Bioproduction

Efficient-Pro Medium and Feeds deliver strong product quality while enhancing productivity in CHO-K1 and CHO-S cells

Introduction

Once a therapeutic antibody is identified and successfully produced at development scales, manufacturers often consider enhancing productivity as the next challenge for scale-up. Although the importance of strong productivity is undeniable, a more crucial challenge can be delivering sustained or improved product quality with productivity enhancements. Having consistent product quality is essential to ensure patient safety and product efficacy. Impurities can affect downstream purification by requiring additional processes and reducing product yields. Therefore, manufacturers are placing greater emphasis on optimizing upstream processes with the goals of minimizing impurities, improving product quality, and decreasing time to market [1]. The early identification of a robust, commercially available, optimized platform medium and cell line–specific feed could be the keys to achieving strong productivity with sustained or improved product quality and delivering product to market more rapidly.

The Gibco™ Efficient-Pro™ Medium and Efficient-Pro™ Feed 1 for CHO-K1 cells and Efficient-Pro™ Feed 2 for CHO-S cells were developed to help address these productivity and quality challenges. To assess the capability of the Efficient-Pro system to deliver improved productivity with strong product quality, 14-day fed-batch microbioreactor studies were conducted. CHO-K1 and CHO-S cell lines producing IgG were cultured with the Efficient-Pro system or another supplier's commercially available medium and two-part feed system. The resulting cell growth, viability, productivity, and product quality attributes including aggregation and fragmentation, charge variants, and N-glycan profiles were evaluated and compared between the two media and feed systems.

Materials and methods

Cell culture

In-house CHO-K1 and CHO-S cells expressing IgG were recovered in banking medium, then adapted in shake flasks for 3 to 6 passages in each test basal medium. Cultures were expanded and set up in triplicate for each condition with a seeding density of 0.3 x 10^6 viable cells/mL in an Ambr™15 microbioreactor (Sartorius). Efficient-Pro Medium (Cat. No. A5322201) and the other supplier's basal medium were supplemented with 6 mM Gibco™ L-Glutamine (Cat. No. 25030149) and 1:100 Gibco™ Anti-Clumping Agent (Cat. No. 0010057AE). The culture parameters of the Ambr 15 microbioreactor were pH 7.05, 50% DO, 37°C, and 1,200 rpm for both CHO-K1 and CHO-S cell lines. Cultures were supplemented daily from days 3 to 13 with 3% Efficient-Pro Feed 1 (Cat. No. A5208801) for CHO-K1 cells and with 2% Efficient-Pro Feed 2 (Cat. No. A5221401) for CHO-S cells. For both cell lines, the other supplier's two-part feed was supplemented daily, from days 3 to 13 at 3% and 0.3%, based on the supplier's recommendation. All cultures were fed glucose to 6 g/L when the measured concentration dropped below 3.5 g/L. Cell counts and viability were evaluated using a Vi-CELL™ XR Analyzer (Beckman Coulter).

(**Note:** The recommended daily feed concentration of Efficient-Pro Feeds 1 and 2 typically ranges from 1.5% to 3% and can be optimized depending on the specific nutritional requirements of each cell line.)

Titer and specific productivity

Antibody titers were assessed using a Cedex™ Bio HT 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 at t0 or t1, and VCD_{t0} and VCD_{t1} represent the total viable cell number at t0 or t1, with t representing time in days.

Equation 1:

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

Protein purification

IgG was purified from supernatant samples using a Thermo Scientific™ POROS™ MabCapture™ A Select Resin. Purified protein samples were buffer exchanged into 20 mM phosphate buffer using Thermo Scientific™ Zeba™ Spin Desalting Plates, 7K MWCO.

Aggregation and fragmentation

Purified IgG samples in phosphate buffer were analyzed for aggregation and fragmentation on a Thermo Scientific™ UltiMate™ 3000 Rapid Separation Dual System with a UV detector using a size-exclusion chromatography (SEC) column and 150 mM phosphate buffer.

Charge variants

Purified IgG samples in 20 mM phosphate buffer were analyzed for charge variants on an UltiMate 3000 Rapid Separation Dual System with a UV detector using a Thermo Scientific™ MAbPac™ SCX-10 column and a Thermo Scientific™ pH Gradient Buffer.

N-glycans

N-linked glycans were prepared from purified IgG in 20 mM phosphate buffer using the Applied Biosystems™ GlycanAssure™ HyPerformance APTS Kit as described in the user guide. The labeled N-glycans were analyzed by capillary electrophoresis (CE) separations performed using the Applied Biosystems™ 3500xL Genetic Analyzer for Protein Quality Analysis.

Results

Growth and productivity

CHO-K1 cells grown in Efficient-Pro Medium and Feed 1 had an average cell viability above 80% to day 14, whereas cell viability using the other supplier's system was lower at an average of 73% (data not shown). Despite lower cell growth with the Efficient-Pro system (data not shown), a comparable titer and 159% average qP was demonstrated relative to the other supplier's system. Additionally, with the other supplier's system, 10% to 15% titer variability was observed later in culture, as well as a decline in the relative average qP. For the Efficient-Pro system, less than 3% titer variability was observed throughout the culture period with a sustained stronger relative average qP (Figure 1).

CHO-S cells grown in Efficient-Pro Medium and Feed 2 demonstrated comparable average cell viability above 80% to day 14 and a 146% peak viable cell density relative to that exhibited with the other supplier's system (data not shown). For the Efficient-Pro system, the relative average titer was 154% and the relative average qP was as high as 142% by day 14 compared to cells grown in the other supplier's media and two-part feed system. Titer variability was low for both systems (Figure 1).

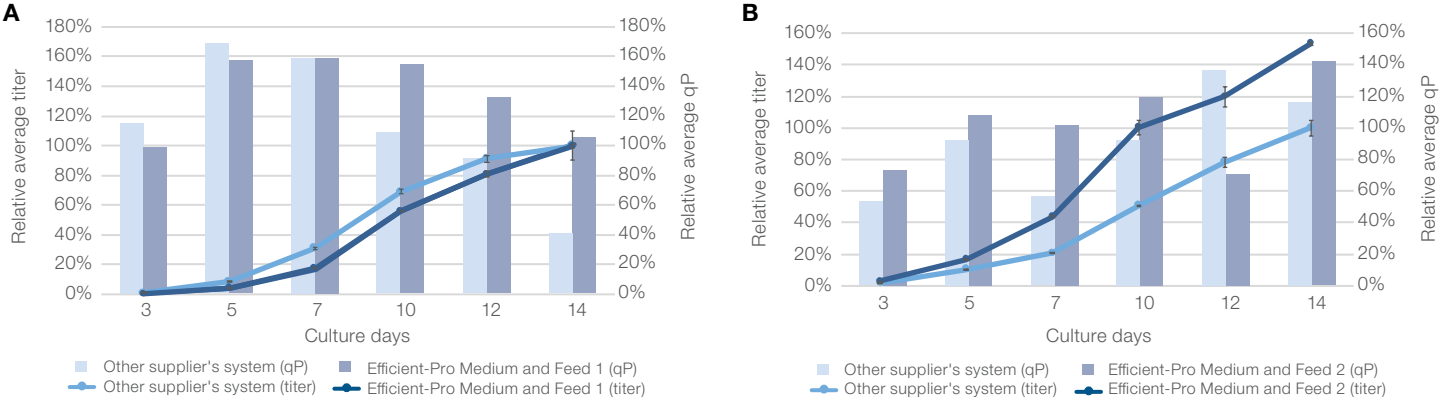

Figure 1. Titer and specific productivity for CHO-K1 and CHO-S cells. (A) CHO-K1 cells grown in Efficient-Pro Medium and Feed 1 demonstrated a comparable relative average titer by day 14 and up to 159% relative average qP when evaluated against the productivity using the other supplier's system. Higher titer variability of 10% to 15% and a decline in qP was observed for the other supplier's system. The Efficient-Pro system resulted in less than 3% titer variability and strong qP values to day 14. **(B)** CHO-S cells grown in Efficient-Pro Medium and Feed 2 produced a 154% relative average titer by day 14 and up to a 142% relative average qP compared with using the other supplier's medium and feeds. (Percent relative average titer was based on the day 14 average titer with the other supplier's system. Percent relative average qP was based on the other supplier's average qP for days 7, 10, 12, and 14.)

Aggregation and fragmentation

The average main peak areas for IgG produced from CHO-K1 and CHO-S cells were comparably high (ranging from 94.5% to 95.6%) when cultured with the Efficient-Pro system or the other supplier's system (Figure 2). For both cell lines, both systems generated comparably low levels of IgG aggregates, with an average high molecular weight (HMW) peak area ranging from 1.8% to 3.6%. For the Efficient-Pro system and the other supplier's system, low levels of fragmentation were also observed for both cell lines with an average low molecular weight (LMW) peak area ranging from 1.9% to 2.9%.

Charge variants

Charge variant analyses of IgG produced by CHO-K1 and CHO-S cells revealed stronger neutral peaks using the Efficient-Pro system compared to the other supplier's system (Figure 3). Using the other supplier's system, relatively higher levels of acidic or basic charge variants were observed.

CHO-K1 cells grown in Efficient-Pro Medium and Feed 1 generated antibodies with an average neutral peak area of 52%, an average acidic peak area of 37%, and an average basic peak area of 10%. IgG produced using the other supplier's system had 39% neutral, 55% acidic, and 6% basic average peak areas. CHO-S cells grown in Efficient-Pro Medium and Feed 2 produced IgG with an average neutral peak area of 46%, an average acidic peak area of 25%, and an average basic peak area of 28%. The other supplier's system yielded IgG with a 29% average neutral peak area, an average acidic peak area of 22%, and an average basic peak area of 49%.

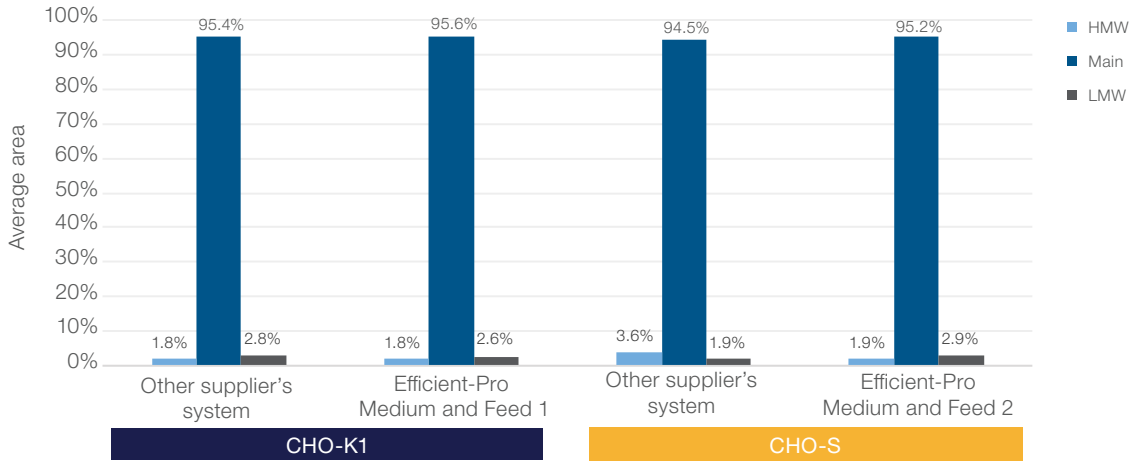

Figure 2. Aggregation and fragmentation for CHO-K1 and CHO-S cells. Using the Efficient-Pro Medium and Feeds or the other supplier's system, CHO-K1 and CHO-S cells produced main peaks with comparably high average areas ranging from 94.5% to 95.6%, low levels of aggregation with HMW peaks with average areas ranging from 1.8% to 3.6%, and low fragmentation with LMW peaks with average areas ranging from 1.9% to 2.9%.

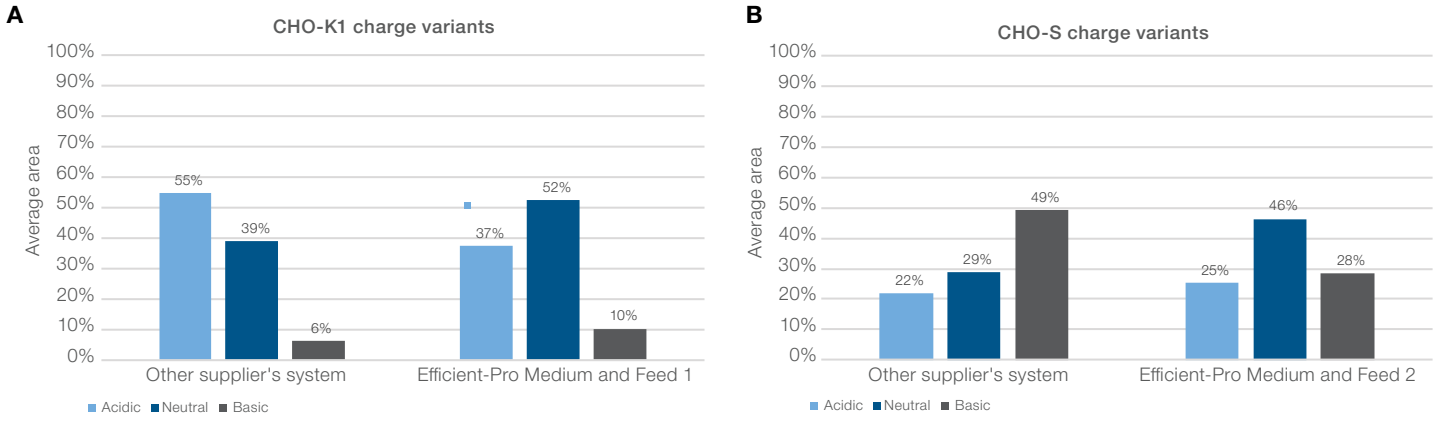

Figure 3. Charge variants for CHO-K1 and CHO-S cells. (A) CHO-K1 cells grown in Efficient-Pro Medium and Feed 1 had an IgG charge variant profile of 37% acidic, 52% neutral, and 10% basic average peak areas. Cells grown with the other supplier's system had an IgG charge variant profile of 55% acidic, 39% neutral, and 6% basic average peak areas. **(B)** CHO-S cells grown in Efficient-Pro Medium and Feed 2 had an IgG charge variant profile of 25% acidic, 46% neutral, and 28% basic average peak areas. Cells grown with the other supplier's system had an IgG charge variant profile of 22% acidic, 29% neutral, and 49% basic average peak areas.

N-glycan profiles

Evaluation of the N-glycan structures of IgG revealed that CHO-K1 and CHO-S cells grown in the Efficient-Pro Medium and Feeds generated higher levels of less differentiated G0F structures (Figure 4). IgG produced using the other supplier's system showed lower levels of G0F and more differentiated G1F1, G1F2, and G2F structures.

CHO-K1 cells grown in Efficient-Pro Medium and Feed 1 generated IgG with structures with the following average areas: 75% G0F, 12% G1F1, 4% G1F2, 2% G2F, and 4% Man5. CHO-K1 cells grown with the other supplier's system had structures with the following average areas: 56% G0F, 26% G1F1, 8% G1F2, 7% G2F, and 1% Man5. CHO-S cells grown in Efficient-Pro Medium and Feed 2 produced IgG structures with the following average areas: 87% G0F, 6% G1F1, 2% G1F2, 1% G2F, and 3% Man5. CHO-S cells grown with the other supplier's system had structures with the following average areas: 70% G0F, 17% G1F1, 5% G1F2, 3% G2F, and 2% Man5.

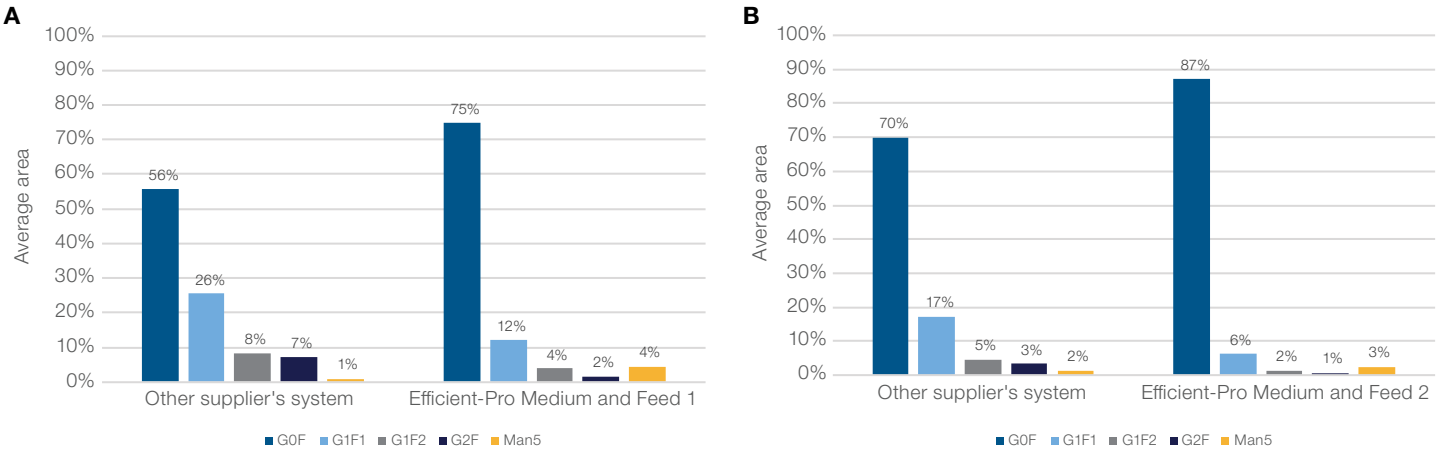

Figure 4. N-glycans of IgG produced from CHO-K1 and CHO-S cells. (A) CHO-K1 cells grown in Efficient-Pro Medium and Feed 1 yielded IgG with average areas of 75% G0F, 12% G1F1, 4% G1F2, 2% G2F, and 4% Man5. Cells grown with the other supplier's system produced IgG with average areas of 56% G0F, 26% G1F1, 8% G1F2, 7% G2F, and 1% Man5. **(B)** CHO-S cells grown in Efficient-Pro Medium with Feed 2 produced IgG with average areas of 87% G0F, 6% G1F1, 2% G1F2, 1% G2F, and 3% Man5. Cells grown with the other supplier's system generated IgG with average areas of 70% G0F, 17% G1F1, 5% G1F2, 3% G2F, and 2% Man5.

Conclusions

The fed-batch studies conducted alongside another supplier's system demonstrated that the Efficient-Pro system provided IgG-specific productivity enhancements of up to 159% for CHO-K1 and 142% for CHO-S cells. Product quality assessments also revealed potential benefits of using the Efficient-Pro system.

Although aggregation and fragmentation assessments showed comparably high main peaks with low levels of aggregation and fragmentation for both systems, IgG charge variant analyses indicated that the Efficient-Pro system supported stronger neutral peaks and lower levels of acidic and basic charge variants. Lower levels of aggregation, fragmentation, and charge variant impurities may potentially reduce downstream purification requirements and improve product yields.

Additionally, for both cell lines, IgG produced with the Efficient-Pro system demonstrated higher levels of less differentiated G0F N-glycan structures that may provide greater potential for N-glycan modulation to a specific desired profile.

As production demands increase and processes are scaled-up, the Efficient-Pro Medium and cell line–specific Efficient-Pro Feed 1 or 2 show strong potential to provide manufacturers with the needed solutions to achieve stronger productivity and maintain or improve product quality.

Reference

1. Xu J, Ou J, McHugh KP et al. (2022) Upstream cell culture process characterization and in-process control strategy development at pandemic speed. *mAbs* 14(1):2060724. https://doi.org/10.1080/19420862.2022.2060724.

Learn more at **thermofisher.com/efficient-pro**