High-Density N-1 Seed Train Expansion Powers Intensified Fed-Batch Processes for Accelerated Biosimilar Production

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Key Takeaways

- OPM-CHO products enabled **N-1 seed train expansion** reaching VCDs ≥40.0 × 10^6 cells/mL within 6 days while maintaining cell viabilities >95%
- **Fed-batch intensification** successfully shortened production process duration (by four days) and additionally enabled continued titer gains through day 16 (~37% increase) relative to traditional fed-batch controls

Introduction

As several originator biologic drug patents near expiration and the market for biosimilars rapidly expands, increasing competition continues to drive down the prices of these critical biotherapeutics[1]. Upstream bioprocess intensification aims to bolster volumetric productivity of biologics manufacturing, thereby boosting cost efficiency and expediting speed to market[2,3]. Intensified fed-batch (IFB) processes, in which a bioreactor vessel is inoculated at a higher initial cell density and then maintained in a fed-batch process, achieves this increase in productivity by reducing the length of the bioreactor lag or growth phase.

Through continuous innovation, OPM Biosciences has developed high-performance, chemically defined cell culture media products to improve upstream process efficiency. In this investigation, we demonstrate that OPM-CHO cell culture media and feeds, including a purpose-built perfusion medium, enable generation of high viable cell densities during N-1 seed train expansion. Furthermore, we establish that OPM-CHO products can further bolster intensification of subsequent N-stage fed-batch processes for production of a biosimilar monoclonal antibody (mAb). These results highlight how integrated N-1 and production media optimization can improve process efficiency and provide meaningful advantages for biosimilar manufacturing.

Materials & Methods

CHOZN GS$^{-/-}$ host cells were obtained from Merck KGaA (Darmstadt, Germany). The CHOZN GS$^{-/-}$ cell line utilized in the following investigation was engineered by the OPM Cell Line Development Department for stable expression of a known immunoglobulin G1 (IgG1) mAb biosimilar.

To generate cell densities sufficient to inoculate intensified fed-batch (IFB) processes at 10.0 × 10^6 cells/mL, N-1 seed trains were expanded either in an enhanced batch process or through regular medium exchanges. In the enhanced batch process, cells were inoculated in 125 mL Nalgene Erlenmeyer shake flasks (Thermo Fisher) in **StarCHO™ Medium**, and cultures were periodically supplemented with 4.0% **StarCHO™ Feed** and 0.4% **CDFS36™** feed supplement. Additional glucose was supplemented as needed to maintain concentrations >1 g/L. For medium exchanges, cells were inoculated in 50 mL TubeSpin Bioreactors (TPP) either in enriched **StarCHO™ Medium** containing 4.0% **StarCHO™ Feed** and 0.4% **CDFS36™** or in **FluxCHO™ Perfusion Medium**. Medium exchanges were performed by centrifuging TubeSpin Bioreactors to pellet cells, replacing the medium supernatant with fresh medium, and then resuspending cells. Rates of exchanges are described in **Table 1**.

Fed-batch processes were performed in 125 mL shake flasks. Cells were inoculated at a density of 1.0 × 10^6 cells/mL for traditional fed-batch (TFB) processes and 10.0 × 10^6 cells/mL for IFB processes. All fed-batch processes were performed in **StarCHO™ Plus Medium** and fed with **StarCHO™ Feed Plus** and **CDFS12™** feed supplement. Additional glucose was supplemented as needed to maintain concentrations >1 g/L. All cultures were maintained in a Multitron incubator shaker (INFORS HT). Cell densities and viabilities were measured with a Vi-CELL BLU Cell Viability Analyzer (Beckman Coulter), glucose and lactate concentrations were measured with an M-100 Biosensor Analyzer (Shenzhen Sieman Technology), and ammonia concentrations and mAb titers were measured using a Cedex Bio Analyzer (Roche CustomBiotech).

Results & Discussion

Table 1 (right). Medium exchange rates for N-1 seed train expansion in vessel volumes per day (VVD).

Day	VVD	Day	VVD
0	0.0	**3**	1.0
1	0.0	**4**	1.0
2	0.5	**5**	1.5

Table 2 (far right). N-1 seed train expansion strategies for generating high cell densities and corresponding media.

Group	N-1 Seed Train Process	N-1 Seed Train Medium
#1	Batch	StarCHO™ Medium
#2	Enhanced batch	StarCHO™ Medium
#3	Medium exchanges	Enriched StarCHO™ Medium
#4	Medium exchanges	FluxCHO™ Perfusion Medium

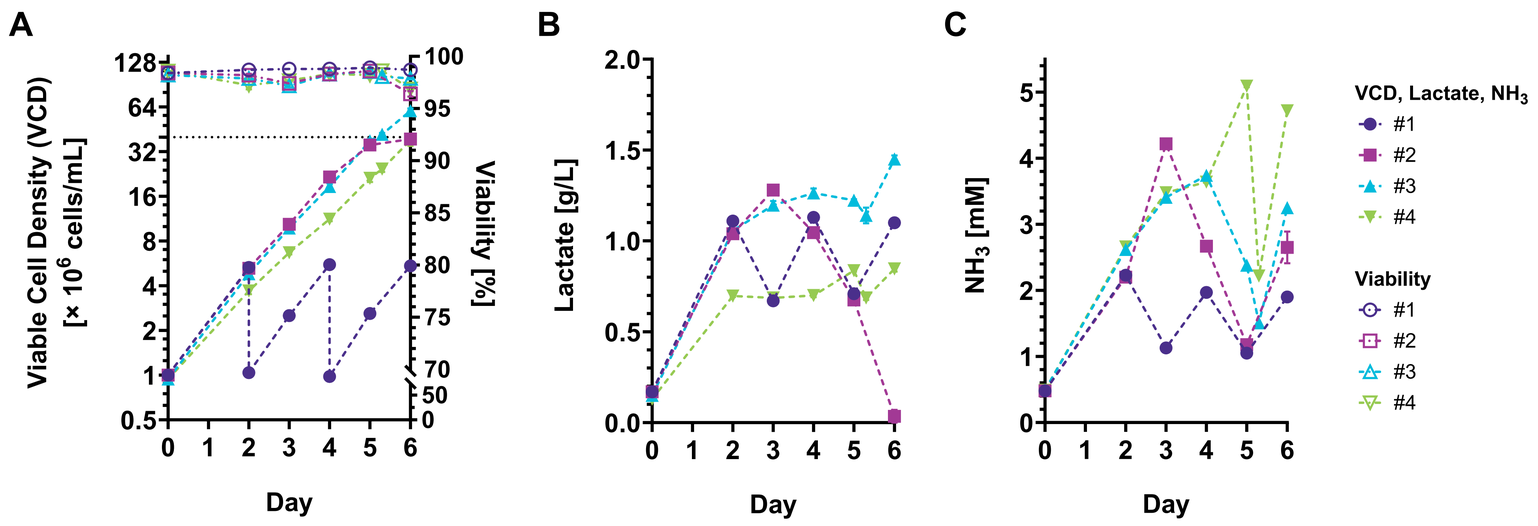


Figure 1 (above). Viable cell density (VCD), cell viability, lactate, and ammonia profiles during N-1 seed train expansion following the strategies in **Table 2**. **(A)** VCDs (closed symbols, left axis with a log-2 scale) and cell viabilities (open symbols, right axis). Cell proliferation in the enhanced batch process began to plateau after day 5 but VCDs reached ~40.0 × 10^6 cells/mL by day 6. Medium exchanges in enriched StarCHO Medium enabled VCDs reaching ~60.0 × 10^6 cells/mL whereas cell growth in FluxCHO Perfusion Medium was slower reaching VCDs of ~40.0 × 10^6 cells/mL by day 6. Viabilities were maintained >95% for all strategies. For all processes, **(B)** lactate concentrations remained <2 g/L and **(C)** ammonia concentrations remained ≤5 mM. Error bars represent mean ± standard deviation of *n* = 2-3 replicates, where applicable.

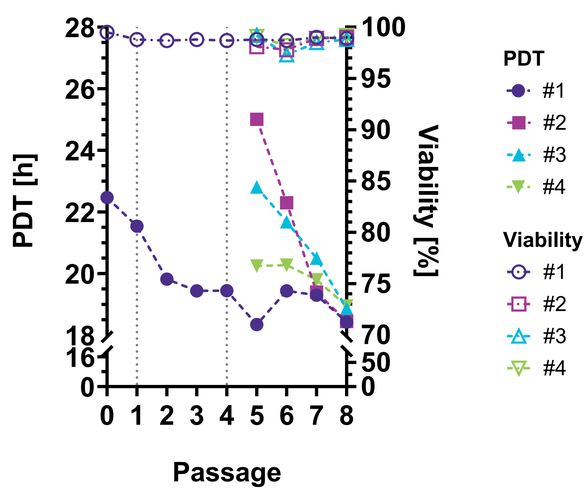
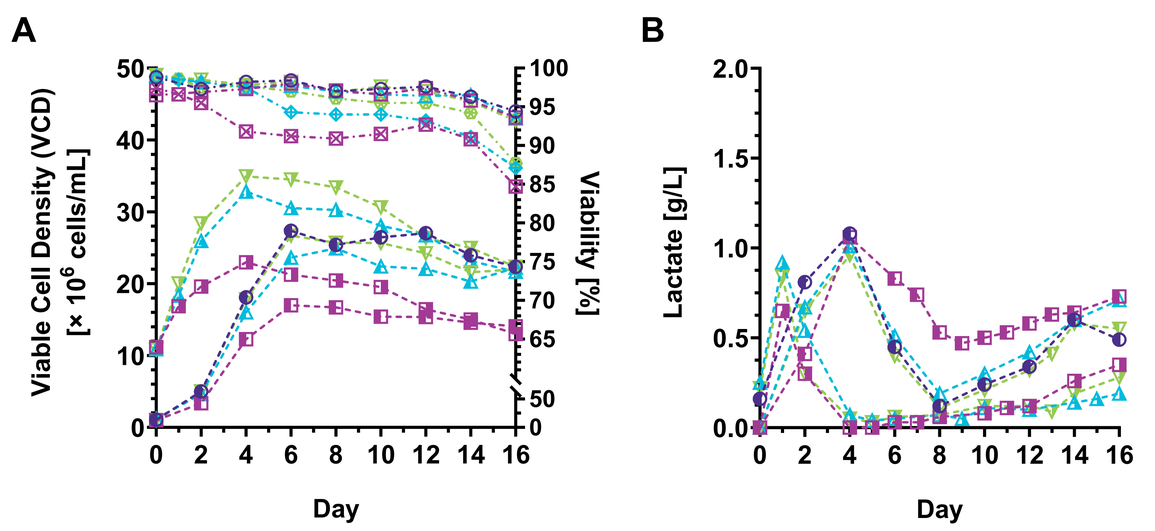
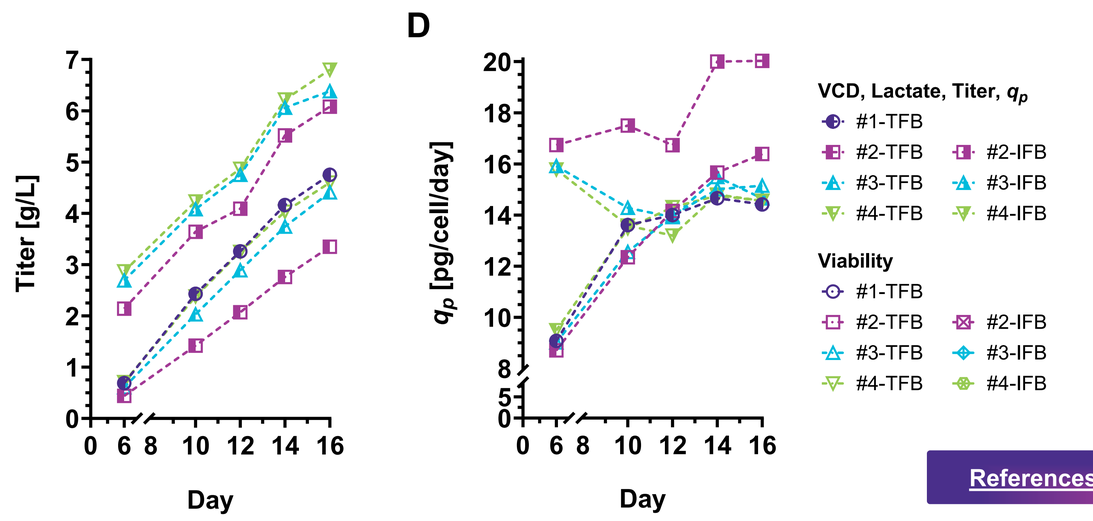


Figure 2 (left). Population doubling times (PDTs, closed symbols, left axis) and viabilities (open symbols, right axis) of cells during batch passaging after N-stage inoculation. N-1 seed train processes were inoculated after passage one, and N-stage fed-batch processes were inoculated after passage four. High-density N-1 processes had increased PDTs on the first passage after N-stage inoculation (passage five) but recovered after two passages.

Figure 3 (below). VCDs, cell viabilities, lactate concentrations, titers, and cell-specific productivities (q_p) for N-stage production fed-batch processes. **(A)** VCDs (closed symbols, left axis) and cell viabilities (open symbols, right axis). The enhanced batch N-1 process resulted in reduced fed-batch VCDs, including in the TFB control, whereas N-1 medium exchanges led to higher fed-batch peak VCDs. Viabilities of all groups, including IFBs, were maintained ≳90% out to day 14. **(B)** Lactate concentrations remained <2 g/L for all processes, both traditional and intensified. **(C)** Titer measurements validated successful process intensification with the N-1 medium exchanges strategy as IFB titers on day 10 were comparable to TFB titers on day 14, allowing for a four-day reduction in process duration with similar efficiency. In addition, extension of these IFB processes out to 16 days demonstrated continued titer gains +1.80 g/L relative to TFB controls (~37% overall increase) and maintained cell-specific productivities sustained by OPM-CHO products **(D)**.





Conclusions & Future Work

Upstream bioprocess intensification can improve facility utilization and reduce cost of goods by increasing process efficiency. Here, N-1 seed train expansion with OPM-CHO products, including a purpose-built perfusion medium, supported generation of high VCDs necessary for inoculation of subsequent IFB processes. Furthermore, OPM-CHO products sustained productivity during IFB operation, enabling shortened production timelines and further achieving greater final titers in a 16-day process relative to TFB controls. Additional investigations are ongoing to characterize harvested protein product quality attributes and confirm comparability of critical quality attributes (CQAs) with the biosimilar reference molecule. Altogether, these results demonstrate that an integrated N-1 seed train expansion and N-stage production medium optimization strategy bolstered by the OPM-CHO portfolio powers upstream bioprocess intensification to increase productivity and improve manufacturing efficiency for biosimilar production.

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References

[1] McCamish M and Woollett G. *mAbs* **3** (2011).
[2] Chen C, *et al*. *Curr Opin Chem Eng* **22** (2018).
[3] Stettler M, *et al*. *Pharm Bioprocess* **6** (2018).

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