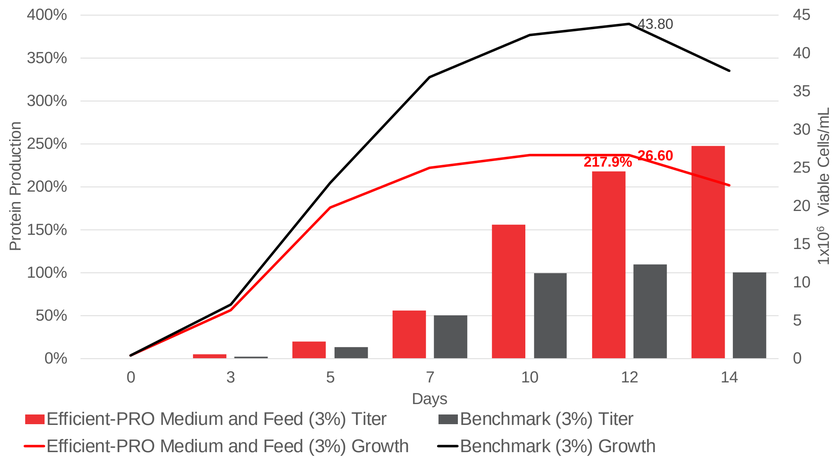
**Cell culture**: CHO-S, CHO-K1, and DG44 cells expressing IgG molecules were grown in Efficient-Pro medium and a commercially available benchmark medium supplemented with 6mM L-glutamine (Gibco™, 25030) and 1% Anti-Clumping Agent (Gibco, 0010057). Culture conditions were maintained at 37˚C, 8% $CO_2$, 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).

**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 was fed at 3% for CHO-K1 on days 3-13. Efficient-Pro Feed 2 was fed on days 3-13 at 2.5% for ExpiCHO-S and at 1.5% for DG44 cells. 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 at 6g/L when measured glucose dropped below 3.5g/L.



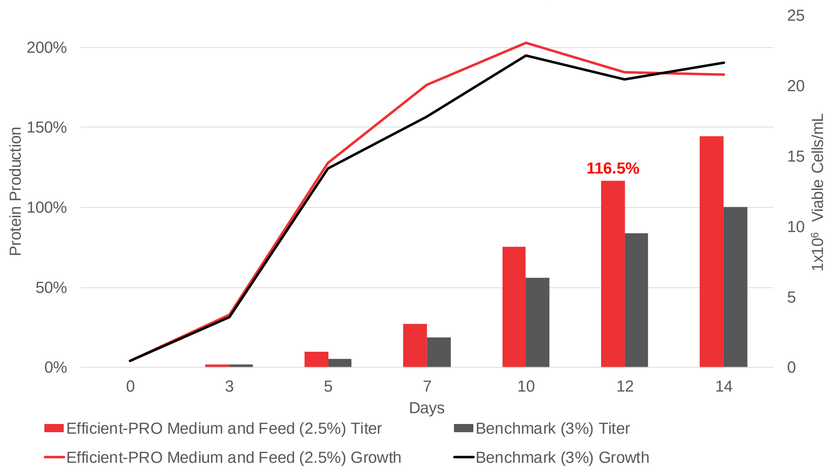

## RESULTS

**Figure 1. Protein Production and Viable Cell Density with Efficient-Pro Medium and Feed 1 with CHO-K1 Cells**



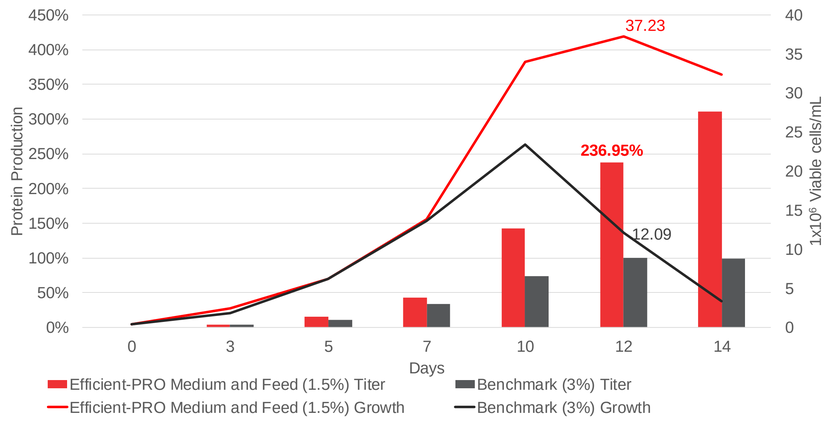

CHO-K1 cells were evaluated in Ambr 15. Bioreactor vessels were set up in triplicates to test Efficient-Pro Medium and Feed 1 for IgG production over 14 days. Efficient-Pro Feed 1 was compared to a commercially available benchmark medium and feed process. Results show that Efficient-Pro Feed 1 enhanced protein production by 147% compared to the benchmark. Efficient-Pro Medium and Feed 1 promoted high viability and lower cell density indicating a higher specific productivity compared to the benchmark medium and feed.

**Figure 2. Protein Production and Viable Cell Density with Efficient-Pro Medium and Feed 2 with ExpiCHO-S Cells**



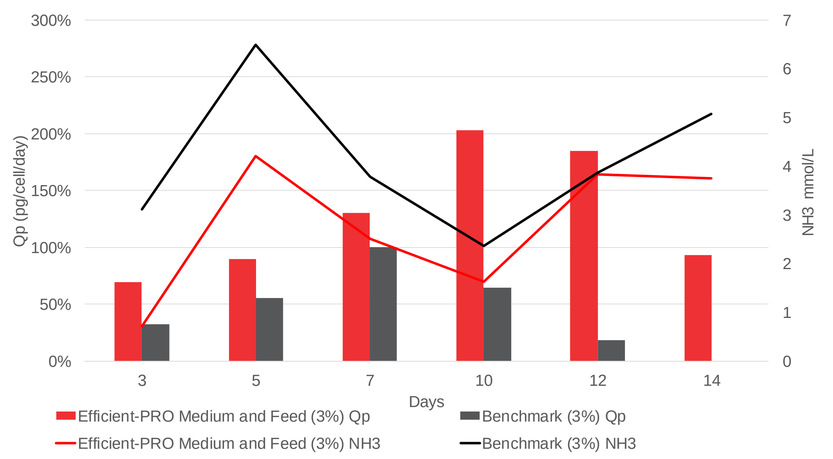

ExpiCHO-S cells were tested in Ambr 15 bioreactors. Triplicate reactors were set up to evaluate Efficient-Pro Feed 2 with ExpiCHO-S cells for IgG production over 14 days. Efficient-Pro Feed 2 resulted in 44% higher protein titer over the benchmark process and maintained viability above 90% during the 14 days with comparable cell density to the benchmark process.

**Figure 3. Protein Production and Viable Cell Density with Efficient-Pro Medium and Feed 2 with DG44 Cells**



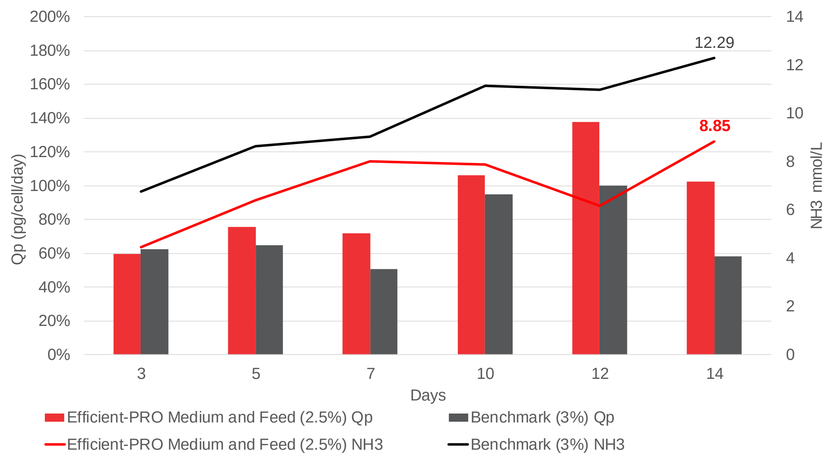

DG44 cells were tested in Ambr 15 bioreactors in triplicate. Efficient-Pro Medium and Feed 2 were evaluated over 14 days. Compared to a benchmark medium and feed process, Efficient-Pro medium and Feed 2 showed 210% higher titer. Efficient-Pro Feed 2 maintained viability above 90% during the 14 days and promoted higher cell density compared to the benchmark process.

**Figure 4. Specific Productivity and Ammonia Accumulation with Efficient-Pro Medium and Feed 1 with CHO-K1 Cells**



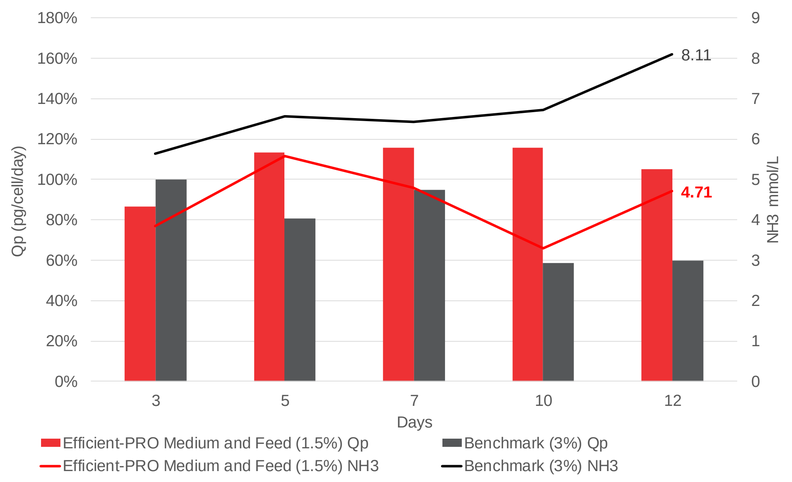

CHO-K1 cells were evaluated in Ambr 15 bioreactors. with Efficient-Pro Medium and Feed 1 and a benchmark medium and feed process. Efficient-Pro Feed 1 promotes a higher specific productivity and lower ammonia accumulation over 14 days.

**Figure 5. Specific Productivity and Ammonia Production with Efficient-Pro Medium and Feed 2 with ExpiCHO-S Cells**




ExpiCHO-S cells were tested in Ambr 15 bioreactors with Efficient-Pro Medium and Feed 2 and a benchmark medium and feed. Efficient-Pro Feed 2 promoted a higher specific productivity and lower ammonia levels compared to the benchmark process.

**Figure 6. Specific Productivity and Ammonia Accumulation with Efficient-Pro Medium and Feed 2 with DG44 Cells**




Efficient-Pro Medium and Feed 2 were compared to a benchmark medium and feed process in Ambr 15 bioreactors. Efficient-Pro Feed 2 promoted higher specific productivity and produced less ammonia over the 14-day experiment.

## CONCLUSIONS

Efficient-Pro Feed 1, when used with CHO-K1 cells, improved protein production compared to a similar benchmark process by 146.9%, with lower cell density, indicating a higher specific productivity.

Efficient-Pro Feed 2, when used with ExpiCHO-S cells, enhanced protein production by 44% over the benchmark process with comparable cell density. Lower ammonia accumulation aligned with higher performance of Efficient-Pro Feed 2 and higher viability, above 90%, during the 14-day performance assay.

Lower ammonia accumulation was also observed with DG44 cells fed with Efficient-Pro Feed 2, which can positively impact cell viability and protein quality.

**Efficient-Pro Medium and Feed Features:**

- Chemically defined medium and feeds
- Available in liquid and AGT format
- Recommended daily feed additions: 1.5 to 3%
- High specific productivity

## 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. PMID: 31066037.

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## TRADEMARKS



Yoana.hammer@thermofisher.com

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