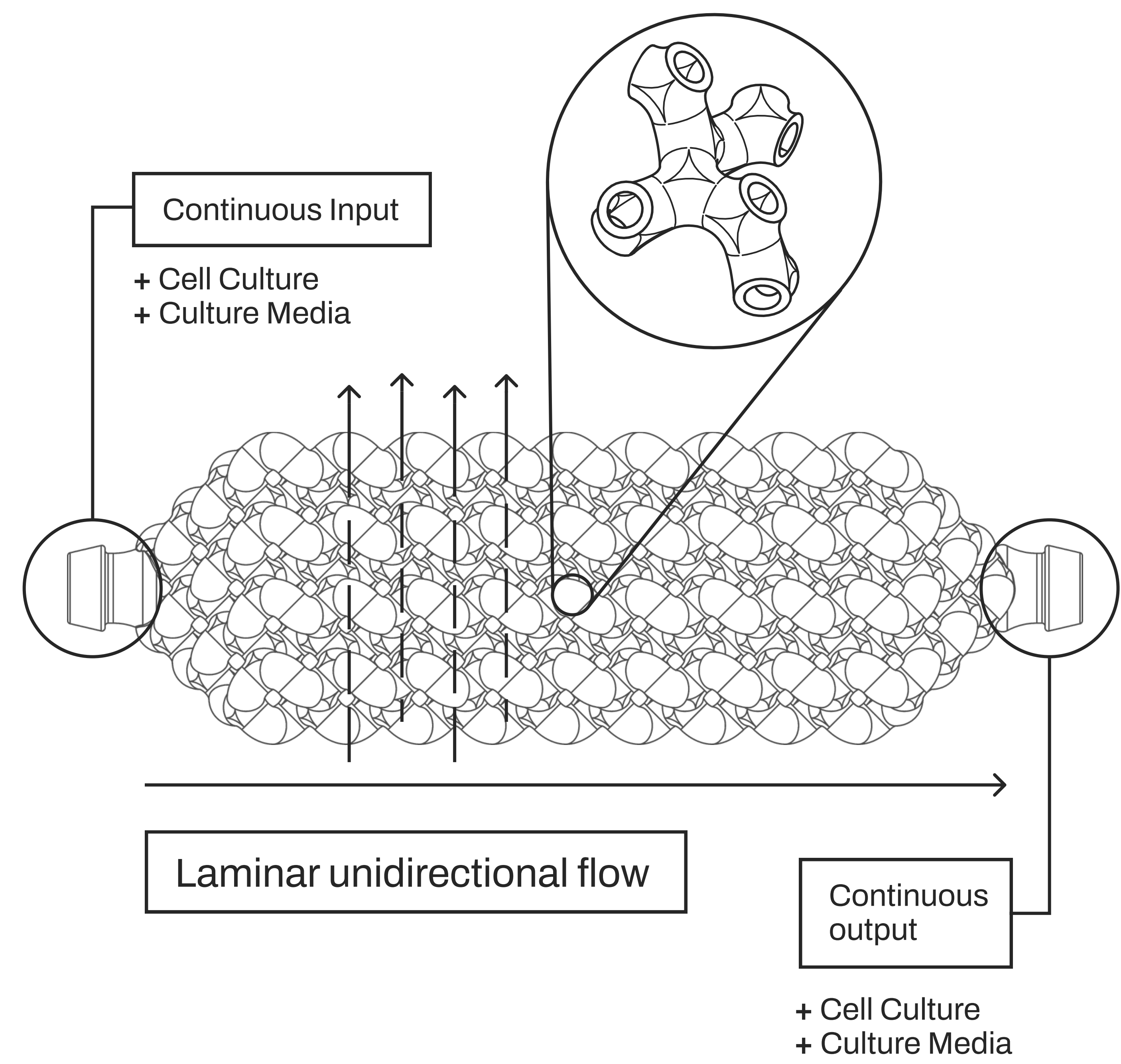


Continuous biosimilar mAb production

in Stamm's bubble-free microfluidic bioreactor

Introduction

Stämm Bio has developed a novel bubble-free bioreactor that has demonstrated significant advantages in space-time yields in high-density mammalian cell culture applications across suspension (CHO, JURKAT) and adherent (HEK, MSC, iPSC) cultures.



Schematic 1. Stämm's 3D-printed Bubble-Free Bioreactor (BFB) cartridge, designed for continuous laminar flow and optimized gas transfer.

Fed-batch processes remain the default approach for monoclonal antibody (mAb) production, driven by historical precedent, simplicity, and reliability. While offering operational familiarity, fed-batch bioprocesses are increasingly constrained by inefficiencies, including downtime during batch transitions, cell density plateaus, metabolite and by-product accumulation, uneven nutrient distribution, and batch-to-batch variability. These limitations underscore the need to advance toward continuous bioprocessing approaches. Perfusion systems offer continuous operation but often introduce cell stress due to constant agitation and gas sparging.

The Stämm platform addresses these challenges through its highly resolved, 3D-printed bubble-free bioreactor (BFB) cartridge (Schematic 1). The BFB enables continuous laminar flow media perfusion and product harvest, operating entirely without the need for antifoam agents.

The BFB enables continuous laminar flow media perfusion and product harvest, operating entirely without the need for antifoam agents. It delivers optimized mass and gas transfer dynamics, allowing cultures to bypass traditional fed-batch plateaus to achieve significantly increased cell densities and space-time yields.

This application note demonstrates the performance capabilities and operational advantages of the Stämm BFB in biosimilar mAb manufacturing with CHO cells.

Material and Methods

mAb-expressing CHO cells, provided by a third party, were cultured and maintained using the proprietary base media of the client's reference fed-batch process. Initial expansion cultures post-thaw were performed with the same base media in shake flasks, starting at 1×10^6 cells/mL in 10 mL and reaching 100 mL at a cell concentration of 6×10^6 cells/mL. This was followed by seeding in a 60 mL Stämm BFB cartridge at an initial inoculum of 6×10^6 cells/mL. Details of the process and materials used are provided in Table 1.

Table 1. Key materials and process parameters for continuous CHO cell culture.

Material / Parameter	Description
Cell Line	CHO
Expressed Protein	Commercial biosimilar monoclonal antibody
Media	Proprietary base media of the reference fed-batch process, with feeds
VVDs	1.0–1.5 reactor volumes/day
Hardware	Stämm Incubator Setup with 60 ml BFB cartridge in a CO2 incubator; 37°C, 5% CO2
Initial inoculum	6×10^6 cells/ml
Run duration	12 days (MT01); 15 days (MT02)

Cells were cultured in a controlled incubator environment at 37°C with 5% CO2. Semi-automated sampling was performed at 24-hour intervals to assess cell concentration, viability, and metabolite consumption. After 12–15 days of continuous operation in the BFB, cells were harvested from the cartridge via automated fluid dispensing and collection.

Cell retention in the BFB is inherent to its structural properties and operating principles, requiring no tangential flow filtration (TFF). Metabolite concentration, dissolved gases, and pH were measured using a BioProfile® FLEX2 (Nova Biomedical). Cell concentration and viability were determined by Trypan blue staining using a Vi-CELL® BLU (Beckman Coulter). The mAb concentration was determined by affinity HPLC on a Protein A column.

Space-time yields (STY) were calculated as described by Bausch et al. (2018), accounting for the active working volume and total process time:

$$STY_i = \frac{Y_i}{V \text{ Bioreactor}^* (t_i - t_0)}$$

(Where: STY [g L⁻¹ d⁻¹], Y = yield [g], t = process time [d], V = working volume [L])

Size variants were determined by HPLC-SEC (size exclusion chromatography), and charge variants were determined by HPLC-IEX (ion exchange chromatography).

Glycan profiling was performed by GLY-UPLC®. For quality attribute assessments, the BFB harvest underwent a single clarification step followed by Protein A purification. Results were compared against a fully purified commercial reference standard.

Results and Discussions

Two simplified, single-replicate continuous production runs (MT01, 12 days; MT02, 15 days) were performed in the Stämm BFB. The client's commercial fed-batch reference protocol was used natively; while the BFB operational and fluidic parameters were optimized between MT01 and MT02, the baseline fed-batch media and biological protocols were not adapted or reformulated.

In the BFB runs, cells achieved a peak total cell density of 67.4 × 10⁶ cells/mL (MT01) and 65.4 × 10⁶ cells/mL (MT02)—an average of 66.4 × 10⁶ cells/mL—with 86–87% final-day viability. This demonstrates a 3.9-fold increase over the 17.0 × 10⁶ cells/mL and 80% viability of the fed-batch reference (Figure 1, Figure 2). Process parameters could be smoothly adjusted during the run and were maintained within normal ranges throughout the continuous operation.

Volumetric productivity plateaued at an average of 0.96–1.01 g/L/d, with peak volumetric productivity reaching 1.10–1.11 g/L/d. This represents an approximately 11.5-fold increase over the fed-batch reference peak (0.096 g/L/d). Cumulative volumetric mAb yield reached 7.86 g/L in 12 days (MT01)—a 6.8-fold increase over the reference process in the same time window—and 11.23 g/L in 15 days (MT02). Across both runs, the average final volumetric yield at harvest was 9.55 g/L (Figure 3, Table 2). Space-time yields consistently achieved 0.65–0.72 g/L/d.

Table 2. Key benchmark results. Stämm BFB runs vs. commercial fed-batch reference

Parameter	MT01 (BFB)	MT02 (BFB)	Fed-batch Reference
Peak Total Cell Density (10 ⁶ cells/mL)	67.4	65.4	17.0
Final viability (%)	86	87	80
Days run	12	15	12
Vol. productivity (g/L/d)	1.10	1.11	0.096
Vol. yield (mg/ml reactor)	7.86	11.23	1.16
STY (g/L/d)	0.65	0.72	0.096

Product quality was assessed using harvested BFB samples following a single Protein A purification step and compared directly with the fully purified fed-batch reference standard. HPLC-IEX and HPLC-SEC profiles closely mirrored the standard, with SEC main peak values of 97.5–97.8% (versus 98.6% for the standard), while IEX profiles presented neutral peaks of 76.15–73.98%, acidic peaks of 12.56–14.59%, and basic peaks of 11.29–11.42% (versus the standard: neutral >68%, acidic <24%, and basic <9.2%)

The product-quality attributes (size, charge, glycans) of the Protein A eluate fell within the historical Drug Substance specification ranges (Figure 4). Glycan profiling by GLY-UPLC® also satisfied specifications, with G0F at 66.6–67.9% (specification ≥64.0%) and Man5 at 2.0–3.9% (specification ≤4.5%). SEC main peak values were 97.7–97.4% (specification >98%), while IEX profiles presented neutral peaks of 76.15–73.98%, acidic peaks of 12.56–14.59%, and basic peaks of 11.29–11.42% (specification: neutral >68%, acidic <24%, and basic <9.2%).

Product quality was assessed using harvested BFB samples following one clarification step by centrifugation (2,000g for 5 min) and a single Protein A purification. These results were compared to the fed-batch reference standard following the same clarification and purification steps as shown in Figure 5.

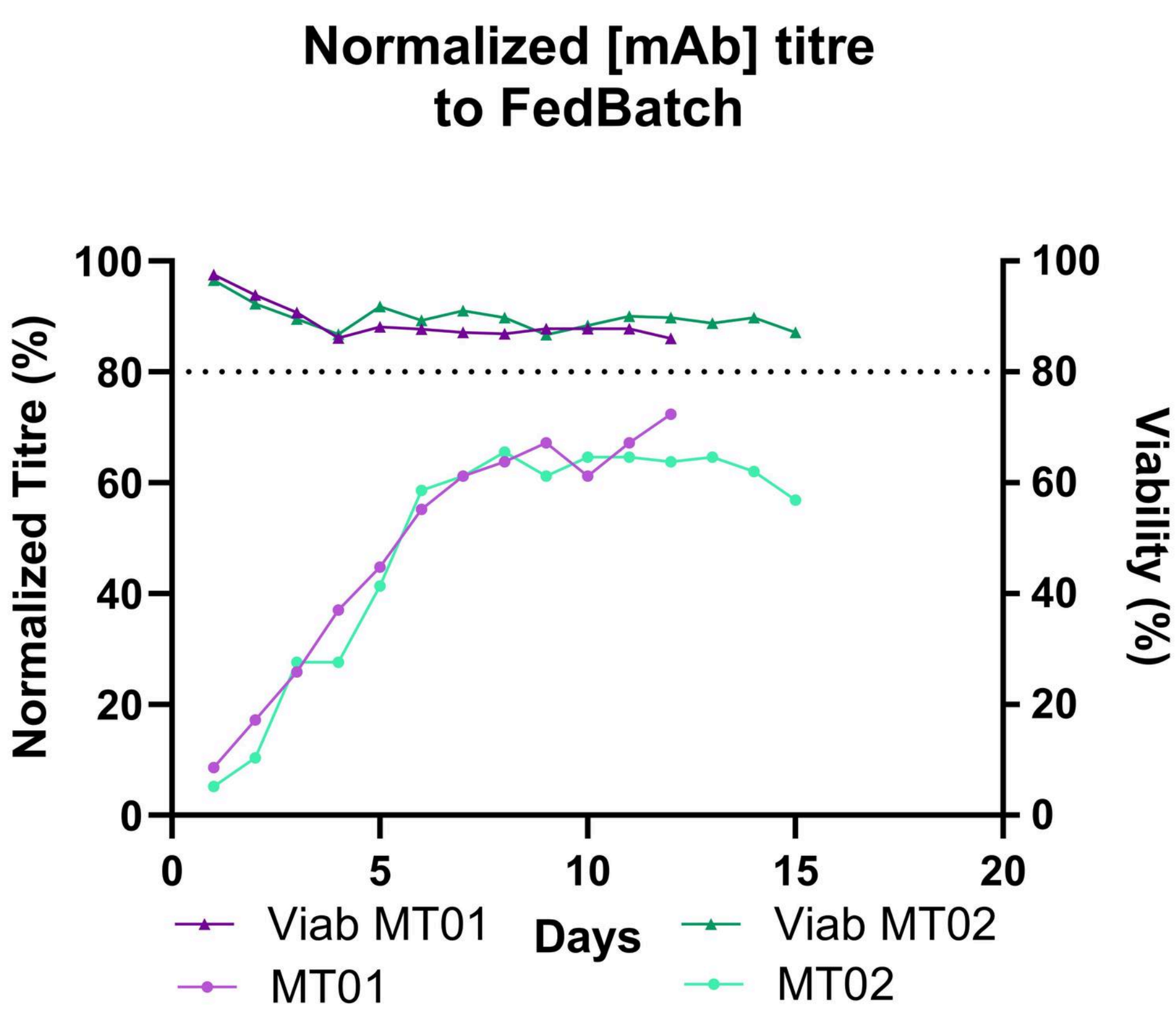


Figure 1. Normalized mAb concentration and viability over time, BFB runs MT01/MT02 vs. fed-batch reference. The Y1-axis represents normalized mAb concentration and the Y2-axis represents cell viability. The dotted line represents final fed-batch viability. MT01 and MT02 represent two separate runs in the BFB for 12 days and 15 days respectively. In both BFBs, titer plateaued at day 7 and maintained mAb concentration until the end of the run (sustained for 6 and 9 days respectively).

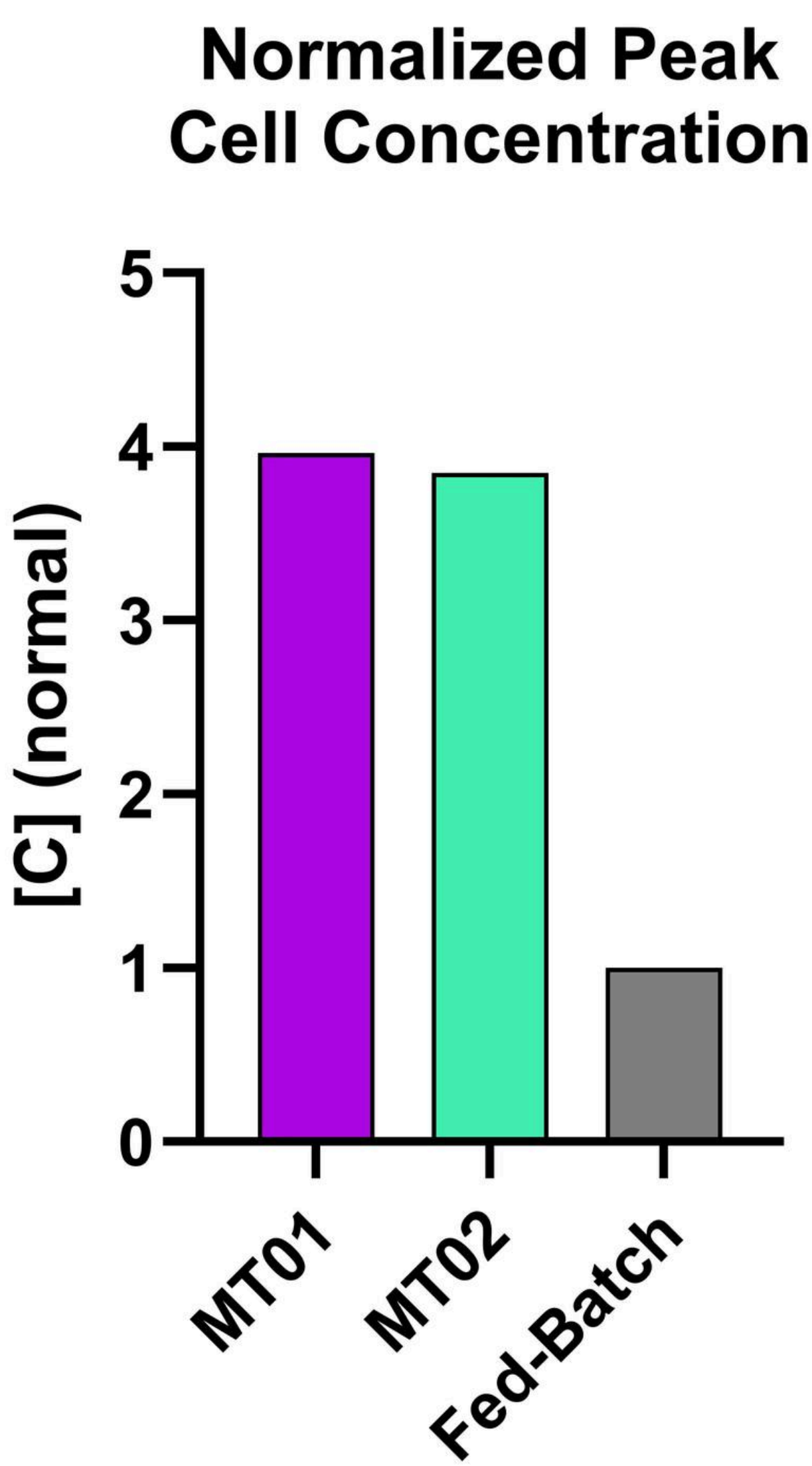


Figure 2. Normalized peak total cell concentration in BFBs vs. fed-batch reference. MT01 CHO cell concentration at the end of the run reached 67.4 × 10⁶ cells/mL and MT02 CHO cells reached a peak cell density of 65.4 × 10⁶ cells/mL.

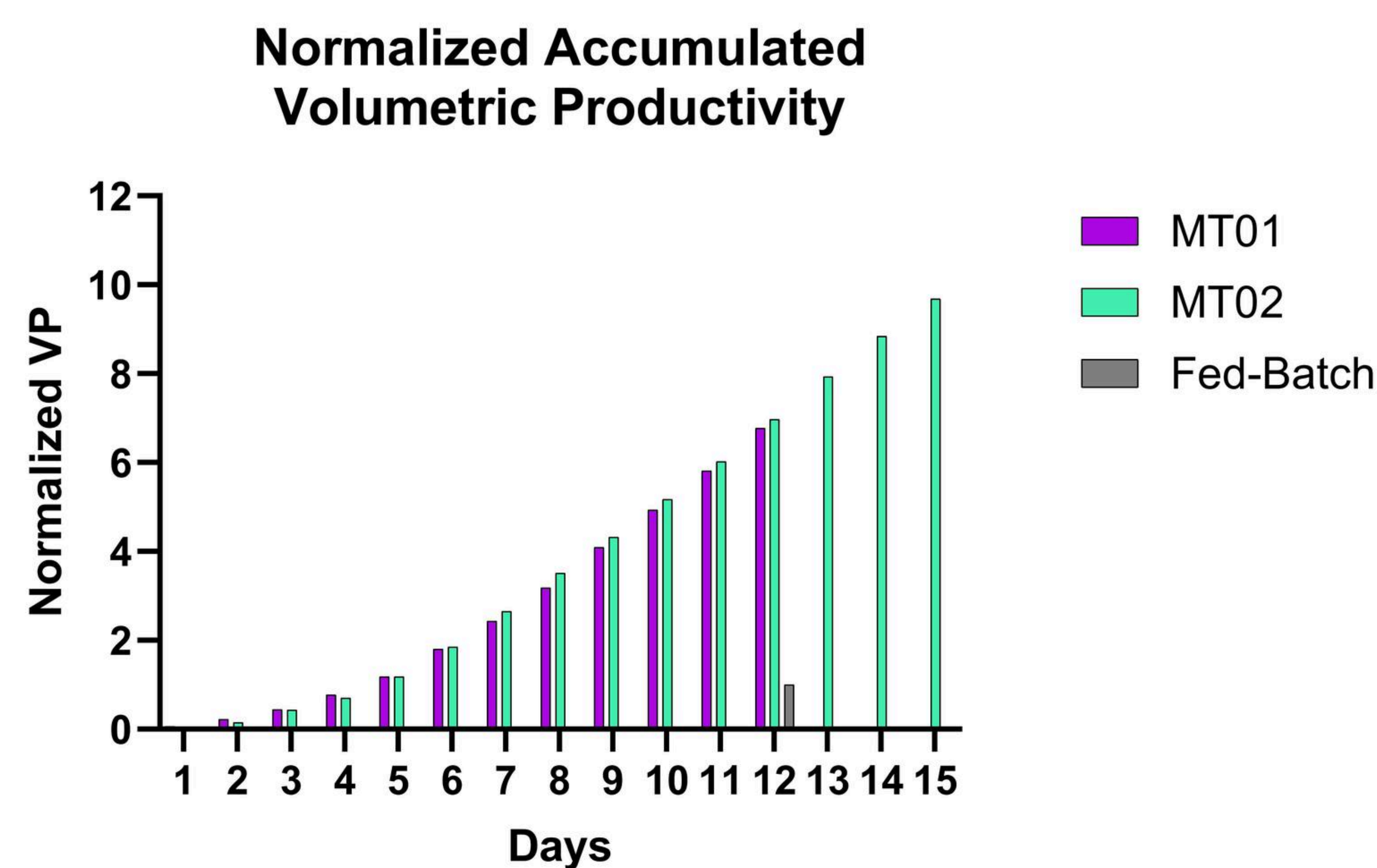


Figure 3. BFBs normalized accumulated volumetric productivity vs. fed-batch reference. The green and purple bars represent BFB runs and the grey bar represents the fed-batch reference at harvest. As indicated by the trend, absolute mAb quantity scaled consistently throughout the assay.

Furthermore, the stringent quality attributes achieved by the BFB samples required only a single clarification step and one affinity purification step, whereas the standard reference required a fully optimized, multi-step downstream process.

This represents a major opportunity to relieving critical facility bottlenecks and enabling reductions in both OPEX and CAPEX across R&D and commercial biosimilar manufacturing scales.

Conclusions

The Stämm BFB outperformed the control fed-batch process, increasing both cell density and volumetric productivity to confirm a distinct performance and operational advantage for continuous manufacturing. Third-party analytical data independently validated that mAbs produced in the Stämm BFB reliably fall within the quality specifications of a commercial biosimilar drug substance.

Key performance improvements included a 3.9-fold increase in maximum cell density (averaging 66.4×10^6 cells/mL), an 8.2-fold increase in average volumetric mAb yield at harvest (9.55 g/L), and an ≈ 11.5 -fold increase in peak volumetric productivity (1.10 vs. 0.096 g/L/d).

Crucially, these yields were achieved "out-of-the-box" using the client's established fed-batch biological protocol. While the hardware operation of the BFB was optimized between runs, no prior adaptation or reformulation of the media was required, leaving vast room for further synergistic process improvements.

References

1. Bausch, M., Schultheiss, C., & Sieck, J. B. (2018). Recommendations for Comparison of Productivity Between Fed-Batch and Perfusion Processes. *Biotechnology Journal*. <https://doi.org/10.1002/biot.201700721>

HPLC-IEX (MT01, MT02 vs standard)

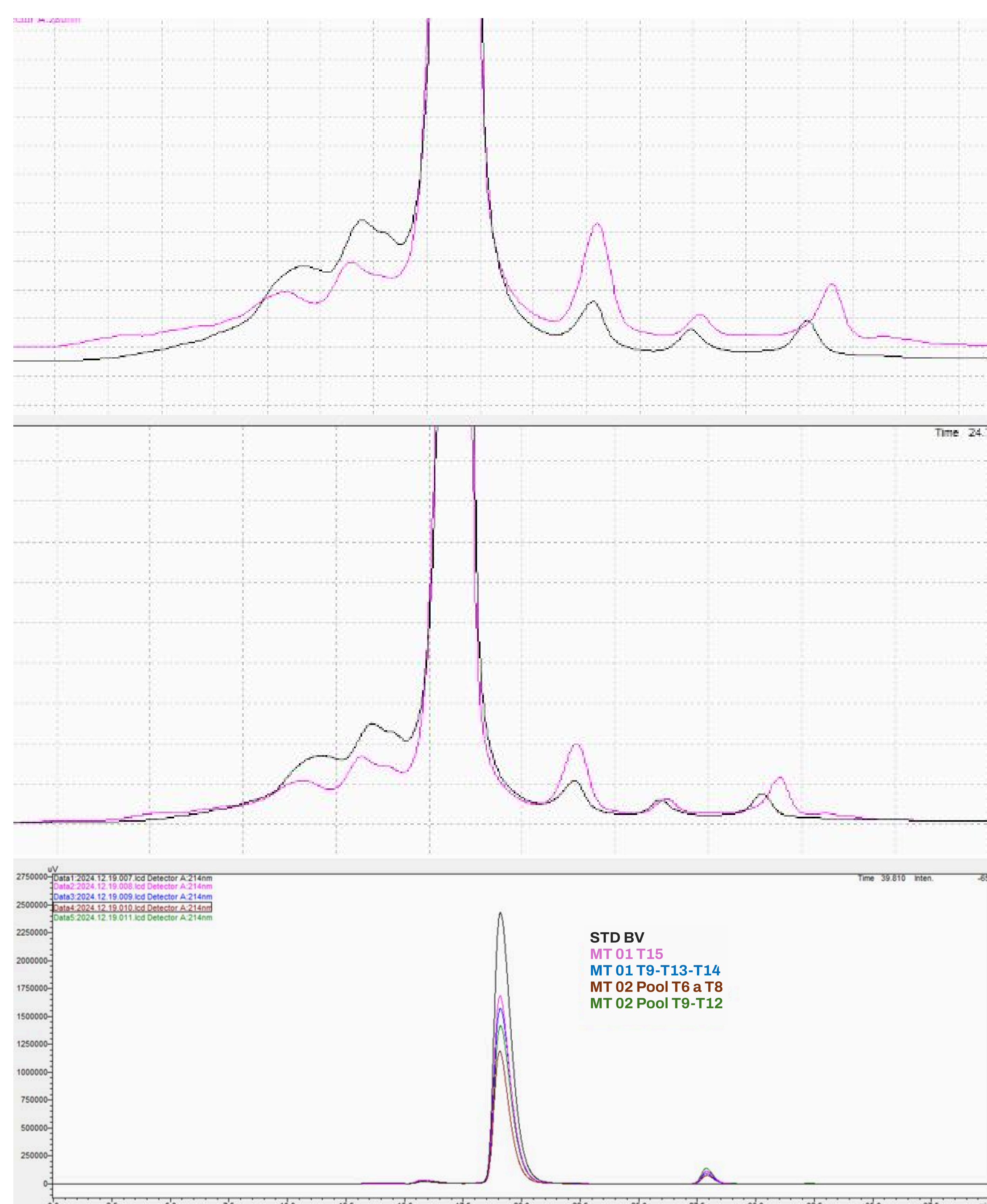


Figure 4. HPLC-IEX and SEC-HPLC profiles. Top/Middle: HPLC-IEX of batched harvest samples (last 3 days) for MT01 and MT02 (pink) superposed against the reference standard (black). Bottom: HPLC-SEC of harvest samples for MT01 and MT02 (colors) superposed against the reference standard (black). Note: Differing injected sample masses naturally affect absolute peak heights; therefore, peak area percentages within each sample are the primary metric of comparison.

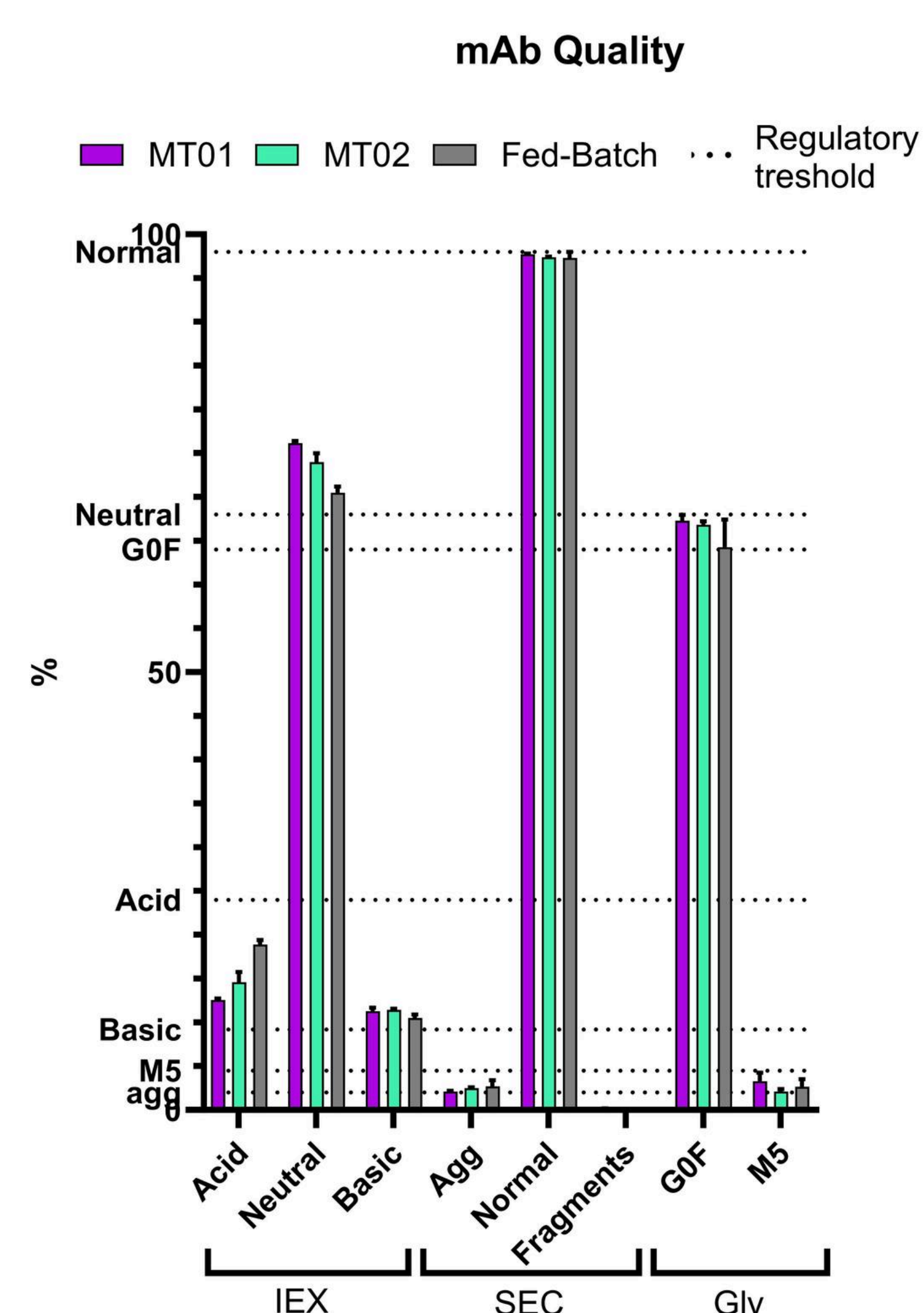


Figure 5. mAb quality attributes for size (normal, aggregates, and fragments), **charge variants** (neutral, basic, and acidic), **and glycans** (GOF (core fucosylated) and M5 (high-mannose-5)) for MT01 (purple), MT02 (green), and fed-batch (gray) without full downstream processing. Dotted lines represent historical specification ranges for each parameter (neutral >68%; acidic <24%; basic <9.2%; main >98%; aggregates <2%; GOF $\geq 64\%$; M5 $\leq 4.5\%$).