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Optimizing AAV8 Production: Addressing Manufacturing Challenges Through Scalable Process Development and Digital Integration

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Abstract

Adeno-associated viral vectors have emerged as increasingly prominent therapeutic modalities in gene therapy, with several serotypes in advanced clinical stages. However, scalable AAV manufacturing presents technical challenges that limit patient access. The fundamental manufacturing constraints include suboptimal vector yields, elevated production costs, inter-batch process variability, and inherent difficulties in scaling transient transfection methodologies from laboratory to industrial scales.

To address these manufacturing bottlenecks, Sartorius collaborated with Matica Biotechnology, a contract development and manufacturing organization specializing in viral vector production, to establish a robust and scalable upstream bioprocessing platform for AAV8 vector manufacturing. This integrated approach leveraged our high-throughput bioreactor systems (Ambr® 250 High Throughput), single-use intermediate-scale platforms (Univessel® SU with Biostat® B-DCU control), and production-scale bioreactors (Biostat STR® 50 L). Advanced process analytical technologies, including MODDE® for statistical experimental design and BioPAT® Process Insights software for predictive scale translation, were implemented to optimize critical parameters and ensure consistency across scales. The FectoVIR®-AAV transfection system provided standardized transfection performance throughout the scale-up.

The collaborative investigation demonstrated successful process scalability across a 200-fold volume range (250 mL to 50 L), maintaining consistent growth kinetics, cell viability, and viral genome titers. This work establishes a systematic framework for reproducible, scalable AAV8 manufacturing that addresses key industry challenges in viral vector production.

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Introduction

Adeno-associated viruses (AAVs) represent a transformative class of gene delivery vectors, distinguished by their favorable safety profiles, capacity for sustained transgene expression, and unique ability to transduce both mitotically active and quiescent cell populations. The therapeutic potential of AAV-based interventions is exemplified by approved treatments for rare genetic disorders, including spinal muscular atrophy (Zolgensma®) and inherited retinal dystrophies (Luxturna®), with numerous additional therapeutic candidates advancing through clinical development pipelines.[1,2]

Among the characterized AAV serotypes, AAV8 demonstrates particularly efficient hepatocyte transduction, positioning it as a preferred vector for liver-directed gene therapies.[3,4] This hepatotropism, combined with reduced immunogenicity compared to earlier AAV serotypes, makes AAV8 an attractive therapeutic platform for treating hepatic metabolic disorders and systemic diseases amenable to liver-based protein expression.

Despite these therapeutic advances, scalable AAV production remains a critical limitation that constrains broader patient access to these transformative therapies.

Modern manufacturing approaches predominantly rely on transient triple-plasmid transfection of HEK293 cell cultures, a methodology that, while established and regulatory-approved, introduces significant process complexities.[5] Key manufacturing challenges include substantial batch-to-batch variability in transfection efficiency, dependence on multi-plasmid expression systems requiring precise stoichiometric control, extended lead times for GMP-grade plasmid DNA production, and difficulties maintaining optimal culture conditions at industrial scales. These factors frequently result in suboptimal vector yields, unfavorable ratios of functional to empty viral particles, and inconsistent process performance during scale-up operations.

Addressing these manufacturing constraints requires a systematic, platform-based approach that integrates advanced bioprocess technologies with robust analytical capabilities. Sartorius has developed a comprehensive technology portfolio specifically designed to overcome AAV manufacturing bottlenecks. This platform encompasses:

- High-throughput automated bioreactor systems (Ambr® 15 and Ambr® 250 High Throughput) that enable rapid, parallelized process optimization and media screening
- Geometrically similar single-use bioreactor systems (Univessel® SU and Biostat STR®) that minimize scale-up risks through consistent hydrodynamic environments
- GMP-grade plasmids, engineering services, and specialized transfection reagents (FectoVIR®-AAV) that enhance transfection consistency and efficiency
- Advanced process analytical technologies (MODDE®, SIMCA®, BioPAT® Process Insights) that accelerate development timelines through statistical modeling and predictive scale-up capabilities.

This investigation, conducted through a strategic collaboration between Sartorius and Matica Biotechnology, demonstrates the practical implementation of this integrated technology platform for upstream AAV8 process development. The primary objective was to establish a reproducible and efficient AAV8 production process capable of seamless scaling across various bioreactor platforms while maintaining consistent product quality and process robustness.

Materials & Methods

The experimental approach employed a systematic three-stage development strategy, progressing from small-scale process characterization to intermediate-scale validation and ultimately to pilot-scale confirmation.

Cell culture and transfection methodology

HEK293 suspension-adapted cells served as the production host due to their established performance in AAV manufacturing applications and regulatory acceptance.[6] Following standard thawing procedures, cells underwent sequential seed train expansion before inoculation into respective bioreactor systems. Transient transfection was performed 48 hours post-inoculation using the FectoVIR®-AAV transfection system (Sartorius Polyplus) with previously optimized plasmid combinations. Cultures were maintained for 72 hours post-transfection to enable viral particle assembly and maturation, followed by standardized cell lysis, nuclease digestion, and harvest procedures for AAV8 recovery.

Bioreactor platform configuration

Small-scale optimization: The Ambr® 250 High Throughput system (Sartorius) enabled parallel operation of 12 independent bioreactors under precisely controlled conditions, facilitating systematic evaluation of critical process parameters, including agitation rate and aeration strategy, through design of experiments (DoE) methodology.

Intermediate-scale validation: Univessel® SU 2 L bioreactors (Sartorius), controlled via the Biostat® B-DCU system (Sartorius), provided dual-reactor capability for process validation and sufficient biomass generation for downstream process development activities.

The Biostat® B-DCU ensured precise monitoring and control of critical process parameters, enabling consistent bioreactor performance and reproducible data capture across development runs. Its alignment with the control strategy used for larger-scale Biostat STR® systems simplified process comparability and supported predictive scale translation.

Pilot-scale demonstration: A 50 L Biostat STR® single-use bioreactor (Sartorius), supported by a Biostat® RM 20 | 50 N-1 seed bioreactor, demonstrated production-relevant scalability while maintaining geometric similarity to smaller-scale systems.

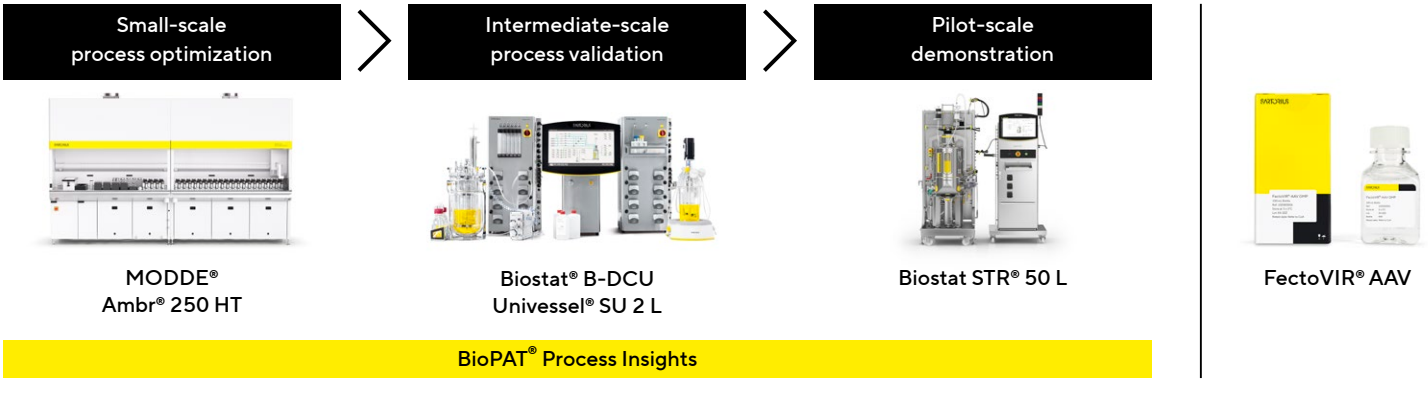
A shake flask culture treated equivalently to the respective bioreactor culture was always run in parallel as a reference control.

Process analytical and scaling technologies

MODDE® software (Sartorius) facilitated the statistical analysis of experimental outcomes, enabling the identification of robust operating parameters within the defined design space. A 2-factor full factorial design was selected. Each factor was tested at two levels, and together with the four center points, 11 experiments were performed. The design was chosen to identify a potential interaction term.

BioPAT® Process Insights software (Sartorius) provided predictive scaling capabilities by calculating equivalent hydrodynamic conditions (stir speed and gassing rate) across bioreactor scales, thereby maintaining consistent mass transfer and mixing characteristics (i.e., proportional gassing rates and power-to-volume ratios) essential for reproducible process performance.

Figure 1: Upstream process development workflow and the tools applied at each scale



Results & Discussion

Process transfer and scale-up strategy

The investigation was based on an established AAV8 production process originally developed in a Univessel® Glass 10 L system at Sartorius, where critical process parameters, including plasmid stoichiometry, plasmid loading, and transfection cell density, had been previously optimized. Building on this foundation, the focus of the joint project was the transfer and further scaling of the process to additional bioreactor formats and volumes. This reference process, characterized by a power-to-volume input of 15 W/m^3 and a proportional gas flow rate of 0.03 vvm, served as the basis for subsequent scale translation activities. Owing to technical constraints, the proportional gas flow rate for the 10 L source bioreactor was limited to 0.01 vvm.

BioPAT® Process Insights software enabled systematic translation of these reference conditions across bioreactor scales by maintaining consistent engineering parameters. Table 1 presents the calculated process setpoints for each bioreactor platform, providing the framework for experimental validation.

Small-scale process optimization

Initial process development at Matica Biotechnology employed the Ambr® 250 High Throughput platform for systematic parameter evaluation through DoE methodology. Twelve bioreactors operated in parallel across a defined design space: agitation rates of 219 – 460 rpm (factor: stir) and gas flow rates of 2.2 – 12.8 mL/min (factor: gas). This experimental design aimed to identify operating conditions that maximized both viral genome and capsid titers while maintaining robust cellular growth.

The results demonstrated that viral genome titers achieved in the Ambr® 250 High Throughput system were comparable to parallel shake flask controls, with optimal performance observed at 319 rpm agitation and 2.2 mL/min gas flow rate (BR1 conditions; Figure 2A). However, while these conditions yielded slightly elevated titers relative to shake flask controls, the corresponding viral capsid-to-genome ratio was suboptimal. Further analysis identified that BR2 conditions (319 rpm, 7.5 mL/min) provided a more favorable capsid-to-genome ratio, leading to the selection of these parameters for scale-up activities.

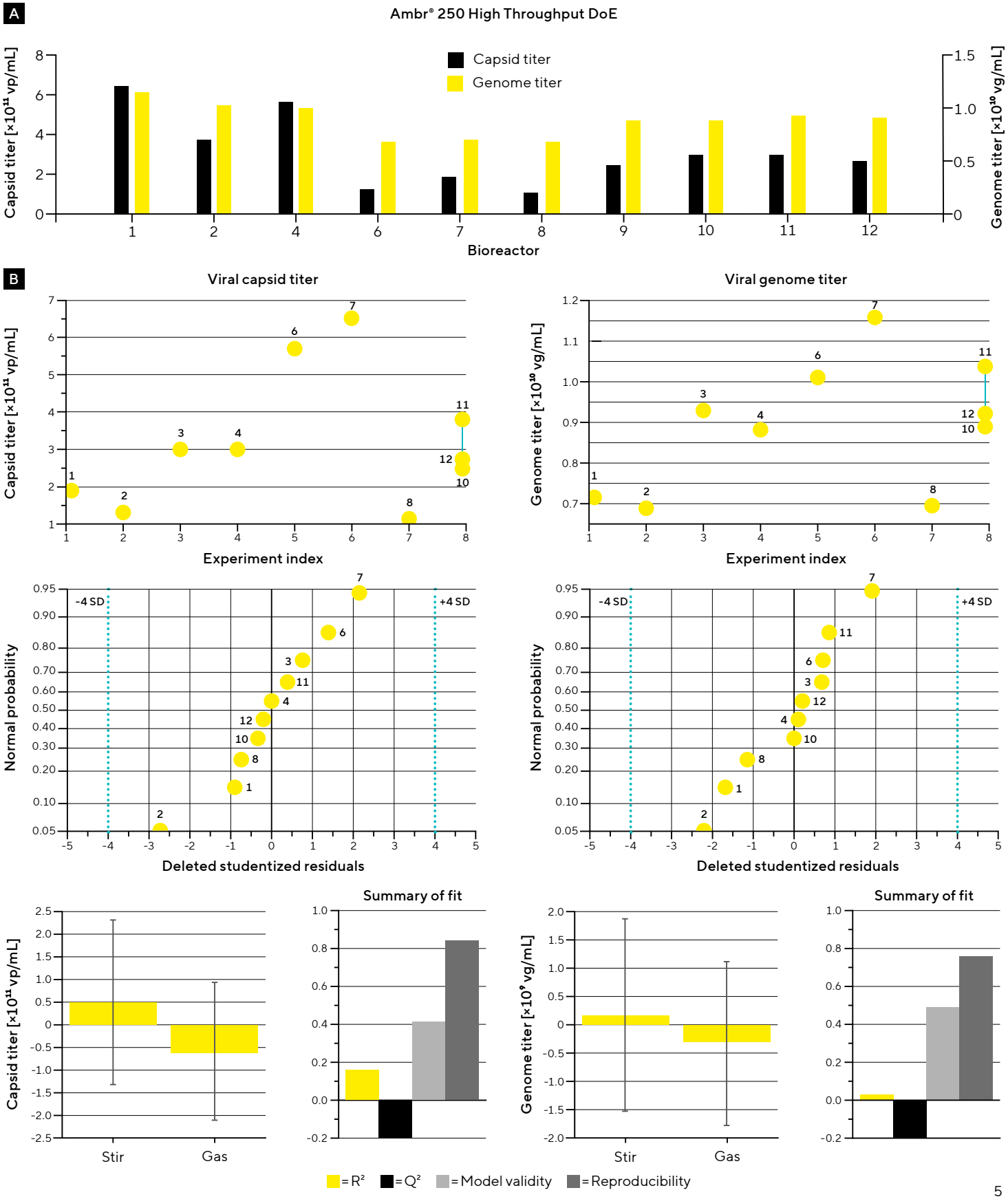
Table 1: Scaled process parameters across bioreactor platforms

Bioreactor system	Proportional gassing rate [vvm]	P/V [W/m^3]	Stir speed [rpm]	Gassing rate [L/min]	kLa [h^{-1}]	Mixing time [s]	Tip speed [m/s]
Ambr® 250 High Throughput	0.03	15	265	0.0075	3.2	7.9	0.36
Univessel® SU 2 L	0.03	15	186	0.06	1.9	10.1	0.56
Univessel® Glass 10 L	0.01*	15	201	0.1	2.8	29.2	0.82
Biostat STR® 50 L	0.03	15	135	1.5	3.7	13.0	1.01

*0.01 vvm was used instead of 0.03 vvm due to technical constraints. Black: constant parameters. Yellow: setpoints for each process scale.

In addition, statistical analysis using MODDE® revealed that neither agitation rate nor gas flow rate was statistically significant within the tested range. Overall, the model diagnostic also shows that there was no significant model found (R^2 below 0.2 and negative Q^2 for both responses), indicating inherent process robustness within the experimental design space with respect to the tested scaling parameters (Figure 2B). This finding provided confidence for scale-up progression, as tolerance to moderate process variations is essential for manufacturing applications.

Figure 2: Viral genome (vg) and viral capsid (vp) titer for each bioreactor of the Ambr® 250 High Throughput run **(A)**, DoE results from analysis of impact of stir speed and gas flow rate on viral capsid and genome titers **(B)**.



Intermediate-scale process validation

Process transfer to Univessel® SU 2 L bioreactors utilized BioPAT® Process Insights software to translate the initially applied 10 L scale and further optimized Ambr® 250 High Throughput conditions to equivalent hydrodynamic parameters. Duplicate experimental runs were conducted to validate process consistency and generate approximately 4 L of harvest material for downstream development activities. The duplicate runs were executed twice, yielding data from four bioreactor runs in total.

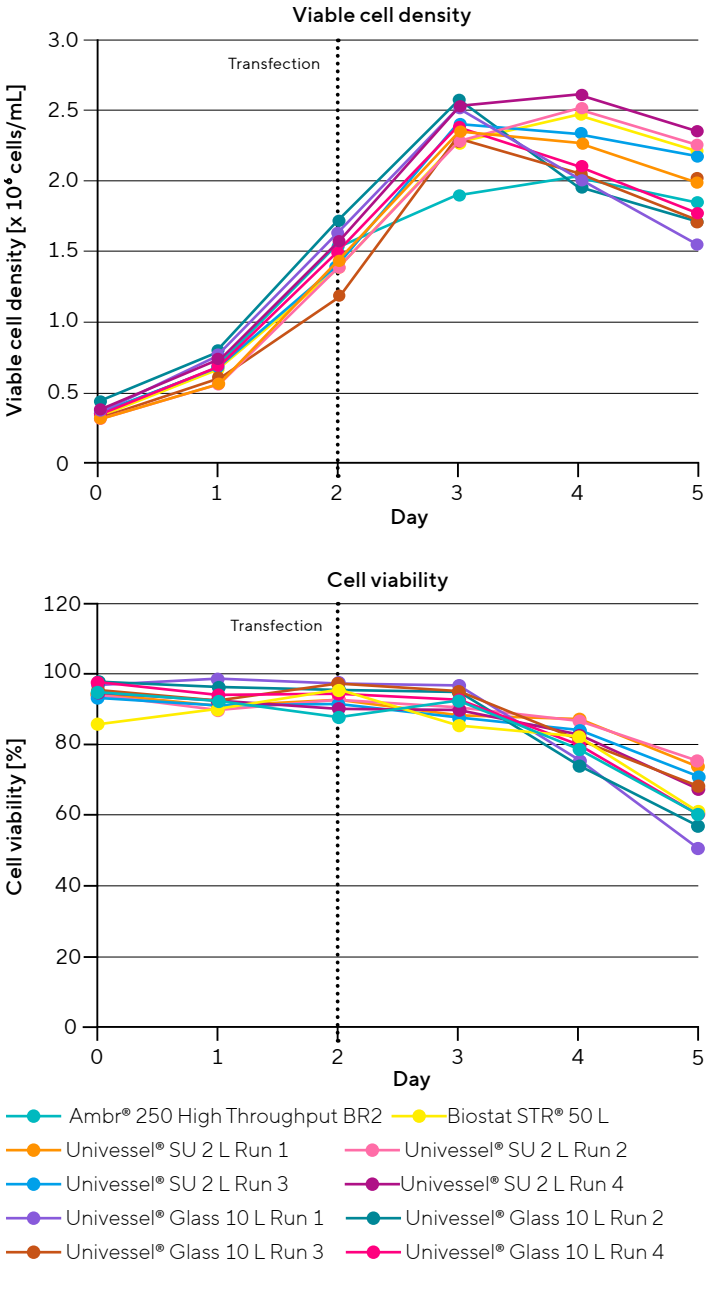
Performance comparison across scales, including data from the Univessel® Glass 10 L source bioreactor, revealed highly consistent viable cell densities throughout the culture period. The 2 L cultures achieved slightly higher maximum cell concentrations than the Ambr® 250 High Throughput system, while demonstrating excellent agreement with the 10 L reference process (Figure 3). Cell viability remained consistently elevated across all bioreactor platforms throughout culture duration, exhibiting the expected modest decline following transfection due to viral production-associated metabolic stress. This consistent viability profile across the 250 mL, 2 L, and 10 L scales provided strong evidence for successful process translation using BioPAT® Process Insights software and effective technology transfer between facilities.

To further characterize process consistency, metabolic parameters including glucose, lactate, and ammonium concentrations were monitored across all bioreactor scales (Figure 4). Glucose consumption profiles demonstrated comparable kinetics across the Ambr® 250 High Throughput, Univessel® SU 2 L, and reference 10 L systems, with similar depletion rates throughout the culture period.
Lactate accumulation patterns likewise showed remarkable consistency between scales, indicating equivalent metabolic states and culture conditions. Ammonium levels remained within acceptable ranges across all platforms, with no significant differences observed that would suggest scale-dependent metabolic stress or suboptimal culture conditions.

The consistency of these metabolic markers across platforms further validated the robustness of the scaling approach and demonstrated that the BioPAT® Process Insights software successfully maintained equivalent physiological environments despite differences in vessel geometry and operating volumes.

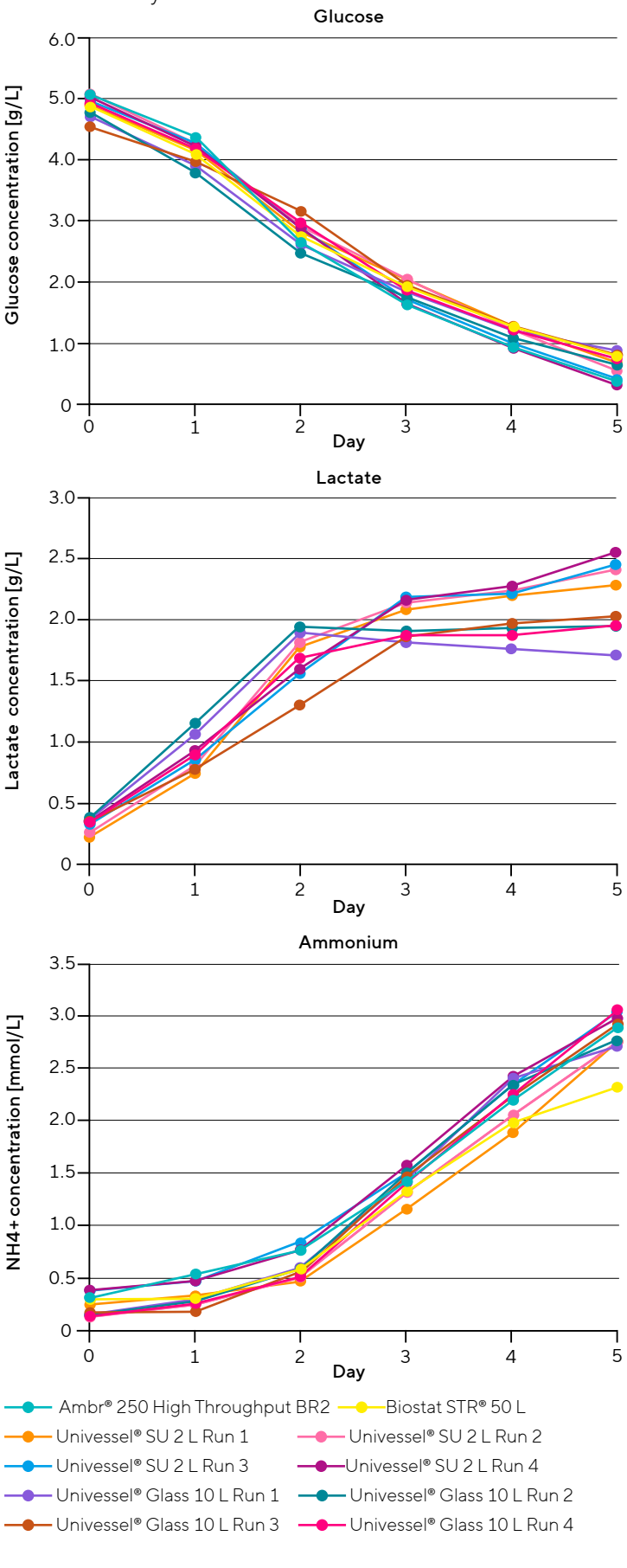
The viral genome titers measured at the 2 L scale were slightly higher than those obtained at the Ambr® 250 High Throughput (Figure 5)—which likely reflects the observed lower cell growth in this system—and significantly higher than the reference shake flask, confirming that the process could be successfully transferred without loss of productivity.

Figure 3: Cell growth and viability profiles across all bioreactor systems



Note. BR = bioreactor

Figure 4: Concentration profiles of major metabolites across all bioreactor systems

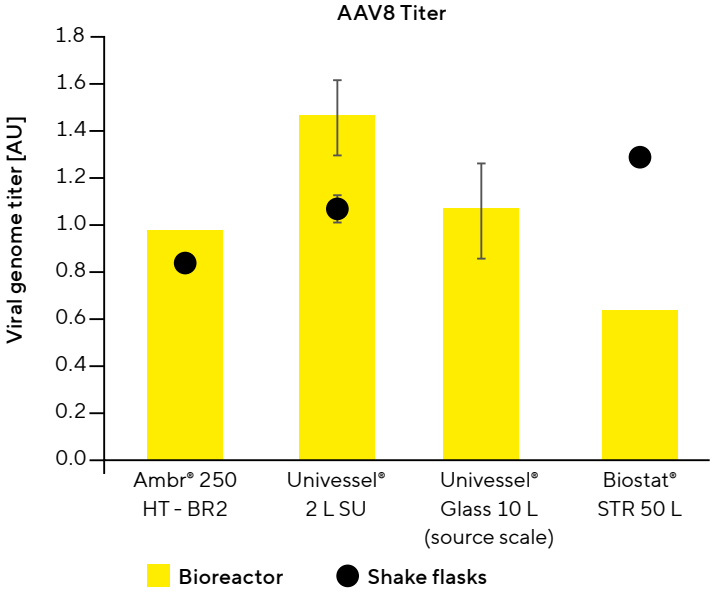


Pilot-scale demonstration

The final validation employed a 50 L Biostat STR® system to demonstrate production-relevant scalability and generate substantial harvest volumes for downstream process development. Cellular growth, viability, and metabolite profiles closely replicated the 2 L and 10 L bioreactor performance characteristics, maintaining high viability until post-transfection with subsequent gradual decline (Figures 3 and 4).

Viral genome titers at 50 L scale remained largely consistent with smaller-scale results, though a modest reduction was observed (Figure 5). This decrease was attributed to operational challenges inherent to initial large-scale execution, particularly transfection at high culture volumes, rather than fundamental process limitations. Continued process repetition and refinement of operator experience are expected to resolve these technical considerations.

Figure 5: AAV8 viral genome titer comparison across all bioreactor systems



Note. BR = bioreactor. Only the best bioreactor from Ambr® 250 High Throughput is shown; mean of N = 4 for Univessel® SU 2 L and Glass 10 L ± StDev; shake flasks were run in parallel to each bioreactor run.

Conclusion

Process robustness and technology transfer

The investigation successfully demonstrated process reproducibility and scalability across a 200-fold volume range while maintaining consistent performance metrics. Technology transfer between Sartorius development laboratories and Matica Biotechnology production facilities proceeded seamlessly, with comparable process performance and product titers achieved across both organizational platforms and bioreactor systems. The consistent Sartorius control architecture from the Biostat® B-DCU at the 2-L and 10-L scales to the Biostat STR® system at 50 L played an essential role in maintaining equivalent process conditions and data structures across scales and sites. This standardization minimized variability and enabled a smooth, data-driven technology transfer process.

These results validate the integrated platform approach, demonstrating that combining high-throughput process development systems, predictive scaling software, optimized transfection reagents, and geometrically similar bioreactor technologies can effectively address key bottlenecks in AAV manufacturing.

This collaborative investigation between Sartorius and Matica Biotechnology provides compelling evidence for establishing reproducible, scalable upstream processes for AAV8 production using integrated bioprocessing platforms. The systematic progression from high-throughput process characterization (Ambr® 250 High Throughput) through intermediate-scale validation (Univessel® SU 2 L) to production-scale demonstration (50 L Biostat STR®) achieved consistent performance across scales with comparable growth profiles and viral titers.

The successful implementation of BioPAT® Process Insights software for predictive parameter scaling, combined with robust transfection performance from Sartorius reagents and consumables, demonstrates the practical value of platform-based approaches in viral vector manufacturing.

Particularly significant was the seamless technology transfer between organizations, highlighting how standardized, systematic methodologies can reduce development timelines and mitigate scale-up risks.

The findings establish a strong foundation for the Sartorius integrated platform as an enabler of industrial-scale AAV manufacturing, providing practical solutions to persistent industry challenges while accelerating the translation of promising gene therapies from development laboratories to clinical and commercial production environments.

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