Bioproduction

Efficient-Pro Medium and Feeds improve productivity in CHO-K1, CHO-S, and DG44 cells

Introduction

Chinese hamster ovary (CHO) cells are the primary cell line for production of biotherapeutic proteins, accounting for up to 70% of recombinant proteins [1]. These cells are the workhorse for bioproduction because they allow for human-like posttranslational modifications and are not susceptible to infection by human viruses [2]. Year after year, biopharmaceutical manufacturers have successfully driven their CHO cells to deliver increased productivity. Despite these advances, the industry continues to strive toward maximizing productivity by streamlining manufacturing processes to reduce costs. To maximize productivity and reduce development time and costs, CHO workflows in the early stages of development require a higher-performing chemically defined (CD) platform with basal media and feeds optimized for specific CHO cell lines.

To help provide solutions for the bioproduction industry, the Gibco™ Efficient-Pro™ Medium and Gibco™ Efficient-Pro™ Feeds 1 and 2 were developed using a traditional and multiomics modeling approach to improve recombinant protein production in CHO cells. The CD Efficient-Pro Medium is supplemented with either of the corresponding single-part feeds designed for optimal performance with specific CHO cell lines. Efficient-Pro Feed 1 is designed primarily for CHO-K1 cells and Efficient-Pro Feed 2 for CHO-S and DG44 cells. The performance of Efficient-Pro Medium and Feeds was evaluated using CHO-K1, CHO-S, and DG44 cells and compared to another supplier's commercially available basal medium and 2-part feed. The cell lines were evaluated in a 14-day fed-batch IgG productivity study with cell growth, viability, titer, and specific productivity assessed at multiple time points. In addition, critical metabolites that are known to impact cell health were evaluated.

Materials and methods

Cell culture

In-house CHO-K1, CHO-S, and DG44 cells expressing IgG were recovered in banking medium, then adapted for a minimum of 3 passages in each test basal medium in shake flasks. Cultures were expanded and set up in triplicate for each condition in an Ambr™15 bioreactor (Sartorius), with a seeding density of 0.3 x 10^6 viable cells/mL. Efficient-Pro Medium (Cat. No. A5322201) and another supplier's basal medium were supplemented with 6 mM L-glutamine and 1:100 Gibco™ Anti-Clumping Agent (Cat. No. 0010057). Culture conditions were set at pH 7.05, 50% DO, 37°C, and 1,200 rpm for all cell lines, except CHO-K1, which was cultured at 1,000 rpm on days 1–9 and 1,200 rpm on days 10–14. The cultures were supplemented daily on days 3–13 with either Efficient-Pro Feed 1 (Cat. No. A5208801), Efficient-Pro Feed 2 (Cat. No. A5221404), or the other supplier's two feeds, as outlined in Table 1. Glucose was fed to 6 g/L when the concentration dropped below 3.5 g/L. Cell counts and viability were evaluated using a Vi-CELL™ XR Analyzer (Beckman Coulter).

Table 1. Feed supplementation strategy.

	CHO-K1	CHO-S	DG44
Efficient-Pro Feed	3% Feed 1	2.5% Feed 2	1.5% Feed 2
Other supplier's feeds	3% Feed A and 0.3% Feed B	3% Feed A and 0.3% Feed B	3% Feed A and 0.3% Feed B

Note: Cultures were supplemented daily on days 3–13. The recommended daily concentration of Efficient-Pro Feed ranges from 1.5% to 3% and can be optimized depending on the specific nutritional requirements of each cell line. The other supplier's feed concentrations were based on the manufacturer's recommendations.

Titer, metabolites, and specific productivity

Antibody titers, lactate, and ammonia metabolites were assessed using a Cedex™ BioHT Analyzer (Roche). Specific productivity (qP) was calculated as IgG produced, in pg/cell/day, based on Equation 1, where $[IgG_{t0}]$ and $[IgG_{t1}]$ represent the IgG product concentration on t0 or t1, and VCD_{t0} and VCD_{t1} represent the total viable cell number on t0 or t1, with t representing time in days.

Equation 1

$$qP = ([IgG_{t1}] - [IgG_{t0}]) / ((VCD_{t1} - VCD_{t0}) / \ln(VCD_{t1}/VCD_{t0}) \times (t1 - t0))$$

Results

Cell growth and productivity

This study showed that the Efficient-Pro Medium and Feeds demonstrated comparable or improved titer and higher specific productivity for the CHO-K1, CHO-S, and DG44 cells compared to the other supplier's medium and feeds. The CHO-K1 cells with Efficient-Pro Medium and Feed 1 presented a comparable relative average titer by day 14 and improved relative average qP up to 170%, compared to the other supplier's medium feed (Figure 1). The CHO-S cells cultured with Efficient-Pro Medium and Feed 2 showed a 144% relative titer on day 14 and up to 180% relative average qP (Figure 2). Lastly, the DG44 cells with Efficient-Pro Medium and Feed 2, demonstrated a 238% average relative titer on day 12 and up to a 168% relative average qP (Figure 3).

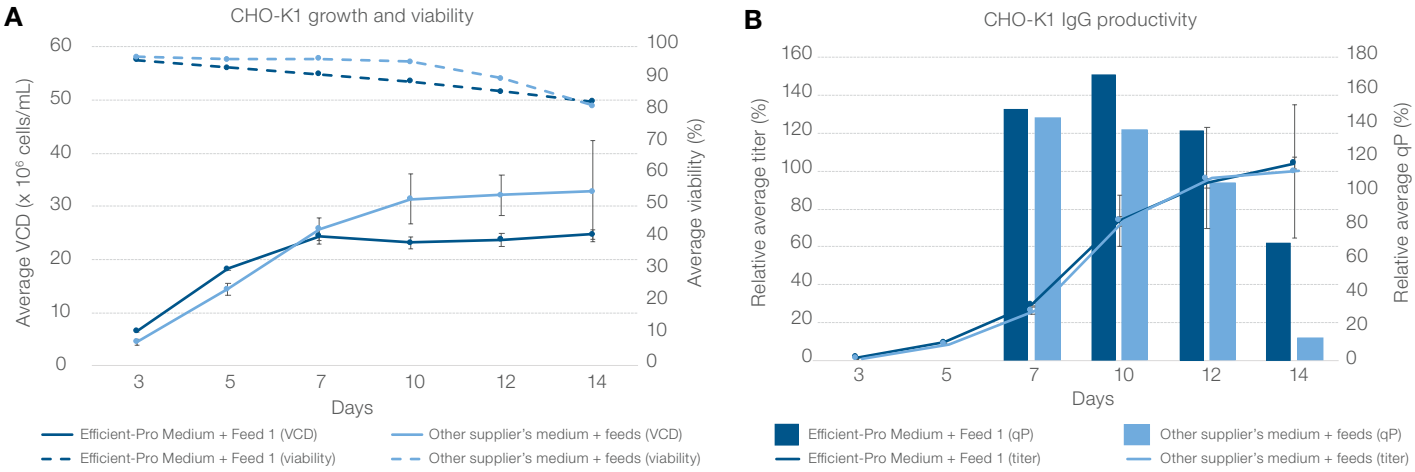

Figure 1. CHO-K1 growth and productivity. (A) CHO-K1 cells had comparable average viability when grown with Efficient-Pro Medium and Feed 1 or with another supplier's medium and feeds, but lower average peak VCD with the Efficient-Pro medium and feed. **(B)** By day 14, relative average titers were comparable in both media, but relative average qP was higher, up to 170%, with the Efficient-Pro medium and feed. Percent relative average titer was based on the other supplier's day 14 average peak titer. Percent relative average qP was based on the other supplier's combined average qP for days 7, 10, 12, and 14. The other supplier's system showed high variability in titer at days 12 and 14 (error bars).

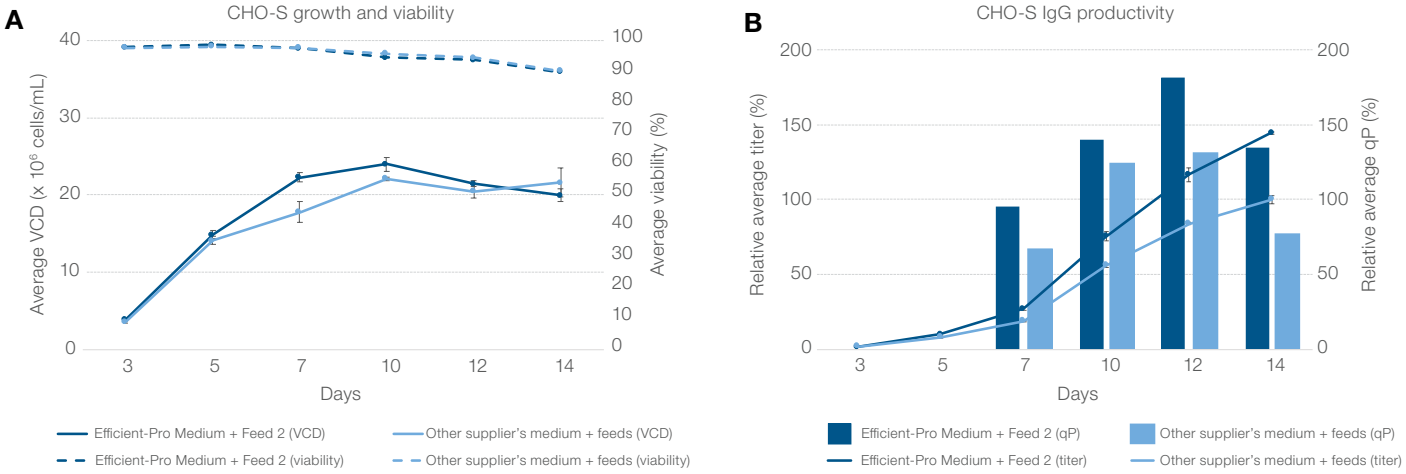

Figure 2. CHO-S growth and productivity. (A) CHO-S cells had comparable average cell viability when grown with Efficient-Pro Medium and Feed 2 or with another supplier's medium and feeds, and higher average peak VCD with the Efficient-Pro medium and feed. **(B)** The Efficient-Pro system produced a 144% relative average titer by day 14, and up to 180% relative average qP. Percent relative average titer was based on the other supplier's day 14 average peak titer. Percent relative average qP was based on the other supplier's combined average qP for days 7, 10, 12, and 14.

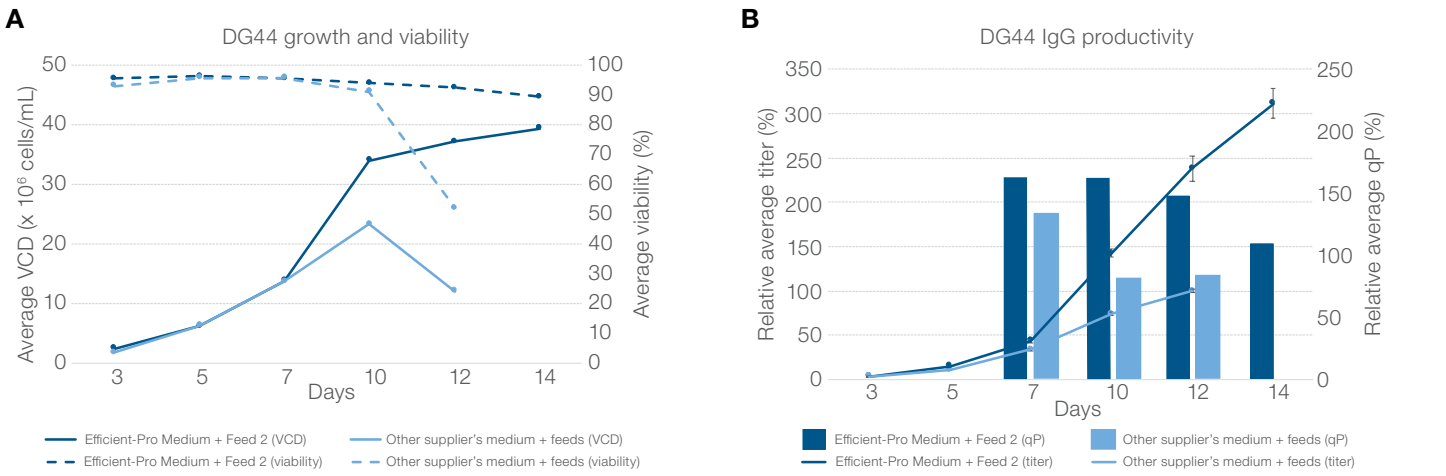

Figure 3. DG44 growth and productivity. (A) DG44 cells grown with Efficient-Pro Medium and Feed 2 had sustained average viability of ≥90% beyond day 12 and higher average VCD than cultures grown with the other supplier's medium and feed, which did not sustain viability beyond day 12. **(B)** The cells grown in the Efficient-Pro medium and feed had a 238% relative average titer by day 12 and 168% relative average qP. Percent relative average titer was based on the other supplier's day 12 average peak titer. Percent relative average qP was based on the other supplier's combined average qP for days 7, 10, and 12, due to cell viability being <60% on day 12.

Metabolites

As shown in Table 2, end-of-culture metabolite analysis of CHO-K1, CHO-S, and DG44 cells showed similarly low levels of ammonia and lower levels of lactate with the Efficient-Pro Medium and Feeds than with the other supplier's system.

Table 2. Key metabolite levels.

	Average ammonia level (mmol/L)		Average lactate level (g/L)	
Cell line	Efficient-Pro system	Other supplier's system	Efficient-Pro system	Other supplier's system
CHO-K1	7	5	1	5
CHO-S	8	11	0	2
DG44	5	7	2	4
Overall	6	8	1	4

Note: Averages for CHO-K1 and CHO-S were based on days 10, 12, and 14 for Efficient-Pro and the other supplier's medium and feed. Averages for DG44 were based on days 10, 12, and 14 for Efficient-Pro Medium and day 10 and 12 for the other supplier's medium and feed, due to <60% viability observed with the other supplier's system.

Conclusions

The results of this study demonstrate that relative to the other supplier's medium and feeds, the Efficient-Pro Medium, with either Efficient-Pro Feed 1 or Feed 2, can support stronger titers of up to 238%, along with improved specific productivity of up to 180%, in key bioproduction CHO cell lines. The Efficient-Pro system was designed using a traditional and multiomics design of experiment (DOE) approach, which resulted in relatively low levels of potentially toxic metabolite production, sustained culture health, and improved productivity. Following these studies, productivity enhancement was shown with a DXB11 cell line (data not shown), indicating the Efficient-Pro system can support improved performance of additional CHO cell lines.

The Efficient-Pro Medium and Feeds system provides an innovative and commercially available platform that can enhance process productivity and reduce your development time, thereby enabling potential reductions in manufacturing costs and accelerating a product's time-to-market. Additionally, the Efficient-Pro Medium and Feeds are available in multiple liquid and Gibco™ Advanced Granulation Technology™ (AGT™) dry formats to streamline CHO workflow transitions from development to commercialization scale-up.

References

1. Zhu MM, Mollet M, Hubert RS, Kyung YS, Zhang GG (2017) Industrial Production of Therapeutic Proteins: Cell Lines, Cell Culture, and Purification. *Handbook of Industrial Chemistry and Biotechnology.* pp 1639-1669. Published 2017 May 3. doi:10.1007/978-3-319-52287-6_29.
2. Li W, Fan Z, Lin Y, Wang TY (2021). Serum-Free Medium for Recombinant Protein Expression in Chinese Hamster Ovary Cells. *Front Bioeng Biotechnol* 9:646363. doi:10.3389/fbioe.2021.646363.

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