Clone-Specific Optimization in CHO: Leveraging OPM Media, Feeds, and Engineered Clones Across Multiple Hosts Panel

Pargol Hashemi, Ph.D.
R&D Senior Scientist I

Abstract

Process performance in CHO is highly clone dependent, and the optimal medium-feed combination can differ substantially even between commonly used CHO hosts. In this application note, we compared fed-batch performance of engineered mAb-producing clones derived from ATCC CHO-K1 and Lonza CHOK1SV GS-KO across multiple OPM basal media and feed combinations. The results showed host-specific ranking of medium-feed pairs and confirmed that the top shake-flask conditions translated into bioreactor, with up to ~30% higher titer in one case. These findings demonstrate that clone-specific optimization is critical for maximizing productivity and process robustness.

Introduction

Chinese hamster ovary (CHO) cells remain the industry standard for recombinant protein and monoclonal antibody (mAb) production (**1**). However, no single medium or feed formulation performs optimally across all CHO clones, even within closely related host backgrounds (**2,3,4,**). Subtle genetic and metabolic differences can lead to meaningful variation in growth kinetics, nutrient utilization, metabolite accumulation, productivity, and process robustness. As a result, media and feed selection should be approached as a clone-specific process development activity rather than a one-size-fits-all catalog choice. OPM Biosciences addresses this need through a broad portfolio of chemically defined basal media and feeds, together with internal access to multiple engineered CHO clones for data-driven evaluation. The objective of this study was to evaluate whether different CHO hosts favor different OPM medium-feed combinations, and whether the top conditions identified in shake flask are maintained in bioreactor scale.

Materials and Methods

Study Design Summary

- 2 engineered monoclonal antibody (mAb)-producing CHO clones
- 2 host backgrounds: ATCC CHO-K1 and Lonza CHOK1SV GS-KO
- Fed-batch shake flask screening followed by fed-batch bioreactor confirmation
- Key readouts: viable cell density (VCD), viability, lactate, ammonia, titer, and specific productivity (Qp)

Cell Lines

Two engineered mAb-producing CHO clones were evaluated in this study: one derived from ATCC CHO-K1 and one derived from Lonza CHOK1SV GS-KO. Both clones were engineered by the OPM Cell Line Development (CLD) Department for stable mAb expression.

Both cell lines were evaluated in fed-batch culture using OPM Biosciences' chemically defined basal media and feeds. Medium–feed combinations were selected from OPM's current catalog portfolio.

Culture Formats

- Fed-batch shake flask screening for rapid comparison of medium–feed combinations.
- Fed-batch bioreactor evaluation to verify performance and process robustness.

Key performance indicators included VCD, viability, metabolite profiles (e.g., glucose, lactate, ammonia), and recombinant protein titer.

Feeding Strategy

- **Fed-batch shake flask experiments:**
  For OPM basal media in combination with OPM feeds, the feeding strategy listed in Table 1 previously optimized in internal studies was applied.

  For benchmark media and feeds, the manufacturers' recommended feeding strategies were followed.

- **Fed-batch bioreactor experiments:**
  Daily feeding started on day 2 was applied. The percentage of daily Feed 1 was determined based on glucose consumption and osmolality changes. Feed 2 (CDFS36™) was added daily (D2–D13) at a ratio of 1/10 of Feed 1.

Glucose Concentration

Throughout the fed-batch process, glucose was supplemented when needed to maintain concentrations above 1 g/L.

Statistics

For statistical analysis, the mean and standard deviation (SD) were calculated from three biological replicates using GraphPad Prism. Statistical significance was assessed using ordinary one-way ANOVA followed by Tukey's multiple-comparison test, as indicated in the figure legends.

Table 1 : Feeding Strategy for Feed# 1 (Every Other Day [EOD])

Feeding Strategy for Feed# 1 (Every Other Day [EOD])							
Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Total % of Feed 1	Feed 2
3%	4%	5%	6%	6%	6%	30%	1/10 of Feed 1

Results and Discussion

Different CHO Hosts Respond Differently to the Same Medium-Feed Conditions

Distinct performances were observed between ATCC CHO-K1 and Lonza CHOK1SV GS-KO clones when cultured under identical basal media and feeding conditions (Figure 1A & B). In the shake flask setting, the ATCC CHO-K1 clone cultured in AltairCHO® and SagiCHO™ media exhibited relatively similar growth profiles over the 14-day fed-batch process (Figure 1A-upper left). In contrast, the Lonza CHOK1SV GS-KO clone showed a distinctly different growth profile when cultured in AltairCHO medium compared with SagiCHO medium, achieving higher VCDs in AltairCHO (Figure 1A-upper right).

With respect to lactate profiles, both clones followed asimilar overall trend, characterized by lactate accumulation during the early phase of the culture followed by a decline, typically around day 8 (Figure 1B). Nevertheless, notable differences were observed among specific medium–feed combinations. For example, lactate levels in cultures using SagiCHO medium supplemented with SagiCHO™ Feed remained close to zero from days 10 to 14 (Figure 1B-upper left). In contrast, cells cultured in the same basal medium but fed with an alternative feed (DenebCHO™ Feed) exhibited higher lactate concentrations toward the end of the process (approximately 1 g/L) (Figure 1B-upper left).

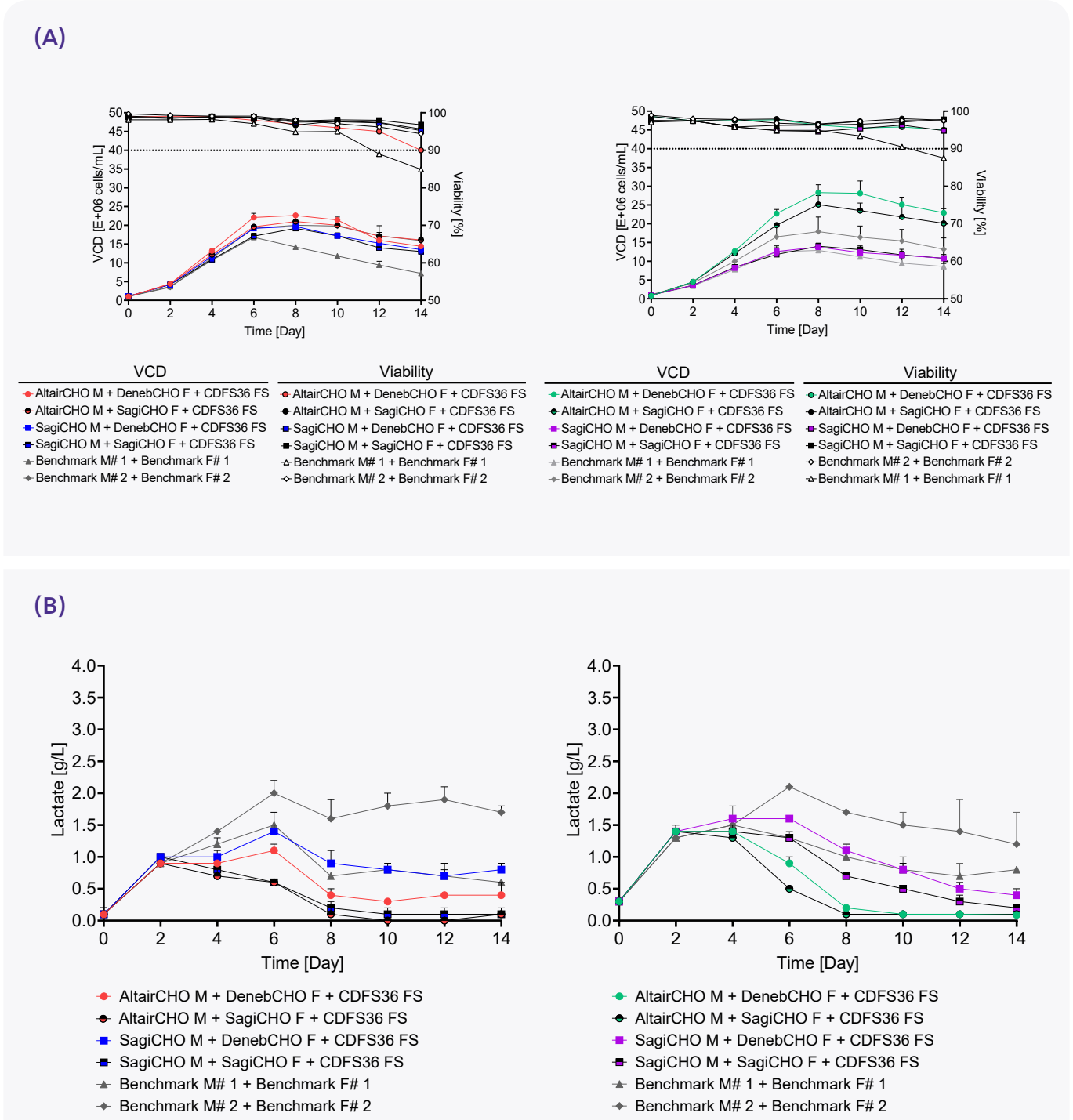

Figure 1. (A) VCD and viability over time for the ATCC CHO-K1 clone (upper left) and the Lonza CHOK1SV GS-KO clone (upper right) in a fed-batch shake flask setting. **(B)** Lactate concentration over time for the ATCC CHO-K1 clone (bottom left) and the Lonza CHOK1SV GS-KO clone (bottom right) in a fed-batch shake flask setting.

In the bioreactor setting, growth trends similar to those observed in shake flask experiments were obtained for both clones (Figure 2A). Minor platform-dependent differences in growth magnitude were observed for the ATCC CHO-K1 clone across the two bioreactor systems (Figure 2A-upper left); the overall performance trends remained consistent. The Lonza CHOK1SV GS-KO clone showed highly comparable growth profiles across both platforms (Figure 2A-upper right). These results support the overall reproducibility of clone-specific medium-feed responses under bioreactor conditions.

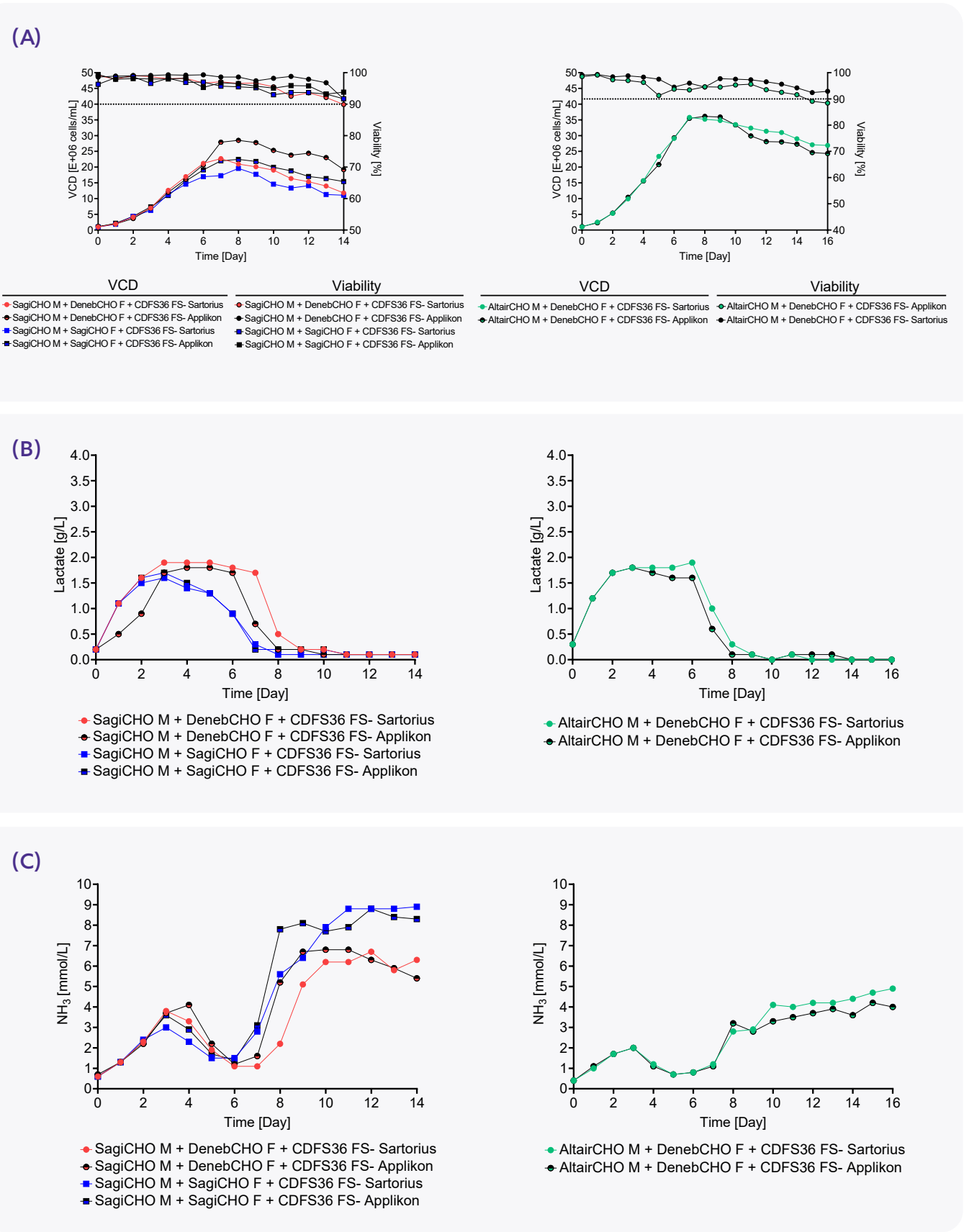

Figure 2. (A) VCD and viability over time for the ATCC CHO-K1 clone (upper left) and the Lonza CHOK1SV GS-KO clone (upper right) in a fed-batch bioreactor setting. **(B)** Lactate concentration over time for the ATCC CHO-K1 clone (middle left) and the Lonza CHOK1SV GS-KO clone (middle right) in a fed-batch bioreactor setting. **(C)** Ammonia concentration over time for the ATCC CHO-K1 clone (bottom left) and the Lonza CHOK1SV GS-KO clone (bottom right) in a fed-batch bioreactor setting.

The Best-Performing Medium-Feed Combinations Are Host- and Clone-Specific

The ranking of medium-feed combinations was not consistent across the two clones (Figure 3A and 3B). For the ATCC CHO-K1 clone, SagiCHO medium combined with either DenebCHO Feed or SagiCHO Feed resulted in the highest titers, reaching up to 8.3 g/L. In contrast, for the Lonza CHOK1SV GS-KO clone, the highest titer, 7.8 g/L, was achieved in AltairCHO medium with DenebCHO Feed. These results show that a condition that performs well in one host background may not be optimal in another, reinforcing the importance of clone-specific optimization.

The differences also suggest that the two host backgrounds vary not only in growth behavior, but also in nutrient utilization, metabolic burden, and tolerance to feed intensity and osmolality. From a process development perspective, this means medium-feed selection should be evaluated together with clone background rather than treated as a standalone product choice.

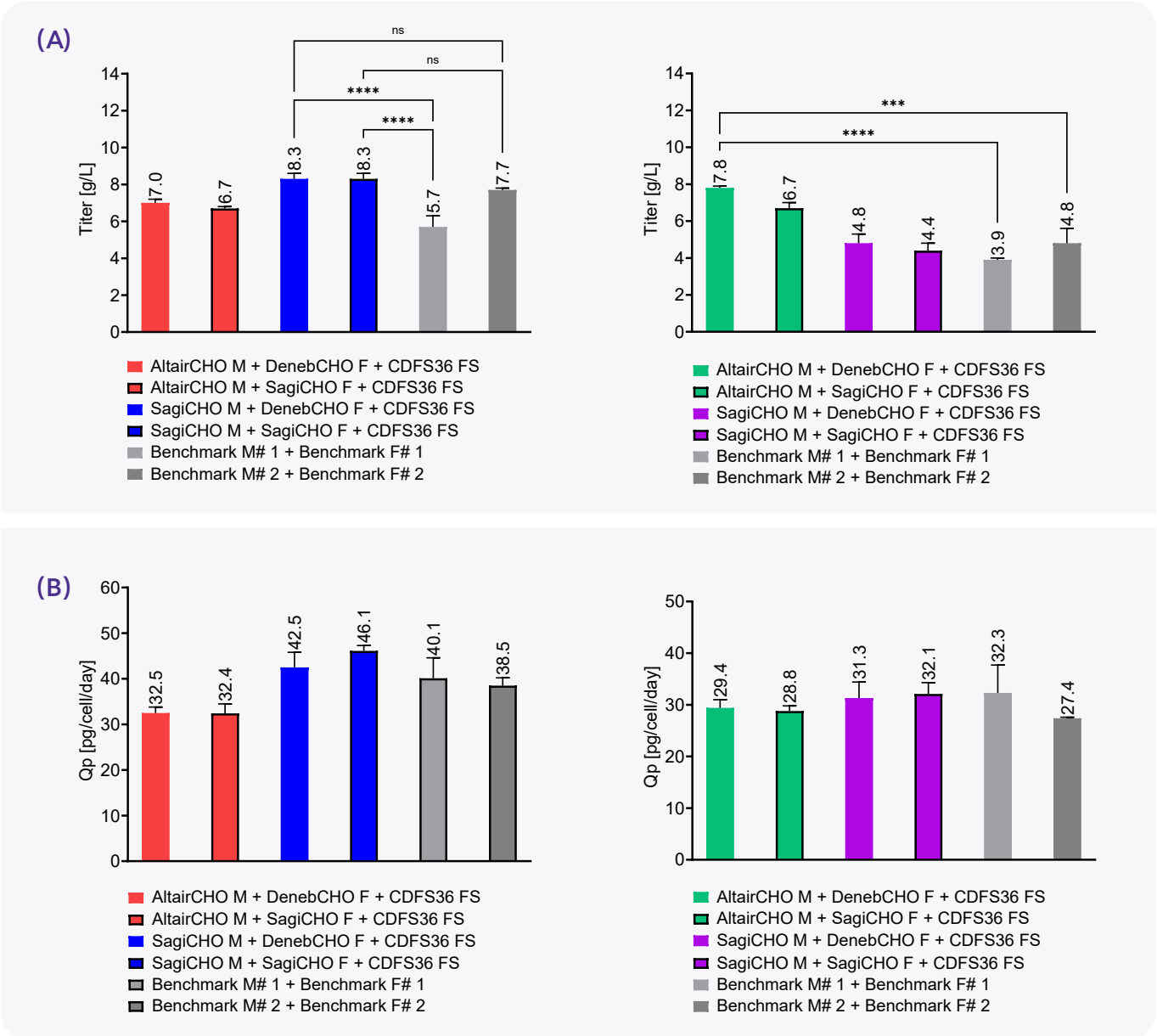

Figure 3. (A) Titer measured on day 14 for the ATCC CHO-K1 clone (upper left) and the Lonza CHO-K1SV GS-KO clone (upper right) in a fed-batch shake flask setting. **(B)** Qp on day 14 for the ATCC CHO-K1 clone (bottom left) and the Lonza CHO-K1SV GS-KO clone (bottom right). ns, not significant; **** $p < 0.0001$; *** $p = 0.0003$.

Top Shake Flask Trends Translate into Bioreactor, with Potential Additional Gains

When the top medium-feed combinations identified in shake flask were evaluated in bioreactor systems, the performance trends were generally maintained and, in some cases, further improved (Figure 4). For example, the Lonza CHOK1SV GS-KO clone cultured in AltairCHO medium with DenebCHO Feed and CDFS36 showed an approximately 30% increase in titer in the Sartorius bioreactor relative to shake flask. These data support the use of shake flask studies for rapid condition ranking while also demonstrating that bioreactor confirmation remains important for capturing platform-specific gains.

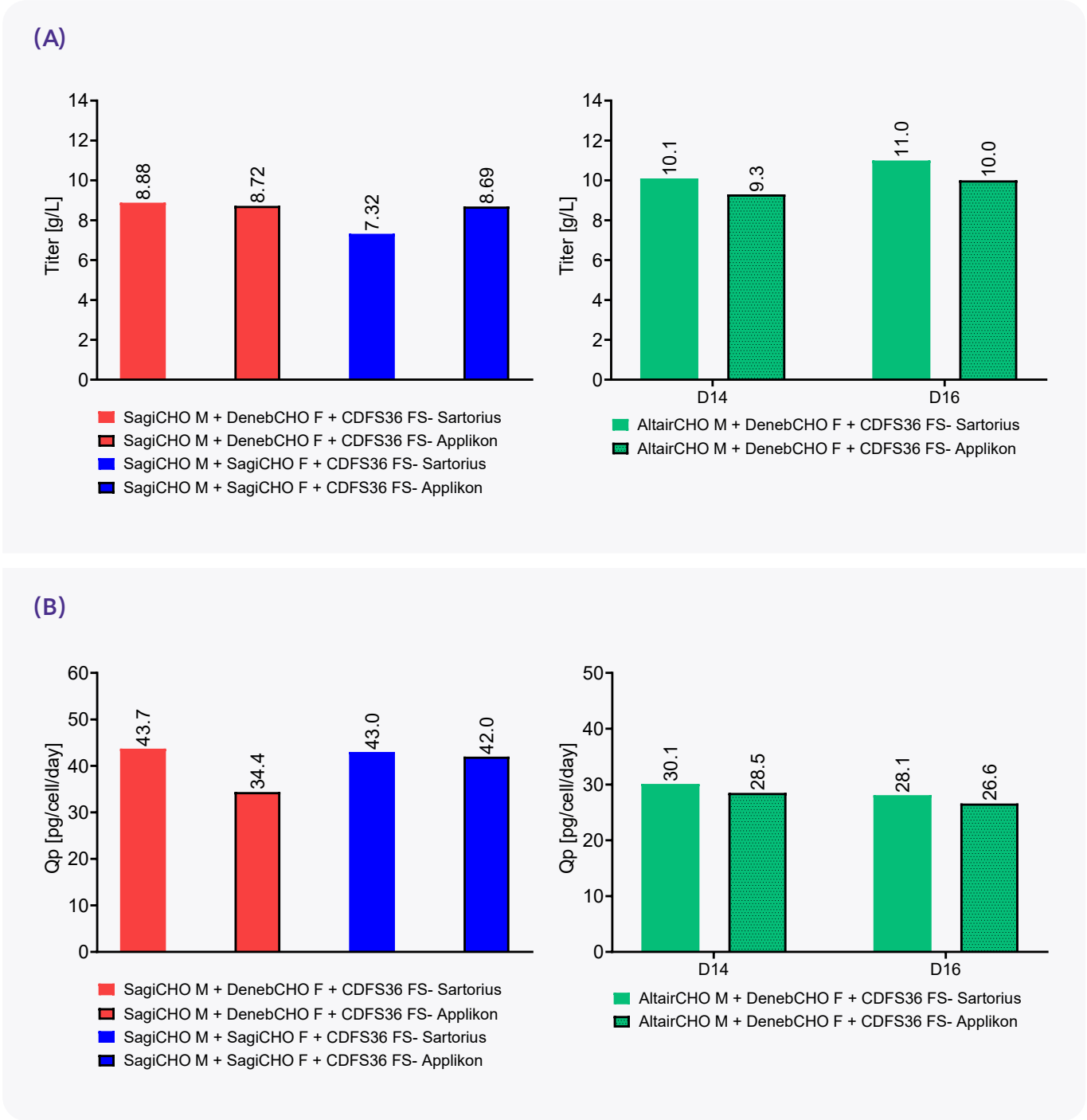

Figure 4. (A) Titer measured on day 14 for the ATCC CHO-K1 clone (upper left) and on days 14 and 16 for the Lonza CHO-K1SV GS-KO clone (upper right) in a fed-batch bioreactor setting. **(B)** Qp calculated on day 14 for the ATCC CHO-K1 clone (bottom left) and on days 14 and 16 for the Lonza CHO-K1SV GS-KO clone (bottom right).

Conclusion

Together, these data show that medium-feed ranking is not universal across CHO hosts and clones. OPM's advantage is not only its chemically defined medium-feed portfolio, but also its ability to evaluate that portfolio in biologically relevant clone backgrounds. This enables faster identification of clone-appropriate conditions and supports more confident process decisions from early screening through bioreactor confirmation.

Value of a Broad Media and Feed Portfolio

OPM Biosciences offers multiple basal media and feeds designed to support diverse CHO phenotypes and process needs. This breadth enables rapid identification of:

- Media conditions supporting robust early-phase growth
- Feed strategies that enhance productivity while maintaining control of key metabolites
- Clone-appropriate osmolality and nutrient delivery profiles

By systematically evaluating different medium-feed combinations, OPM can identify the most suitable pairing for each clone, leading to stronger overall process performance.

Advantage of CLD/CDMO-Enabled Access to Relevant Clones

By combining access to diverse CHO clones with integrated CLD and CDMO (contract development and manufacturing organization) capabilities, OPM Biosciences can evaluate basal media and feed combinations in biologically relevant clone backgrounds. This strengthens confidence in interpreting clone-specific performance trends, improves the quality of early process development decisions, and helps connect media optimization with more robust scale-up strategies across different CHO production systems.

References

1. Walsh, G. *Biopharmaceutical benchmarks 2018.* Nature Biotechnology, 36, 1136–1145 (2018).
2. Kim, J. Y. et al. *CHO cells in biotechnology for production of recombinant proteins: current state and further potential.* Applied Microbiology and Biotechnology, 93, 917–930 (2012).
3. Wurm, F. M. *Production of recombinant protein therapeutics in cultivated mammalian cells.* Nature Biotechnology, 22, 1393–1398 (2004).
4. Sellick, C. A. et al. *Metabolite profiling of CHO cell cultures: pathways and process impacts.* Biotechnology and Bioengineering, 108(10), 2460–2472 (2011). Biotechnology, 22, 1393–1398 (2004).

opmbio.com

Contact : opmus_sales@opmbiosciences.com
OPM Biosciences, Inc.• 5653 Stoneridge Dr., Suites 117 & 118, Pleasanton, CA 94588 | NA113A.