Cell Culture Media-Feed Co-Optimization Empowers Clonal-Dependent Extended Fed-Batch Performance in CHOZN GS$^{-/-}$ Cells For Biosimilar Production

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Introduction

Biopharmaceuticals and biotherapeutics based on recombinant proteins offer clear advantages over many traditional small-molecule drugs. Their mechanisms of action are often more specific, which can translate into fewer off-target effects and improved tolerability, and they have advanced treatment options across a broad range of diseases and indications[1]. Most recombinant protein therapeutics are manufactured in mammalian cell culture-based systems. This is because these systems can produce complex proteins with proper folding and assembly, and they support human-like post-translational modifications that are often required for functionality and reduced immunogenicity[2]. Among mammalian host cells, Chinese hamster ovary (CHO) cells are the dominant production platform, and it is estimated that nearly 70% of recombinant protein therapeutics are produced in CHO cells[3]. Within this landscape, antibody-based products represent a major class of recombinant protein therapeutics. Monospecific immunoglobulin G (IgG) molecules, which fall within the broader category of monoclonal antibodies (mAbs), account for approximately 74% of all approved antibody therapeutics[4].

Cost Considerations in Biomanufacturing

Despite the substantial success of antibody-based biotherapeutics, their broad adoption and patient access remain limited by high costs, especially in low- and middle-income countries (LMICs)[5]. A prominent incentive driving down both the cost of goods (COGs) and overall prices of mAbs is the expanding biosimilars market, which continues to accelerate as originator biologic manufacturing patents expire and competition increases[6]. In this context, while market dynamics ultimately determine the commercial price at which a therapy may sell, COGs set the lower boundary for sustainable pricing[5]. Although platform manufacturing processes for mAbs vary across organizations, most processes share a similar end-to-end workflow from cell line development through upstream production and downstream purification[7]. As a result, improvements in process efficiency can directly contribute to lower COGs. In recent decades, upstream productivity gains have been a major contributor to significant reductions in COGs. For example, typical mAb titers in the late 1990s were often 1-2 g/L or below, whereas titers of 5-8 g/L are routine today[4]. Among the levers available to improve upstream process efficiency, cell culture media and feed optimization remains one of the most impactful and scalable approaches.

OPM's Cell Culture Media Products

OPM Biosciences is an integrated solutions provider of cell culture media products and services. Through our products, we optimize cell culture processes to accelerate biotherapeutic discovery and manufacturing, enabling greater affordability and accessibility of these life-saving therapeutics. OPM Biosciences achieves this through a portfolio of high-performance, chemically defined media products specifically optimized for upstream cell culture process development and commercial manufacturing. Our OPM-CHO media portfolio, tailored for stable CHO cell protein expression,

can support high production-phase cell viabilities that enable longer process durations, promote greater viable cell densities (VCDs), or bolster cell-specific productivities that result in product volumetric titer gains to reduce drug substance and overall COGs.

Furthermore, with experience in biosimilar development, OPM's products and services are backed by technical expertise supporting the development of processes aligned with biosimilar critical quality attributes (CQAs) necessary for comparable clinical performance and regulatory approval.

Materials & Methods

CHOZN GS$^{-/-}$ parental/host cells were obtained from Merck KGaA (Darmstadt, Germany). Two CHOZN GS$^{-/-}$ clones, clone #1 and clone #2, were engineered by the OPM Cell Line Development Department for stable expression of known immunoglobulin G4 (IgG4) biosimilars mAb1 and mAb2, respectively. Cells were thawed from research cell banks, routinely passaged in basal medium via dilution, and maintained in an incubator shaker at 37.0 °C, 8.0% CO_2, 80% relative humidity, and 170 RPM with an orbital diameter of 25 mm or 120 RPM with an orbital diameter of 50 mm. Unless otherwise indicated, fed-batch processes were performed in 125 mL Erlenmeyer shake flasks and maintained in an incubator shaker at the same parameters, with the exception of process-dependent temperature downshifts. Fed-batch cultures were inoculated at a density of 1.0×10^6 cells/mL in basal medium, fed with feed medium and feed supplement, and supplemented with glucose to maintain concentrations >1 g/L.

Combinations of OPM-CHO basal media, feed media, and feed supplements tested are displayed in **Table 1.** Shake flask feeding was performed every other day beginning on the second day of the process, and the total feed medium percentage was between 30-40% of the initial culture volume for a 16-day process. Feed supplement volume was maintained at a ratio of 10% of the feed medium volume. Process performance was benchmarked against global competitor products, one basal medium and two different feed media, following the manufacturers' recommendations. Bench-scale bioreactor (BRX) validation was performed using an Applikon my-Control 3 L Glass Vessel (Getinge) with a pH setpoint of 6.95 ± 0.25, dissolved oxygen (DO) setpoint of 40%, and agitation rate of 300 RPM. Bioreactor feeding was performed daily beginning on the second day of the process, and the total feed medium percentage was 41% of the initial culture volume for an 18-day process. Cell densities and viabilities were measured with a Vi-CELL Cell Viability Analyzer (Beckman Coulter), glucose and lactate concentrations were measured with an M-100 Biosensor Analyzer (Shenzhen Sieman Technology) or a Cedex Bio Analyzer (Roche CustomBiotech), and mAb titers were measured using a Cedex Bio Analyzer. Error bars, where present, represent mean ± standard deviation for $n = 3$. Selected product quality was evaluated by the OPM Analytical Department after a one-step purification and compared against the known reference molecule.

Group	Basal Medium	Feed Medium	Feed Supplement
OPM #1-1	StarCHO™ Medium	StarCHO™ Feed Plus	CDFS12™
OPM #1-2	StarCHO™ Medium	VectorCHO™ Feed	CDFS12™
OPM #2-1	StarCHO™ Plus Medium	StarCHO™ Feed Plus	CDFS12™
OPM #2-2	StarCHO™ Plus Medium	VectorCHO™ Feed	CDFS12™

Table 1. Combinations of OPM-CHO basal media, feed media, and feed supplements tested. Performance was benchmarked against global competitor products following the manufacturers' recommendations.

Results & Discussion

Cell Passaging & Population Doubling Times

For clone #1 (**Figure 1A**), population doubling times (PDTs) were similar in all basal media with average PDTs of ~20 h. Cell viabilities were also comparable, generally ≥99.0% in all basal media. In comparison, clone #2 had greater variability in PDTs (**Figure 1B**).

For clone #2, the average PDT was lowest in OPM #1 (StarCHO Medium) and highest in the competitor medium. Average cell viabilities for clone #2 were ≥98.0% in all basal media.

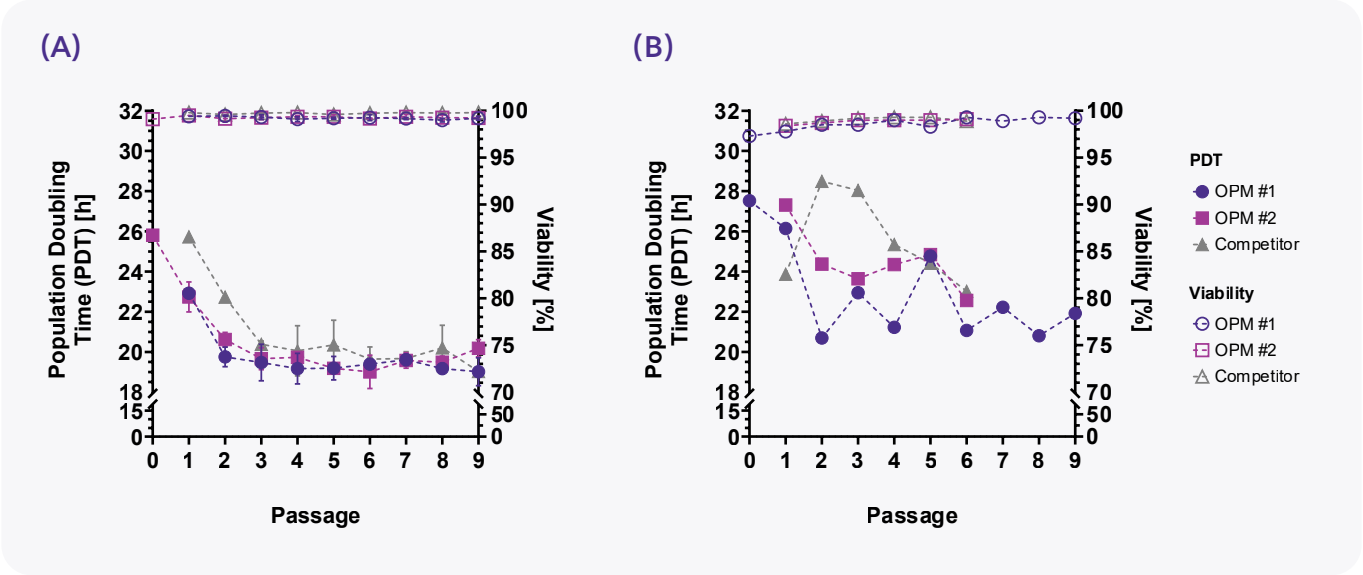

Figure 1. Population doubling times (PDT, closed symbols, left axes) and cell viabilities (open symbols, right axes) of (**A**) clone #1 and (**B**) clone #2 during passaging and seed train expansion.

Fed-Batch Process Results

Combinations of OPM-CHO media and feeds supported VCD profiles similar to or greater than competitor products for both clones investigated (**Figure 2A, 2D**). For clone #1 (**Figure 2A**), peak VCD typically occurred on day 8 and ranged from 25-50 × 10^6 cells/mL depending on the basal media and feed media/feed supplement combination as well as process parameters. For clone #2 (**Figure 2D**), peak VCD typically occurred on day 8 at ~30 × 10^6 cells/mL with OPM-CHO products versus ~20 × 10^6 cells/mL with competitor products. These inter- and intra-clonal differences correlate with the PDTs discussed previously (**Figure 1**) where clone #1 exhibits higher peak VCDs than clone #2 due to the shorter doubling times, and clone #2 exhibits higher peak VCDs in OPM basal media than competitor basal media for the same reason.

Similar to VCDs, OPM-CHO products maintained cell viabilities similar to or better than competitor products for both clones (**Figure 2B, 2E**). In particular, OPM #2 (StarCHO Plus Medium) maintained clone #1 viabilities ≥95.0% by day 14, which enabled process extension, and viabilities remained ≥90.0% even by day 18 (**Figure 2B**). In comparison, clone #1 viabilities with competitor products were ≤90.0% by day 14 and rapidly declined with competitor #2 where process extension was investigated. Due to the superior viability maintenance of StarCHO Plus Medium for clone #1, OPM combination #1-2 with StarCHO Medium was not investigated for this clone; OPM #2-1 was further supported through a validation run in a bench-scale bioreactor (OPM #2-1 BRX). For clone #2, all groups maintained cell viabilities >80% by day 14, which allowed process extension to day 16 when cell viabilities remained >70% at the time of harvest (**Figure 2E**). Specifically, for OPM combinations #1-2, #2-1, and #2-2, cell viabilities were >90% through day 14, and remained ≳90% by day 16. For both clones, high production-phase cell viabilities may support further fed-batch process extension with continued titer gains.

Regarding detrimental metabolites, lactate concentrations for both clones did not exceed 2 g/L at any time for any experimental groups (**Figure 2C, 2F**). For clone #1 (**Figure 2C**), OPM-CHO products promoted superior lactate metabolism during the production phase in comparison to the competitor products. For clone #2 (**Figure 2F**), slightly higher lactate concentrations with OPM-CHO products may be attributed to the greater VCDs relative to the competitor groups (**Figure 2D**).

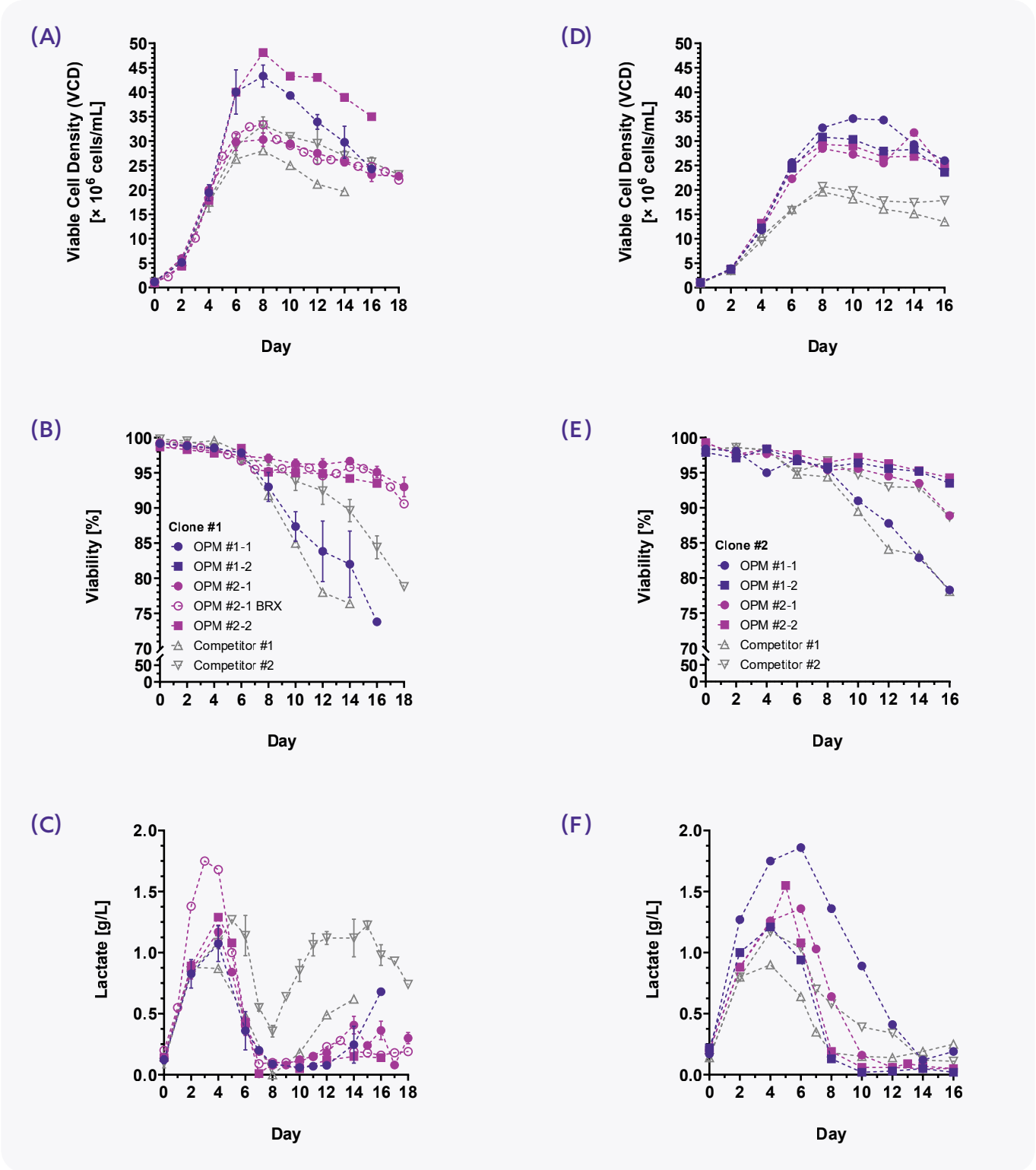

Figure 2. Viable cell density (VCD), cell viability, and lactate profiles during fed-batch processes. (A) VCDs, (B) cell viabilities, and (C) lactate concentrations for clone #1 (14- to 18-day process durations). Combination OPM #2-1 was validated in a bench-scale bioreactor for clone #1 (open colored symbols). (D) VCDs, (E) cell viabilities, and (F) lactate concentrations for clone #2 (16-day process durations).

Volumetric titer measurements demonstrated similar or better process performance with OPM-CHO products in comparison to competitor products (**Figure 3**). OPM #2-1 promoted the greatest production of mAb1 in clone #1 (**Figure 3A**) and the highest cell-specific productivity during the production phase of 13-14 pg/cell/day (**Figure 3B**). Process extension, enabled by the high cell viabilities maintained during the production phase, led to continued titer gains. This resulted in performance superior than the leading competitor products. Notably, since OPM #2-1 and Competitor #2 had similar VCD profiles (**Figure 2A**), this improved process performance for clone #1 can be attributed to enhanced cell-specific productivities. Clone #1 performance with OPM #2-1 was subsequently validated in a bench-scale, fed-batch bioreactor process (**Figures 2 and 3**). In comparison, amongst OPM-CHO products for clone #2, all combinations promoted similar mAb2 titers and cell-specific productivities; however, OPM-CHO products promoted considerable titer gains of 40+% relative to competitor products with comparable or better cell-specific productivities (**Figure 3C**). Highlighting clonal differences, since OPM groups had similar cell-specific productivities to Competitor #2, this improved process performance for clone #2 can be attributed to greater VCDs (**Figure 2D**).

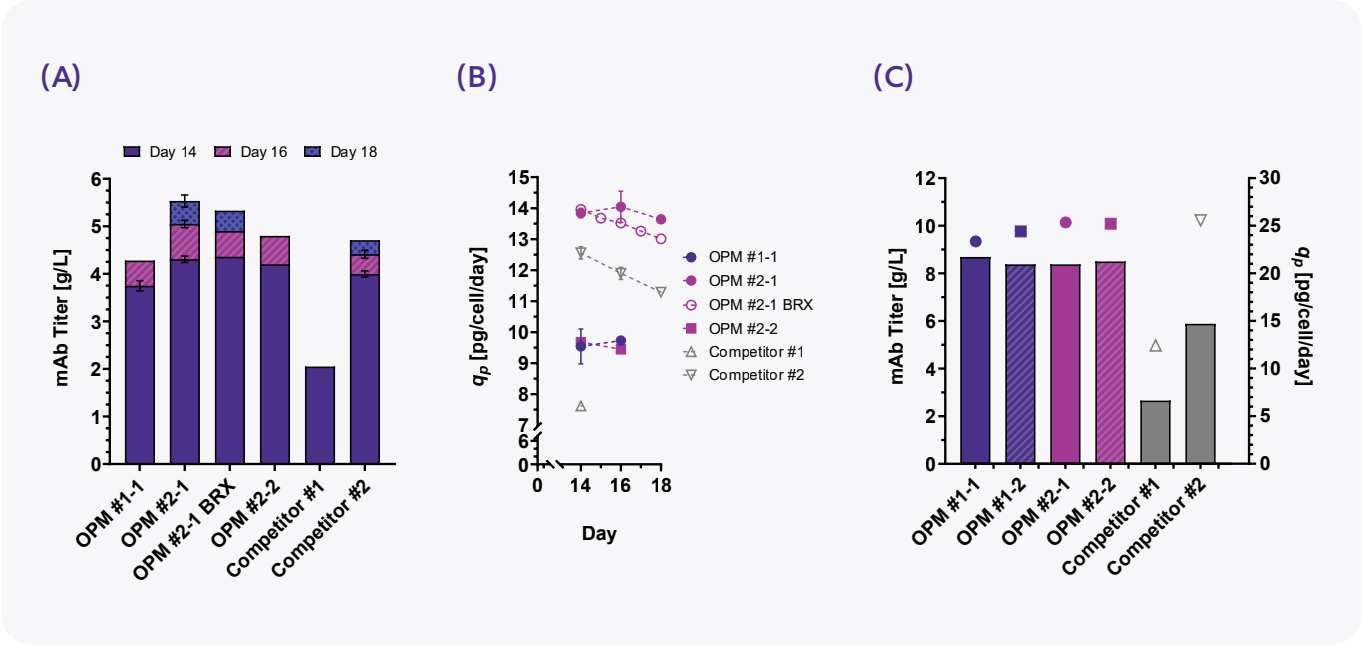

Figure 3. Volumetric titers and cell-specific productivities (q_p) of fed-batch processes. (**A**) mAb1 titers expressed by clone #1 measured at multiple timepoints (e.g., days 14, 16, and 18) and corresponding (**B**) cell-specific productivities. (**C**) Final mAb2 titers (bars, left axis) expressed by clone #2 and corresponding cell-specific productivities (symbols, right axis) after 16-day fed-batch processes.

Product Quality Analysis

Analytical characterization of mAb2 (**Figure 4**) demonstrated minimal product fragmentation or aggregation (**Figures 4A, 4B**) and a similar charge variant distribution to the reference molecule (**Figure 4C**). N-glycan profile analysis revealed some variation from the mAb product obtained in comparison to the reference molecule; however, it is important to emphasize that for IgG4 therapeutics, such as the target molecule in this investigation, the glycan profile is not a CQA. This is because IgG4 isotype molecules do not rely on fragment crystallizable (Fc) region effector functions such as antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) as part of their mechanism of action[8], and therefore the therapeutic efficacy of the molecule is not heavily reliant on the glycosylation profile of the molecule[9].

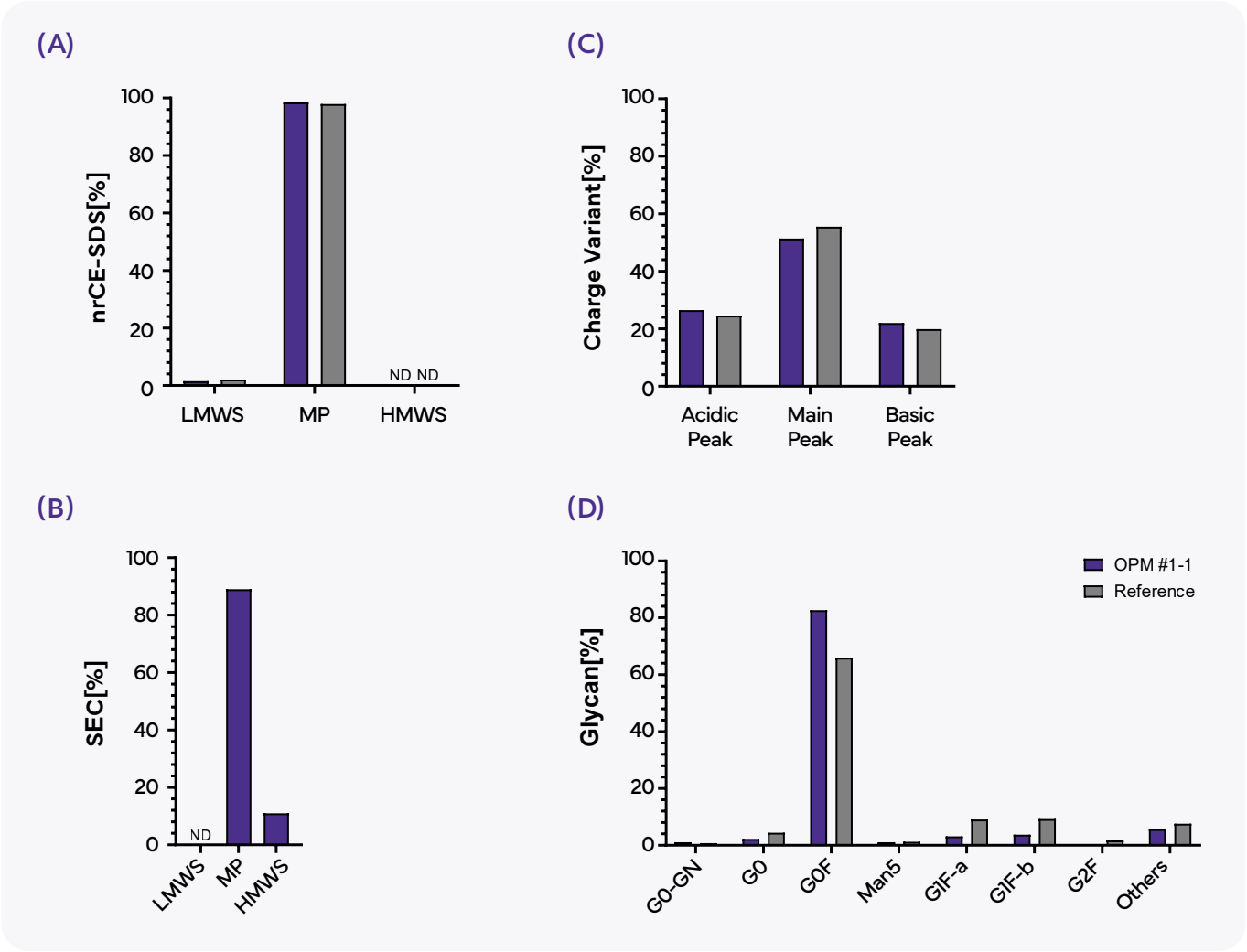

Figure 4. Analytical characterization of mAb2 purified from OPM combination #1-1 after a 16-day fed-batch process and the reference molecule for comparison. Size variant analysis via (**A**) non-reduced capillary gel electrophoresis sodium dodecyl sulfate (nrCE-SDS) and (**B**) size-exclusion chromatography (SEC). LMWS: low molecular weight species. MP: main peak. HMWS: high molecular weight species. ND: not detected. (**C**) Charge variant analysis via imaged capillary isoelectric focusing (icIEF). (**D**) N-glycan profile analysis via ultra-performance liquid chromatography with fluorescence detection (UPLC/FLD).

Conclusions & Future Directions

OPM Biosciences offers a wide range of catalog and customized cell culture media solutions for the development and manufacturing of biotherapeutics. Here, we demonstrated that selected products from the OPM-CHO media portfolio supported comparable or higher VCDs while maintaining strong production-phase cell viabilities, enabling extended fed-batch process durations. As a result, OPM-CHO media and feed combinations delivered greater volumetric titers than benchmark commercial media products under the evaluated process conditions.

Notably, the primary driver of performance improvement was clone-dependent: clone #1 expressing mAb1 benefited from enhanced cell-specific productivity whereas clone #2 expressing mAb2 benefited from increased VCDs with OPM-CHO products. These results highlight an important advantage of OPM's broad media and feed portfolio: productivity gains can arise through different biological mechanisms or process conditions depending on clone-specific metabolism, growth behavior, and productivity profile. This reinforces the value of pairing media screening with clone-level process understanding, rather than relying on a single universal formulation strategy. Finally, characterization of product obtained from clone #2 (mAb2) revealed

product quality attributes comparable to the reference molecule, demonstrating the utility of the OPM-CHO platform in enabling extended fed-batch processes while maintaining critical product quality attributes. Driven by continuous innovation, future work will focus on advancing intensified and extended fed-batch strategies enabled by OPM's robust media and feed platform, with continued emphasis on improving titer, productivity, and product quality alignment. Whether your molecule of interest is an innovator biologic, biosimilar, or biobetter, OPM offers flexible, data-driven solutions designed to meet your productivity and CQA requirements.

Acknowledgements

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