STRATYX™ Delivers Superior Viable Cell Density and Increased mAb Titer Compared with Conventional 250 mL Bioreactors in Fed-Batch CHO Culture

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Abstract

Scale-down models are critical tools in biotherapeutic process development allowing higher-throughput experimentation outside of manufacturing scale. This study evaluates a side-by-side comparison of two 250mL bioreactor platforms running a fed-batch CHO cell culture process. We evaluated both systems at two agitation power inputs (19 and 30 W/m^3) with all other process parameters held constant and bioreactors were harvested on day 14 post inoculation. Both platforms demonstrated comparable VCD profiles and metabolite trends. Interestingly, the Stratyx system yielded higher cell viability and IgG titer at both conditions. Glutamine and glutamate levels also differed between platforms at different power density conditions. Importantly, product quality was determined to be equal across all vessels. These results suggest that the Stratyx 250 is a viable and potentially advantageous scale down model platform for fed-batch CHO processes. Performance improvements were likely due to the Stratyx 250's independent gassing architecture and precise feeding. These findings support the Stratyx 250 as a viable scale-down model for fed-batch CHO processes and illustrate Bionova Scientific's ability to deploy advanced bioreactor technologies to generate high-quality, decision-enabling data that de-risks development and accelerates progression toward manufacturing.

Introduction

The commercialization of biotherapeutics requires efficient and robust process development, which can translate from laboratory to manufacturing scale. Achieving this consistency relies on well-defined bioreactor characteristics between bioreactor scales, regardless of vendor or geometry. Scale down models (SDMs) are the workhorses for process optimization, characterization, and evaluating manufacturing changes. SDMs reduce resource consumption and high-throughput data generation via parallel operation. Organizations adept at managing diverse reactor geometries and control systems accelerate technology transfer, directly improving development timelines. The strategic use of state-of-the-art bioprocess equipment is key to achieving optimal process robustness, high yield and high quality, which in turn enhances operational efficiency and patient outcomes.

Bionova Scientific applies a phase-appropriate, platform-based upstream process strategy to rapidly generate comparable, decision-grade data across diverse bioreactor formats while maintaining a consistent control strategy and analytics package. Standardized platform elements including seed train, fed-batch schedule, set points, and sampling which enable efficient scale down model execution and accelerate translation to manufacturing by leveraging prior knowledge and a risk based development mindset. This approach aligns with ICH guidance emphasizing process understanding and robust control strategies as the foundation for consistent performance and streamlined lifecycle management. Using this platform process, we executed a controlled, side-by-side evaluation of Stratyx 250 and Ambr® 250 to isolate bioreactor-specific effects (e.g., mass transfer and control implementation) from process and cell line variability.

To establish process comparability and aid in optimization, we evaluated two distinct 250 mL scale down bioreactor platforms, running an identical biotherapeutic production process, with a variation in power density. These systems serve as reliable scale down models for larger manufacturing reactors. We conducted a parallel run comparing the industry-standard Sartorius Ambr® 250 (representative of larger CHO fed-batch processes) with the Culture Biosciences Stratyx 250. The Stratyx system features advanced, cloud-based control that facilitates real-time monitoring and integration with artificial intelligence and machine learning workflows. Both platforms were operated with the same seed train and process. Comprehensive in-process analytics were performed on samples, including measurements for viable cell density, viability, dissolved gases, metabolites, and product titer/quality attributes.

This paper compares the process performance and product quality metrics generated from the side-by-side operation of the Stratyx 250 and Ambr® 250 systems. The findings contribute to validating system equivalence and optimizing the utilization of next generation scale down tools in bioprocess development.

Materials and Methods

Study design
We performed a parallel, small-scale fed-batch comparison between Sartorius Ambr® 250 vessels and Culture Biosciences Stratyx 250 bioreactors. Two agitation power inputs (P/V) were evaluated to test platform comparability across gas-liquid mass transfer and mixing regimes while keeping all other parameters constant.

Cell line and media
NISTCHO cells expressing a recombinant IgG[1] were thawed and expanded per standard procedures. In short, cell bank was thawed into a T75 flask. When confluent, cells were resuspended in shake flasks for a total of 6 passages. Cultures used Excell Advanced CHO Fed-Batch Medium (Millipore Sigma) with supplements of Cellboost 7a and Cellboost 7b (Cytiva). Bioreactors were inoculated at 0.7e6 VC/mL and the process was run for 14 days under fed-batch control.

Parameter	Description		
Cell line	CHO-K1		
Culture Start Volume	190 mL		
Passage 6 Day 3 Count	5.4e6 viable cells/mL		
Temperature	36.5°C, Shift to 34°C on Day 7		
Target Density	0.7e6 viable cells/mL		
Feed Strategy	Cell Boost 7a: 4.2% of CSV on Days 2, 4, 6, 8, 10, and 12 Cell Boost 7b: 0.42% of CSV on Days 2, 4, 6, 8, 10, and 12		
Glucose Strategy	If less than 2 g/L pre-feed, bring up to 5 g/L.		
Harvest	Day 14		
Media	Excell Advanced CHO Fed-Batch Medium		
Condition Tested	Condition 1: 19 W/m3 Condition 2: 30 W/m3		
pH	7.00 ± 0.20		
pH control	Upper Deadband: CO2, as required Lower Deadband: 1M Sodium Carbonate, as required		
Dissolved Oxygen, DO	30%		
Agitation	**P/V** 19 W/m3 30 W/m3	**Ambr®250 (RPM)** 366 428	**Stratyx (RPM)** 328 377
Gassing Strategy	Overlay: N/A Air sparge through Macrosparger: 0.5 mL/min		

Sampling and analytics

Daily samples were taken for VCD/viability, metabolites, and product titer.

- VCD/viability: Vi-CELL Blu (Beckman Coulter)
- Chemistry (glucose, lactate, ammonium): CEDEX BioHT (Nova Biomedical)
- IgG titer: CEDEX BioHT (Roche Diagnostics)
- Gas/pH: RapidPoint 2000 Blood Gas Analyzer (Siemens)

Statistical methods used were two tailed t-test assuming unequal distribution comparing the equipment and agitation.

Results

Cellular viability and viable cell density (VCD) were quantified for both bioreactor platforms under each power input condition (Figure 1). On Day 14, cell viability was significantly higher in the Stratyx system compared to the Ambr system ($p=0.0017$). The 30 W/m^3 condition on Stratyx demonstrated statistically higher VCD. No statistically significant difference was observed in VCD under the 19 W/m^3 condition.

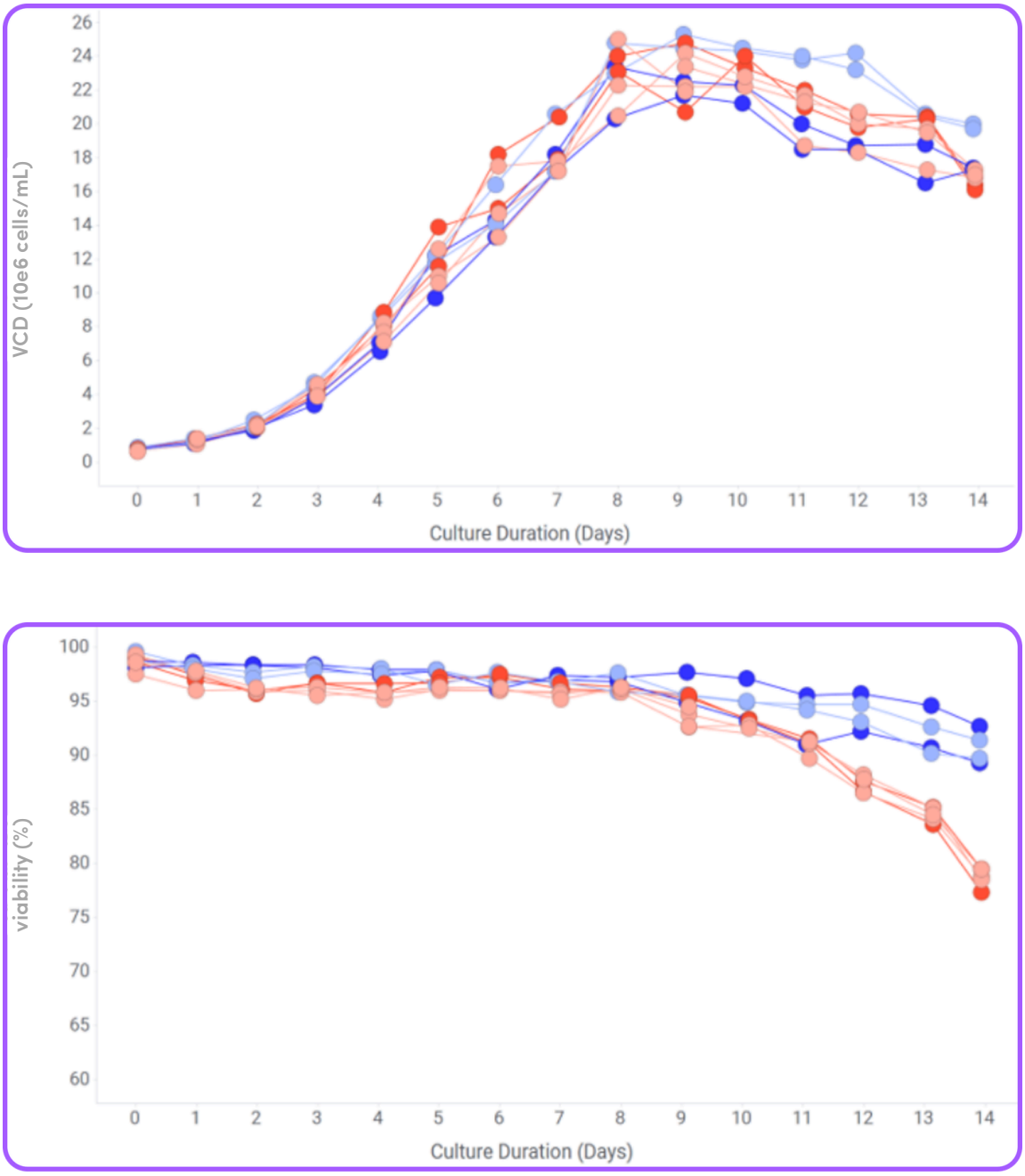
Glucose and Lactate samples on day 14 were not statistically different between either condition (Figure 2). This suggests that even with previously observed differences in cell viability, the CHO cells entered lactate production and consumption as expected.

Glutamine and Glutamate concentrations at day 14 were not significantly different in the 19W/m3 condition, but there was a significant difference in both amino acids in the 30W/m3 condition (Figure 3, $p=0.0009$ and $p=0.0308$ respectively). Stratyx Bioreactors demonstrated higher Glutamine and lower Glutamate compared to Ambr bioreactors. Ammonia levels were not significantly different between the two groups.

ProA product titer was determined to be statistically significant between both equipment at both conditions (Figure 4, $p=0.0028$ and $p=0.0033$ for 19 and 30 W/m3respectively). Stratyx resulted in higher product titer in both agitation conditions at day 14.

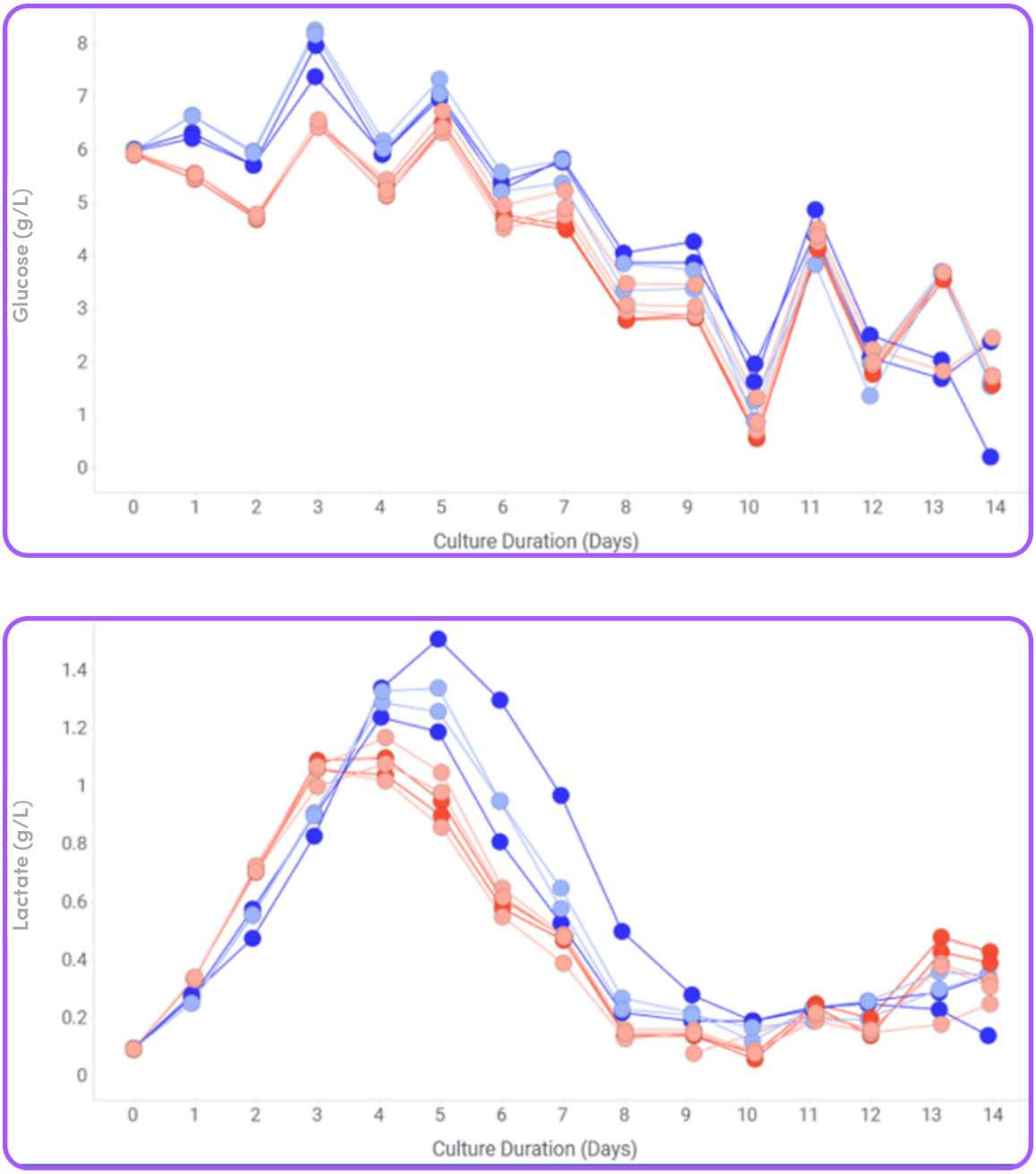
Harvested product from day 14 was assayed for product quality via CE-SDS and SEC analytical methods (Figure 5). All products were within the expected range for the assays evaluated, with limited fragmentation and expected light and heavy chain fragments.

Figure 1: Viable cell density and Viability



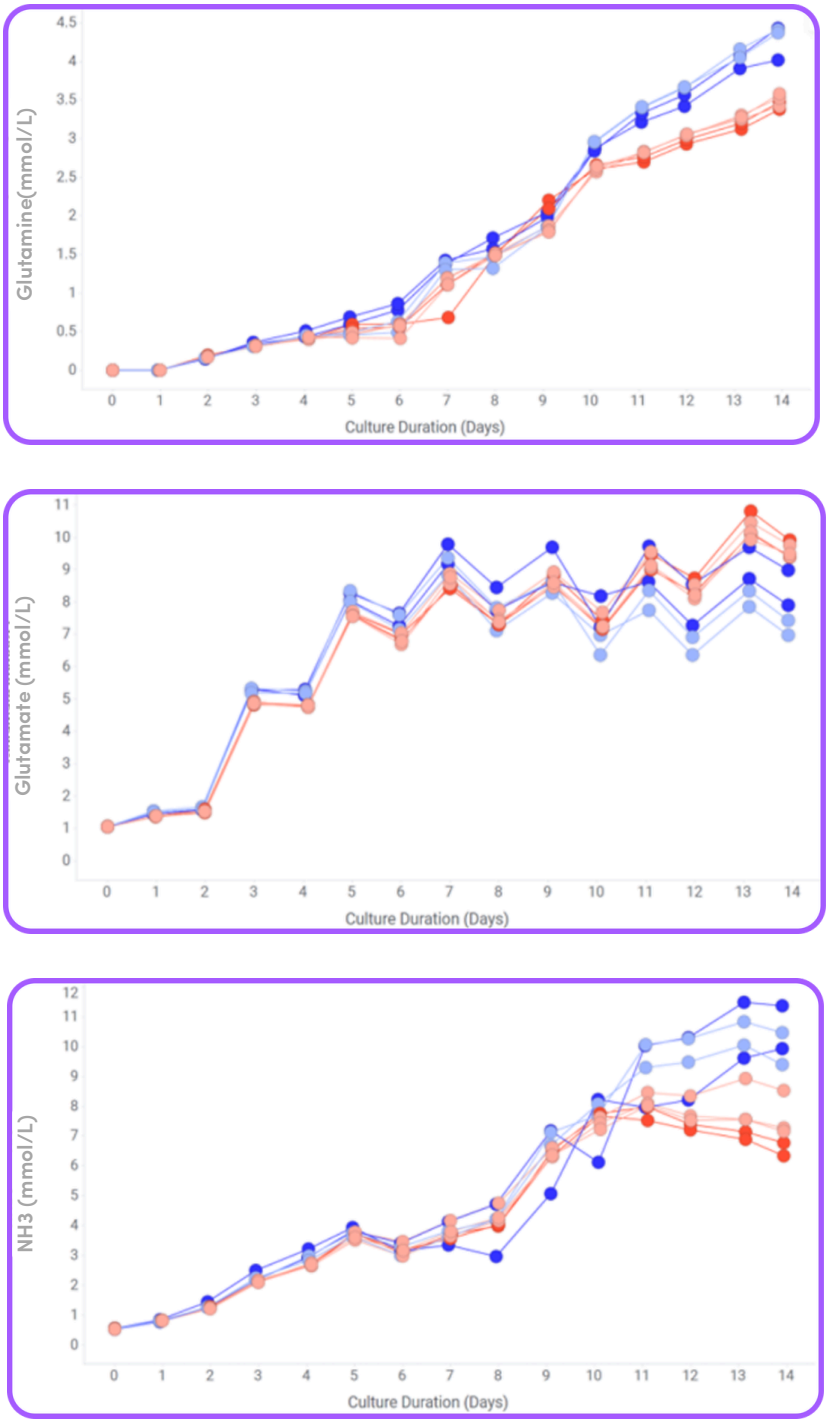
Viability and viable cell density (VCD) were assayed daily. Stratyx dark blue = 19W/m3, Stratyx light blue = 30W/m3, Ambr dark red = 19W/m3, Ambr light red = 30W/m3, each dot represents an independent bioreactor.

Figure 2: Glucose and Lactate



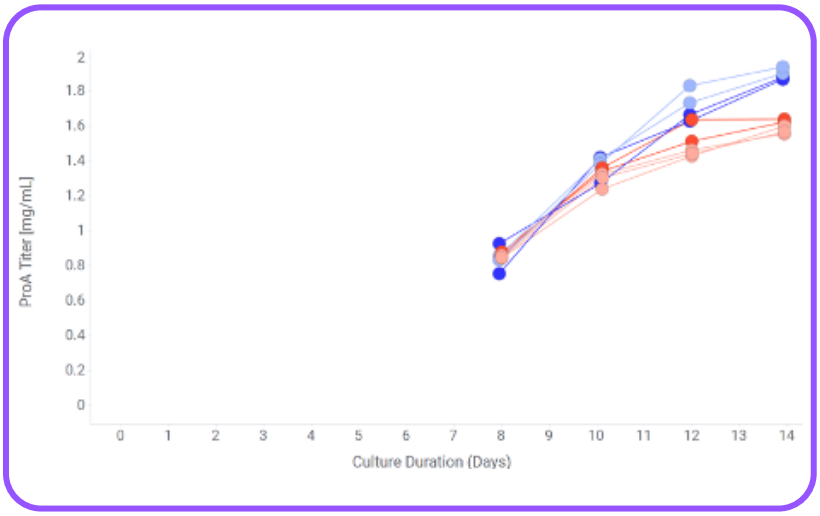
Glucose and Lactate were assayed daily. Stratyx dark blue = 19W/m3, Stratyx light blue = 30W/m3, Ambr dark red = 19W/m3, Ambr light red = 30W/m3, each dot represents an independent bioreactor.

Figure 3: Glutamine, Glutamate, Ammonia



Glutamine, Glutamate, Ammonia were assayed daily. Stratyx dark blue = 19W/m3, Stratyx light blue = 30W/m3, Ambr dark red = 19W/m3, Ambr light red = 30W/m3, each dot represents an independent bioreactor.

Figure 4: Product Titer



Product titer was assayed via Protein A at days 8, 10, 12, and 14. Stratyx dark blue = 19W/m3, Stratyx light blue = 30W/m3, Ambr dark red = 19W/m3, Ambr light red = 30W/m3, each dot represents an independent bioreactor.

Table 2: Product quality assays

		CE-SDS Non-Reduced	CE-SDS Reduced	SEC
Vessel	**Power**	%CPA IgG	%CPA IgG	% Main Peak
Stratyx	19	92.37	98.45	99.72
	30	92.15	98.54	99.70
Ambr®250	19	93.46	98.36	99.69
	30	93.11	98.40	99.73

Product quality was determined by Capillary Electrophoresis Sodium Dodecyl Sulfate (CE-SDS) non-reducing and reducing and as size exclusion chromatography at harvest (day 14). Data presented as average of all bioreactors in each condition.

Discussion:

Here we show a comparison of CHO fed-batch processes executed on 250mL bioreactor systems. We show that runs comparing two different small scale bioreactor systems were largely comparable with a few notable exceptions: Stratyx system at higher power per volume had increased cell viability (Average Stratyx 90.55%, 78.93%), higher Glutamine (Stratyx 4.39 mmol/L, Ambr 3.51 mmol/L) and lower Glutamate levels (Stratyx 7.2 mmol/L, Ambr 9.55 mmol/L) at day 14. Processes run in the Stratyx system also produced higher ProA product titer at both conditions (Stratyx 1.896g/L, Ambr 1.596g/L) while maintaining similar per cell production rates (Average Stratytx 0.1023 g/ 10^6 cells, Ambr 0.0956 g/ 10^6 cells). The product quality measurements that were taken showed no differences between the two equipment suggesting the increase in product titer did not coincide with changes in product quality.

Together this data shows that with an experienced process development team you are able to evaluate multiple process equipment and process parameters at the same time. This is advantageous as it allows you to select the best scale down model for your process. While there were differences between the equipment, in general processes run on Stratyx equipment resulted in higher cell viability and product titer without changing product quality measurements. A process that has higher cell viability and product titer would likely be better for downstream processing as impurity levels would likely be lower. The changes observed may be the result of fully independent gassing and more precise syringe-based feeding that are only available in the Stratyx bioreactor systems.

Conclusions:

We observed

- **Improved performance**. Both the Stratyx 250 and Ambr® 250 vessels displayed similar VCD trends; however, Stratyx consistently maintained significantly higher cell viability throughout the culture process.
- **Robust under stress**. Fed-batch CHO process showed similar glucose and lactate profiles in both systems
- **Scalable alternative**. Stratyx proved to operate comparably to the Ambr® System.

Citations:

1. Dahodwala H, Hodzic I, Slesarev A, Cutak B, Kuzin A, Lal R, Liu J, Mahon J, Narasimhan RL, Onuska J, Ravellette J, Reger K, Sakurada S, Stewart F, Borgschulte T, Cote C, Lee KH, O'Dell WB, Kelman Z, Anderson BA. Development and Characterization of the NISTCHO Reference Cell Line. Biotechnol J. 2025 May;20(5):e70012. doi: 10.1002/biot.70012. PMID: 40346766; PMCID: PMC12064881.