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Scaled-Down Perfusion Optimization With the Ambr® 250 High Throughput System

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Abstract

Innovation and improvement in bioprocessing are essential to enhancing global access to and affordability of advanced biotherapeutics. Using the Ambr® 250 High Throughput system in perfusion mode, rather than traditional bench-scale perfusion setups, can reduce the cost, time, and effort required to develop product-representative scale-down models.

Through a series of three experiments over 25 days, we evaluated various critical process parameters and processing strategies while verifying the perfusion capabilities of the Ambr® 250 High Throughput system. The study confirms that high productivity and product quality can be achieved with minimal resource use and facility space when the influence of key process conditions is well understood. Additionally, the study included a comprehensive clone screening to identify top-performing clones at the 250 mL scale, focusing on key metrics such as titer, specific productivity, growth rates, peak viable cell density, and viability.

The Ambr® system demonstrated its ability to maintain performance levels comparable to historical processes while offering significant cost and space savings, which are crucial for enhancing biotherapeutic production. This advancement supports more efficient and affordable biotherapeutic production and further emphasizes the potential for innovation in bioprocessing.

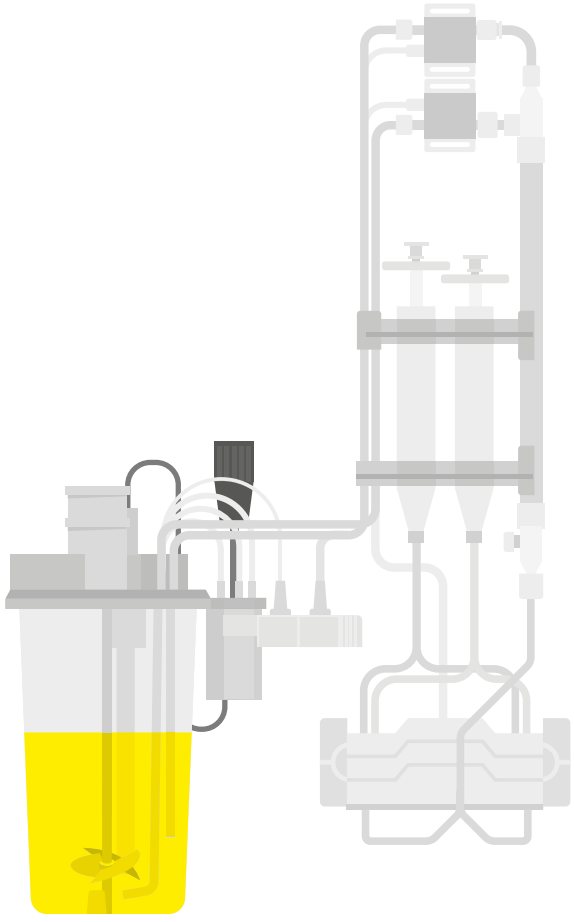
Introduction

Optimizing processes to enhance productivity and reduce costs drives innovation and advancements in bioprocessing. The development and manufacturing of transformative biologic solutions using benchtop bioreactor systems in continuous operation mode, specifically through perfusion operation, enhances productivity and reduces cost of goods. In a traditional benchtop setup, there are inherent limitations on throughput and footprint, along with higher costs for resources such as media, feeds, consumables, and labor. However, smaller-scale bioreactors, available on the Ambr® 250 High Throughput system, reduce footprint and capital expenditure, while offering the flexibility and scalability required for higher-throughput production within the same facility.

The Ambr® 250 High Throughput system can be operated in perfusion mode (Figure 1), providing a scalable model for evaluating or transitioning established processes to perfusion. By migrating to the Ambr® 250 High Throughput system, users gain nearly threefold more experimental conditions per square foot of lab footprint, while the sevenfold reduction in working volume per bioreactor drives proportional savings in reagent and consumable costs. Ambr® multi-parallel bioreactors offer additional features, including process automation, liquid handling, advanced software control, single-use capabilities, and integrated analytical devices, which reduce overall labor cost and development time.

We collaborated with Just-Evotec Biologics, an innovative CDMO for complex biologics and leader in end-to-end continuous manufacturing, to evaluate the performance of their class-leading upstream perfusion process in the Ambr® 250 High Throughput system. In total, three experiments with 21 Ambr® 250 bioreactor conditions were conducted over a 25-day period and compared to Just-Evotec's historical 3 L benchscale platform data. The design of experiments (DOE) conditions included varying agitation speed, crossflow rates, foam mitigation strategies, bleed methods, and cell clones, which are key levers in determining the success of this scale-down trial.

Figure 1: Ambr® 250 High Throughput perfusion vessel

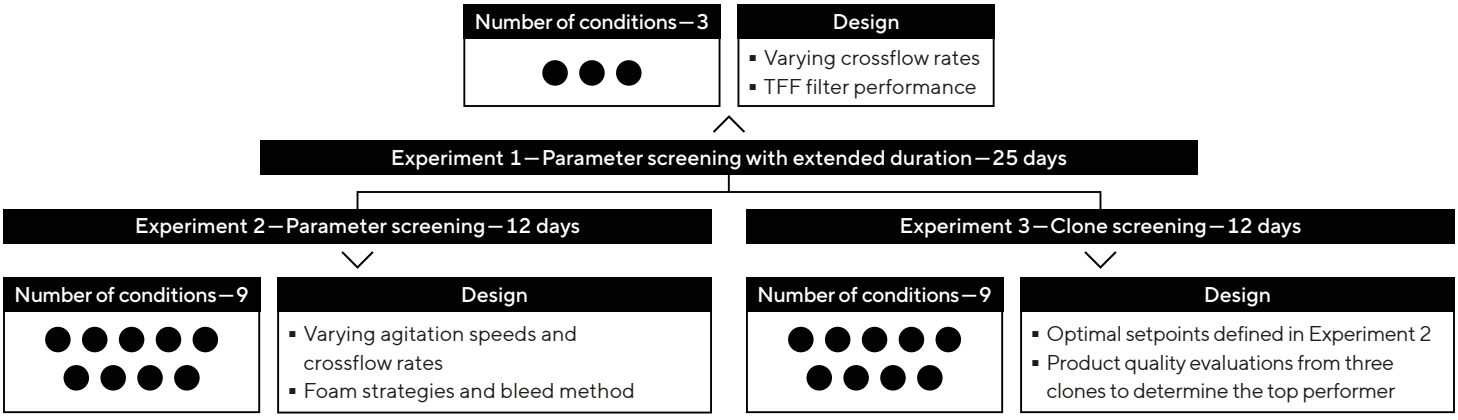


Materials and Methods

Overview of experimental design

The experimental design is presented in Figure 2. During Experiment 1, three bioreactors were operated for 25 days under the conditions outlined in Table 1. For Experiment 2, we tested a variety of conditions over 12 days across nine bioreactors (Table 1). The optimal setpoints for these experiments were used for Experiment 3, where three separate clones were tested across nine bioreactors (Table 3).

Figure 2: Visual overview of the experimental design



Note. TFF = tangential flow filtration

Ambr® 250 High Throughput 12-way perfusion system

Over a 25-day period, the Ambr® 250 High Throughput 12-way perfusion system, integrated with BioProfile® FLEX2 and the Ambr® Analysis Module, was used to evaluate the performance of perfusion processes using a CHO cell line and specific medium. The perfusion strategy is an established process at Just – Evotec Biologics. DOE conditions were defined for three experiments (Figure 2) comprising a total of 21 bioreactors using MODDE® software. The definition of the Ambr® process focused on incorporating the scaled-down agitation speed, derived from tip speed and power per volume, within the design space.

Daily samples were taken from each bioreactor to measure viable cell density (VCD), viability, various metabolites (glucose, glutamine, glutamate, lactate, potassium, sodium, and calcium), electrolytes, and osmolality using an integrated BioProfile® FLEX2. pH was monitored daily using the integrated Ambr® Analysis Module. Titer measurements were initiated on Day 2 of perfusion using the Octet® R8 system. During perfusion, daily permeate samples were collected manually, and titer measurements were taken.

Sartorius software

DOE conditions were defined in MODDE® software, and included varying agitation speeds, crossflow rates, foam mitigation strategies, bleed methods, and cell clones. The selection of agitation speeds was based on historically relevant bioreactor scaling parameters, including power per volume, tip speed, and kLa. An expanded view of the DOE is provided below. Results from each of the three experiments were compared to historical data provided by Just – Evotec Biologics using batch evolution analysis in SIMCA® software to confirm comparable performance by the Ambr® system.

Setup of the DOE and Experiments 1 and 2

An overview of the conditions in each bioreactor from Experiments 1 and 2 is shown in Table 1. Further details of the conditions are shown in the following sections.

Table 1: DOE setpoints for bioreactors in Experiments 1 and 2

Bioreactor	Perfusion strategy	Crossflow rate	Agitation speed	Foam mitigation strategy	Bleed method
1 (Experiment 2)	Just—Evotec Biologics	A (low)	D (low)	G	J
2 (Experiment 2)	Just—Evotec Biologics	A (low)	F (high)	G	J
3 (Experiment 2)	Just—Evotec Biologics	B (medium)	E (medium)	G	J
4 (Experiment 2)	Just—Evotec Biologics	B (medium)	F (high)	G	J
5 (Experiment 2)	Just—Evotec Biologics	C (high)	E (medium)	G	J
6 (Experiment 2)	Just—Evotec Biologics	C (high)	E (medium)	G	J
7 (Experiment 2)	Just—Evotec Biologics	C (high)	E (medium)	G	J
8 (Experiment 2)	Just—Evotec Biologics	C (high)	E (medium)	G	K
9 (Experiment 2)	Just—Evotec Biologics	C (high)	E (medium)	H	J
10 (Experiment 1)	Just—Evotec Biologics	A (low)	E (medium)	G	J
11 (Experiment 1)	Just—Evotec Biologics	B (medium)	E (medium)	G	J
12 (Experiment 1)	Just—Evotec Biologics	C (high)	E (medium)	G	J

Foam management strategies

We compared two distinct foam management strategies, Strategy G and Strategy H. Bioreactors 1–8 and 10–12 operated under Strategy G, while bioreactor 9 utilized Strategy H (Table 1):

- Strategy G: A 3% antifoam solution was dosed only when necessary to minimize total antifoam usage. This approach employed a multi-level control loop designed to introduce antifoam when the optical foam sensor reached a predetermined value for a certain duration.

- Strategy H: A semi-continuous dosing methodology was employed, in which an antifoam dose was administered regardless of the current foam levels, four times a day. The dose amount varied per day; as process time increased, the dose also increased until it reached a maximum volume.

Bleed strategies

We compared two bleed strategies, Strategy J and Strategy K. Bioreactors 1–7 and 9–12 operated under Strategy J, while bioreactor 8 utilized Strategy K (Table 1).

- Strategy J: The Ambr® 250 control software has the option of 4 different VCD control modes (Table 2). For this study, control method 4 was selected. Historical data were used to determine a suitable growth rate, and fail-safes were built into the protocol to limit the impact of rogue high or low VCD measurements from the integrated BioProfile® FLEX2 analyzer. As bleed control is process-dependent, please contact your local Sartorius specialist to discuss the best VCD control strategy for your process.

- Strategy K: This strategy relied on an empirically derived control formula that tracks cell growth from previous measurements and past bleeds to adjust current bleed output.

Table 2: VCD control modes available on the Ambr® 250

VCD control mode	Description	Comments
1. Fixed bleed volume [%]	User defines the daily bleed as a percentage of vessel volume.	Simple, easy to calculate, but lacking in adaptability.
2. Bleed in response to offline | at-line VCD	User enters measured VCD, system will calculate bleed required, replacing volume lost with fresh media.	Simple, more adaptable, and accurate than mode 1. No fail-safe against large fluctuations.
3. Bleed in response to online VCD measurement	Bleed can be initiated from a number of online proxy measurements pulled from system variables like O_2 flow. Control logic constructed from user-selected variables.	Higher responsiveness than modes 1 and 2, as well as inherent resistance to inaccurate VCD measurements. Accuracy based on proxy and empirically derived formulae.
4. Bleed using target cell density control loop	System will calculate bleed based on VCD and user-defined or system-calculated growth rate. Predictive | feedforward control based on VCD feedback.	Potential for high accuracy and high process adaptability. More complex logic can be challenging to set up for undefined and | or new processes.

Evaluation procedure

With the support of Sartorius experts and tools, an established industrial 3 L perfusion process was successfully transferred to the Ambr® 250 High Throughput system to optimize parameters and ensure consistent results. The procedures that followed are outlined below.

Setup of Ambr® 250 High Throughput system and inoculation for Experiments 1 and 2

The initial phase focused on preparing the Ambr® 250 High Throughput system and inoculating the bioreactors for Experiments 1 and 2. This included completing essential startup procedures, such as finalizing the protocol, executing clean-in-place (CIP) protocols, installing perfusion assemblies, priming pumps, filling media, and conducting any necessary analyzer maintenance. Additionally, the seed expansion passages were completed, culminating in the automated inoculation of 12 bioreactors using the Ambr® liquid handler.

Sampling, protocol updates, and data analysis

During the next phase, the focus shifted to sampling (bioreactors and their permeate), collecting retentates, and updating certain aspects of the protocol. Protocol updates included adjusting control loops for dissolved oxygen (DO), pH, and antifoam. PID parameters were optimized to accommodate high cell densities. Preliminary data analysis of the experiments was also conducted to determine the setpoints for Experiment 3.

Transition to Experiment 3

Based on the performance of key bioreactor setpoints, the protocol for Experiment 3 was designed to reflect the optimal conditions, determined primarily by cell growth and health metrics. Similar to the initial phase, a typical Ambr® system setup, including finalizing the protocol, priming pumps, and filling media, was performed. Notably, bioreactor stations 1 – 9 were reused by loading a new protocol into the software, enabling adaptation to revised new requirements. The transition between experiments was completed within one day, with Experiment 2 concluded and Experiment 3 initiated on the following day. This short turnaround time for nine vessels demonstrates efficient plug-and-play operation of the Ambr® system and supports rapid progression between experimental runs.

Nine new bioreactors were inoculated with three different cell clones (Clone X, Clone Y, and Clone Z) as per the DOE strategy (Table 3). Each cell line was run in triplicate, leveraging the best setpoint conditions from Experiment 2. During this time, Experiment 1 was still underway, and the sampling and data analysis routine continued.

Completion and data processing

The final phase involved completing the sampling process and all necessary Ambr® takedown procedures, such as unloading bioreactors, sterilization-in-place (SIP) protocols, and decontaminating the system. This also included the start of data compilation, sharing, and analysis, setting the stage for potential future experiments and evaluations.

Table 3: Clones for Experiment 3

Bioreactor	Clone
1	X
2	X
3	X
4	Y
5	Y
6	Y
7	Z
8	Z
9	Z

Note. The parameters for Experiment 3 were determined based on the data from Experiment 2 (see Results).

Results and Discussion

Experiment 1

Experiment 1 focused on optimizing bioreactor performance and evaluating the filter's capacity limits. Through careful monitoring of transmembrane pressure (TMP) and VCD, the experiment aimed to achieve operational stability and sustain effective crossflow rates without requiring filter replacement.

After 18 – 19 days, all bioreactors operated under low viability conditions as depicted in Figure 3. As illustrated in Figure 4 (right), only bioreactor 11 — which used a medium crossflow rate — exhibited an increase in TMP around days 19 – 20 of the process. Despite the steady rise, the TMP remained within a manageable range. The bioreactor continued to operate effectively and maintained the intended crossflow rate until the end of the experiment, without requiring a filter change. The flux was approximately equal to that of the historical process used for comparison.

In addition to focusing on the primary DOE parameters, specific control loops were optimized to achieve precise performance. Initially, the DO trends displayed significant noise (Figure 5). However, by fine-tuning the control PID settings, the DO levels successfully stabilized, ensuring reliable control over the setpoint.

Results from Experiment 1 facilitated the process of determining the initial setpoints for Experiments 2 and 3.

Figure 3: VCD and viability trends for Experiment 1

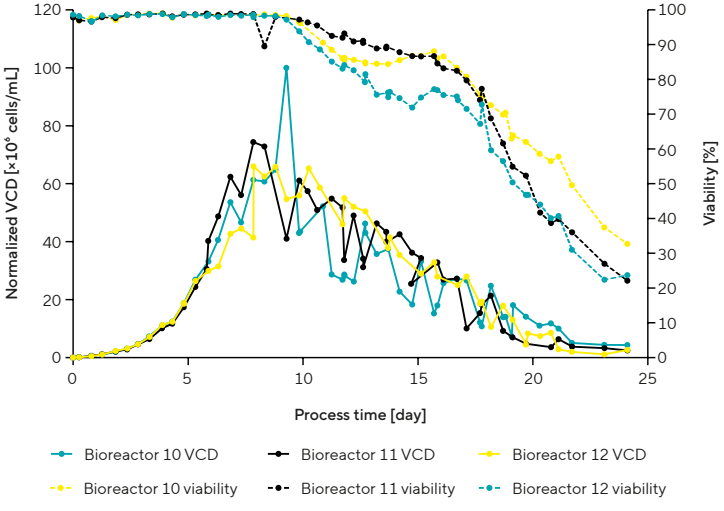
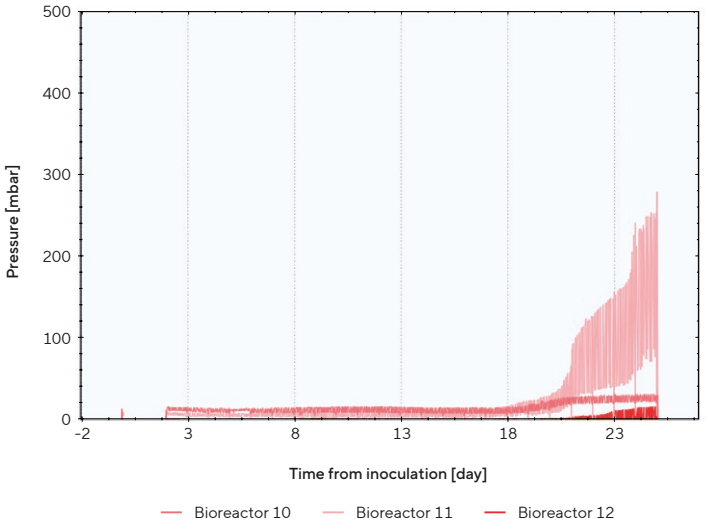
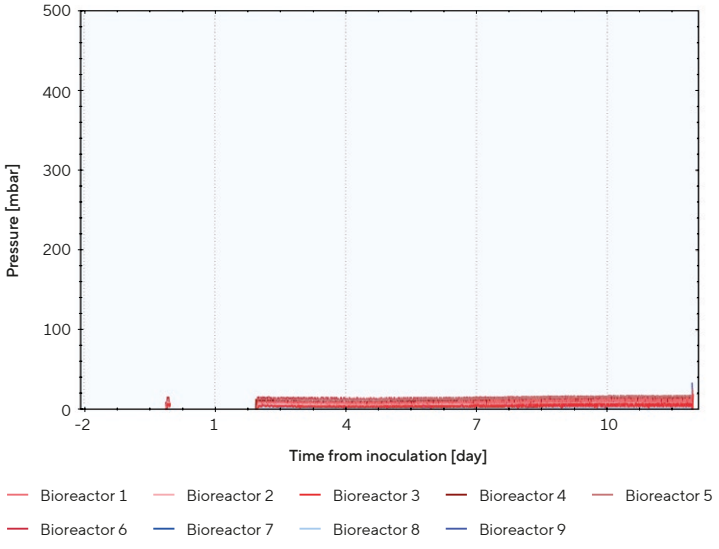


Figure 4: TMPs for Experiments 1 – 3

Experiment 1



Experiment 2



Experiment 3

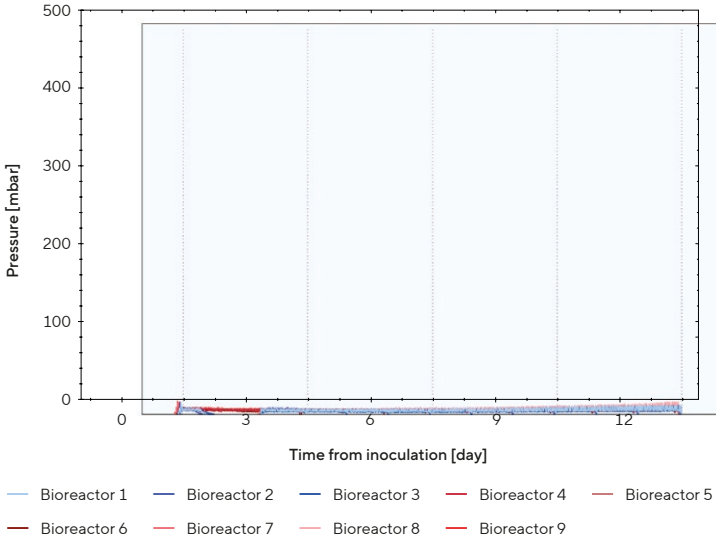
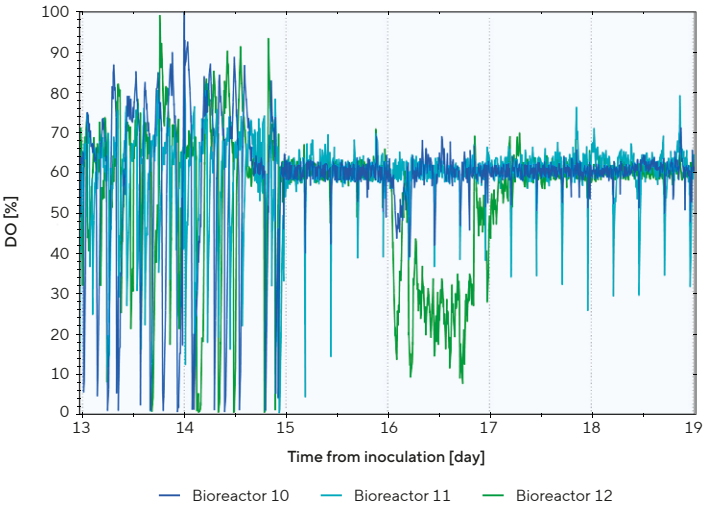


Figure 5: Dissolved oxygen trends for the three bioreactors in Experiment 1



Experiment 2

Experiment 2 aimed to explore a variety of DOE conditions, examining a range of parameters across nine bioreactors (also summarized in Table 1):

- Crossflow rates: low (A), medium (B), and high (C)
- Agitation speeds: low (D), medium (E), and high (F)
- Foam strategies: antifoam addition via a control loop (Strategy G) and semi-continuous addition (Strategy H)
- Bleed strategies: target cell density control loop (Strategy J) and an empirically derived control formula (Strategy K)

The VCD and viability data for Experiment 2 are shown in Figure 6. The trends were similar to the larger-scale 3 L and 500 L GMP runs (data not shown), matching the client data within 10%, specifically when examining peak VCD.

Antifoam strategy

Foam control strategies involved monitoring the foam sensor readings (Strategy G), as illustrated in Figure 7, and a bolus feed of antifoam every few hours (Strategy H). When the optical-based foam sensor reading exceeds a specific threshold value for a specified duration, it triggers. To evaluate the effectiveness of the two foam strategies, it is crucial to compare the bioreactor pressure, TMP, and foam sensor readings between bioreactors 5 and 9, which share identical conditions except for the foam strategy. This comparison is important because increased amounts of antifoam can potentially lead to membrane fouling, and excessive foaming in the bioreactor could result in elevated bioreactor pressure.

As seen in Figure 7, when comparing bioreactors 5 and 9, the foam sensor reading for bioreactor 9 was rarely activated, most likely caused by excessive antifoam addition. Even if bioreactor 9 experienced an excess amount of antifoam, there was no negative impact on TMP (Figure 4), VCD (Figure 8), titer (Figure 9), or specific productivity (Figure 10).

As explained in the next section, bioreactor 9 required an increase in agitation speed for approximately three days to maintain the DO setpoint. The only other bioreactor with the same agitation speed that required this shift in control was bioreactor 8, which employed a different bleed strategy.

This shift in bioreactor 9 is most likely due to the increased amounts of antifoam, as this strategy dispenses antifoam regardless of the presence of foam. For bioreactor 9, the agitation speed did not reach its maximum allowable level, indicating that the controller was not operating at its upper limit, and suggesting that this condition remained a potentially viable option for consideration in Experiment 3.

Agitation speed

Varying the agitation speeds showed no notable impact on VCD and viability (Figure 6). However, further examination of the effects of agitation revealed several observations related to titer concentration, DO control, and specific productivity.

Regarding specific productivity, the medium agitation speed bioreactors (bioreactors 3 and 5–9) most closely matched the historical data, indicating favorable performance at this agitation level (Figure 10). Compared to the historical data, the bioreactors with low (bioreactor 1) and high (bioreactors 2 and 4) agitation speeds underperformed (Figure 10). While medium-rate bioreactors initially displayed higher titers than historical runs, they aligned with controls as the study progressed. In terms of DO control (Figure 11), the low-rate bioreactors required an increase in cascade level to maintain the DO setpoint. Interestingly, the only medium-rate bioreactors that required this level increase were bioreactors 8 and 9, which varied in bleed and foam strategies. This aspect will be further explored in subsequent sections.

Crossflow rate

Although an increase in crossflow rate was expected to have some effect due to the elevated shear rate—a critical parameter in this process—the results in Figures 9, 10, and 11 indicate that high crossflow (bioreactors 5–9) did not appear to negatively impact cell culture performance throughout the range tested in Experiment 2. This is further supported by the results in Figure 18 (discussed in more detail later).

Bleed strategy

The bleed strategies employed were designed to maintain target cell density and ensure accurate growth rates. Despite the effectiveness of the current approach, additional optimization is needed to further enhance precision and adaptability.

Figure 6: VCD and viability trends for Experiment 2

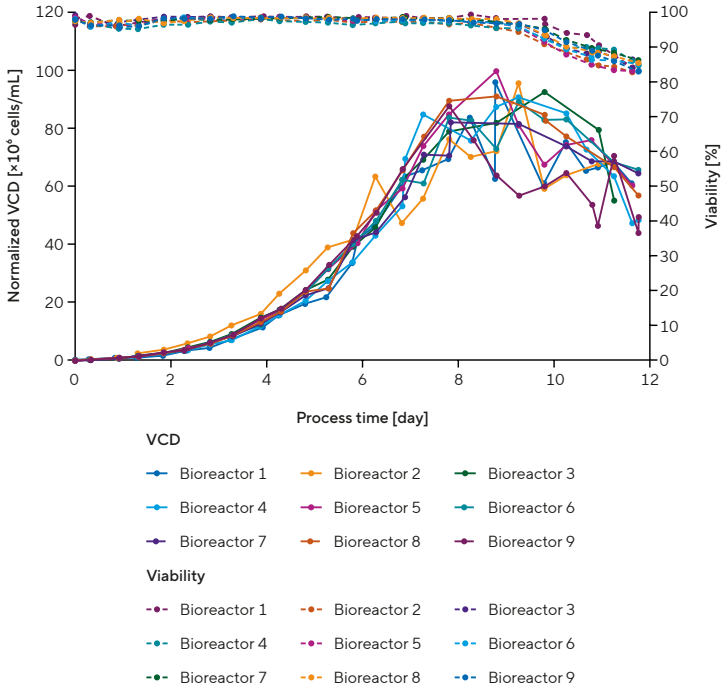


Figure 7: Foam sensor readings for Experiment 2 (bioreactors 5 and 9)

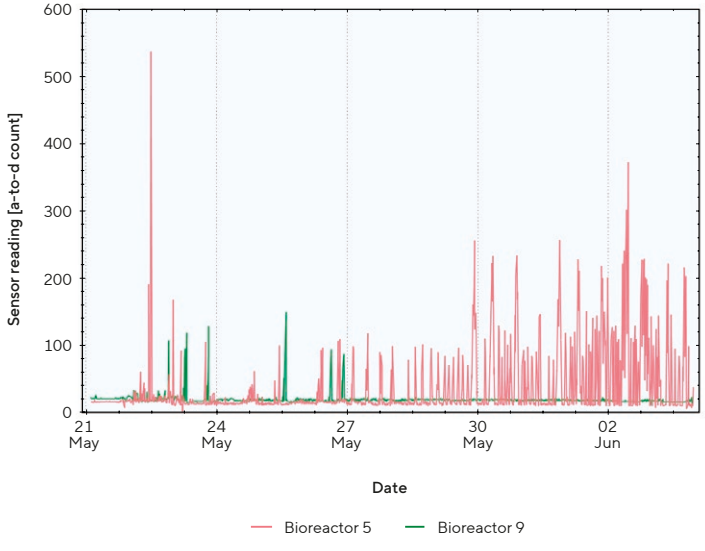


Figure 8: Impact of foam addition on VCD in bioreactors 5 and 9 (Experiment 2)

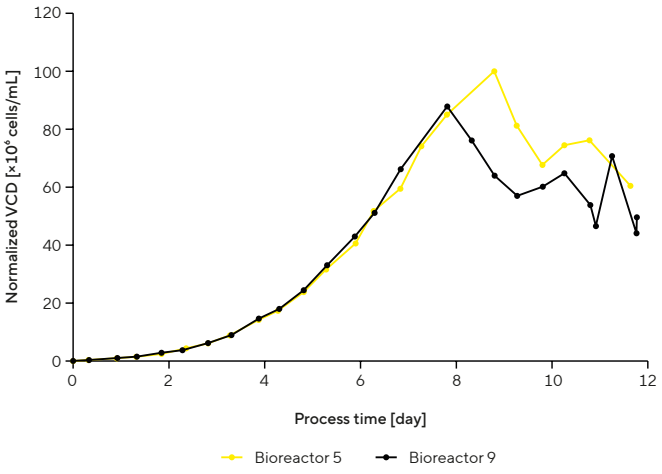


Figure 9: Experiment 2 titers (measured by Octet® R8 system)

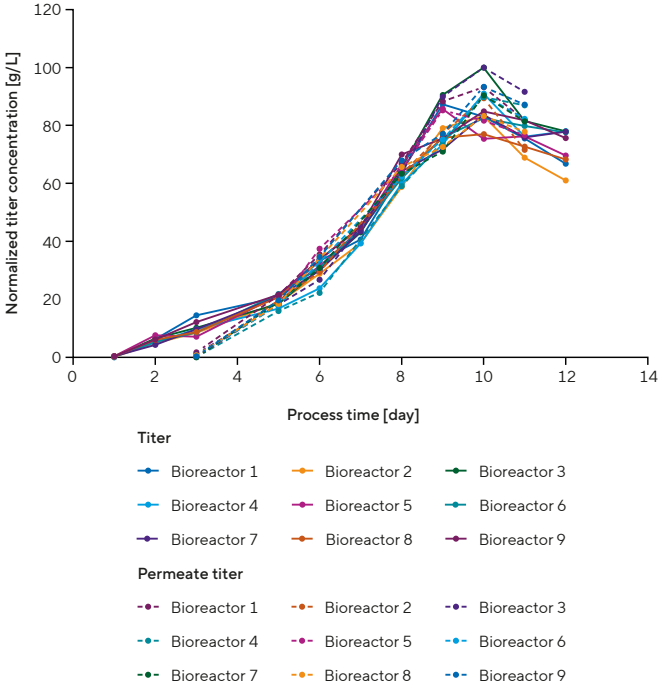


Figure 10: Specific productivities (qP) for Experiment 2

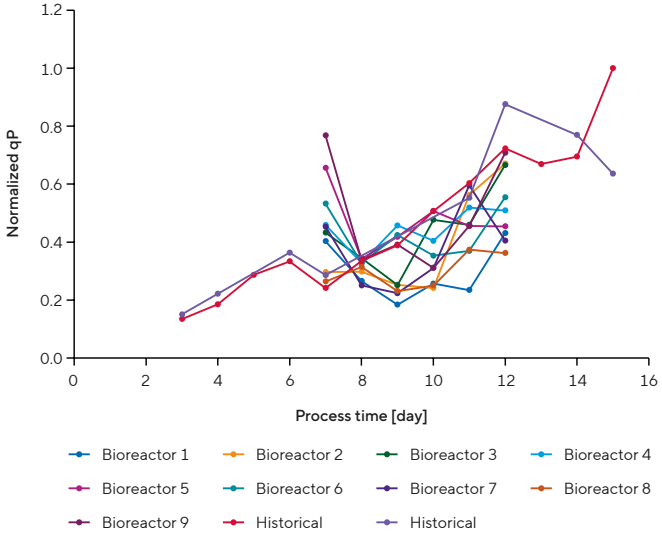
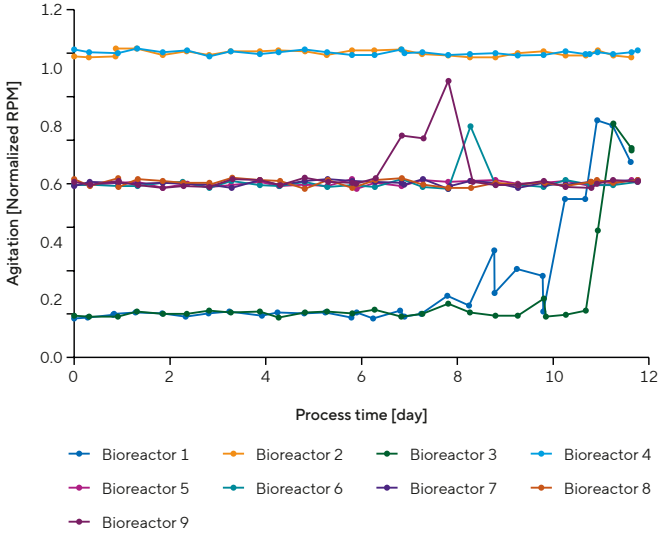


Figure 11: Effect of agitation speed on DO control in Experiment 2



Note. Agitation ramped up to meet DO demands for both low (bioreactor 1) and some medium (bioreactor 3 and 5–9) speed vessels.

Experiment 3

Experiment 3 aimed to determine whether the top-performing clones in Just–Evotec Biologics' 3 L process (Clone X, Clone Y, and Clone Z) also excelled at the Ambr® 250 scale. Based on the findings from Experiment 2, optimal setpoints for agitation, crossflow, and the appropriate foam and bleed strategies were determined for Experiment 3. In summary:

- **Crossflow rate:** With no significant performance differences observed between the different rates, the medium rate (B) was selected.
- **Agitation speed:** The agitation speeds showed no impact on VCD; however, the high agitation speed (F) optimized the titer, DO, and specific productivity more | than the others and was therefore selected.
- **Foam strategy:** Despite needing to elevate DO control to maintain the setpoint, Strategy G was used since there was no significant impact on TMP, titer, or specific productivity. Strategy G also avoided any risk of bioreactor overpressure or potential foaming.
- **Bleed strategy:** Strategy J, which relied on the Ambr® 250 control software, was selected.

In general, the results aligned with Just–Evotec Biologics' 3 L historical data. Early on in this run, bioreactor 4 (Clone Y) and bioreactor 9 (Clone Z) experienced some technical issues with faulty pH probe connections and were therefore excluded, leading to a final dataset of three replicates for Clone X, two replicates for Clone Y, and two replicates for Clone Z. Additionally, it is important to note that the crossflow was active during inoculation, leading to the decision to maintain its setpoint.

Before perfusion began, VCD and viability were consistent between the three clones (Figure 12). Clone X exhibited the fastest growth but reached the lowest peak VCD. Clone Y maintained growth throughout the end of the experiment, whereas Clone Z appeared to be nearing its peak on the final day of the experiment. In terms of final viability, clones cultured in the Ambr® 250 system outperformed their counterparts at the 3 L scale.

The titers of bioreactor and permeate samples indicate a 1–2 day delay in production rate compared to the 3 L process (Figures 13 and 14). Figure 14 shows that overall performance was statistically similar between the two scales. However, during inoculation, crossflow was unintentionally active, which could be linked to the delay.

Figure 12: VCD and viability trends for Experiment 3

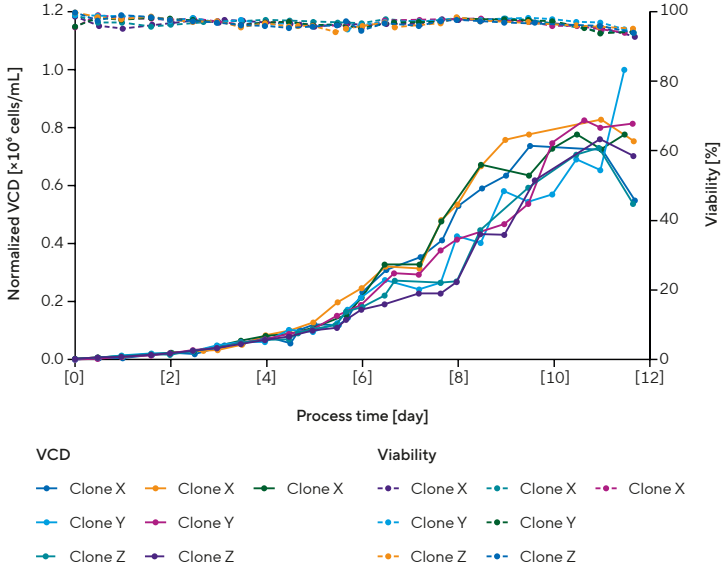


Figure 13: Normalized permeate titers for Experiment 3

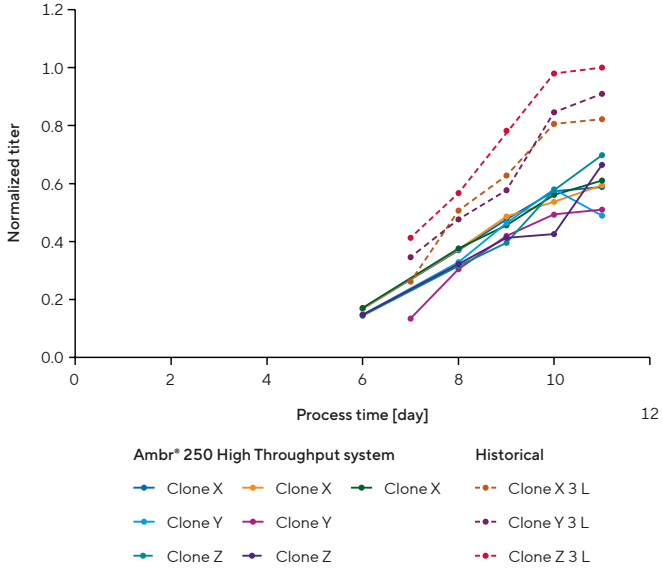


Figure 14: Comparison of Experiment 3 data to historical data from Just—Evotec Biologics

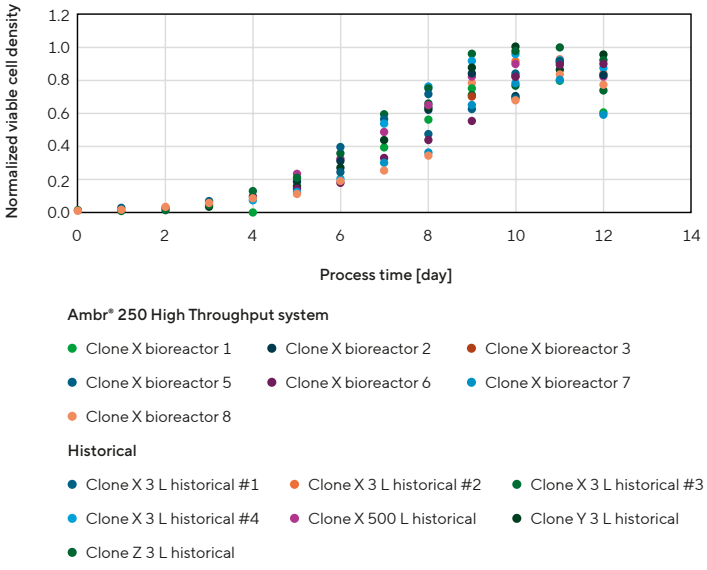
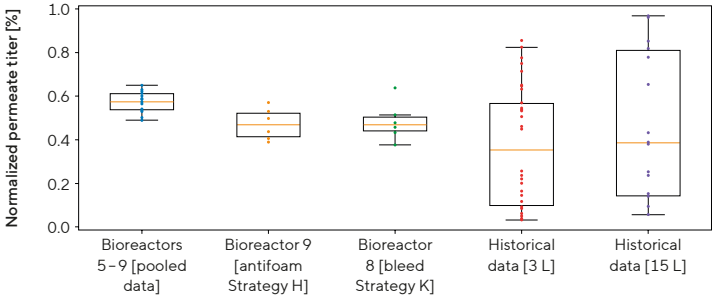


Figure 15: Cell-specific productivity and titer results for Experiment 2 are statistically similar to historical data

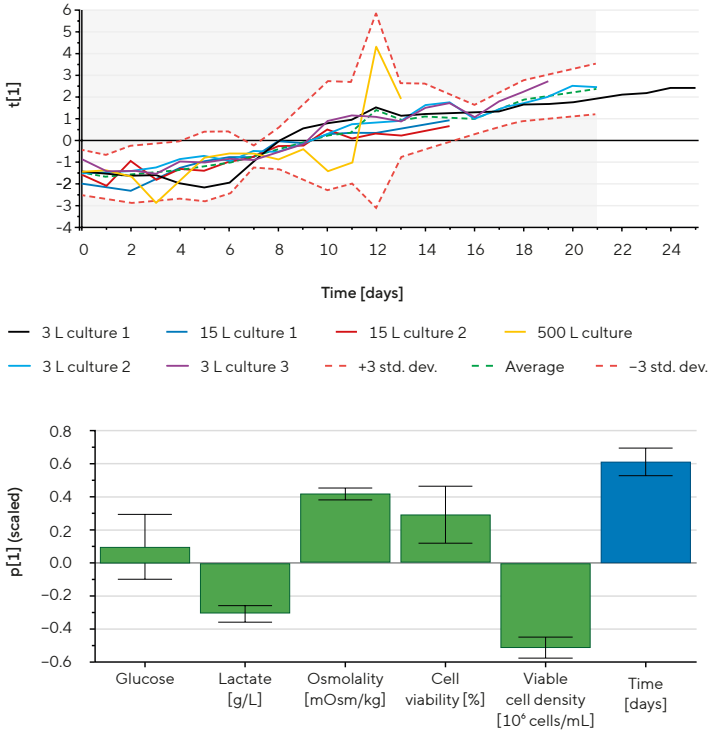


Comparison of Ambr® 250 High Throughput and historical processes using SIMCA®

The performance of the Ambr® 250 High Throughput system was compared to historical data using batch evolution models (BEM) in SIMCA® software. Batch evolution analysis uses orthogonal partial least squares (OPLS®) regression to fit batch variables to the time they were observed in the batch. Using this analysis, an expected trajectory from representative batches can be fit and used to assess comparator batches, or batches in progress. Scale-independent process data from seven batches of Clone Z at various scales (3 – 15 L) were provided by Just – Evotec Biologics for comparison (Figure 16). These batches were used to fit a BEM, which served as the baseline and comparison for Ambr® 250 High Throughput system batches.

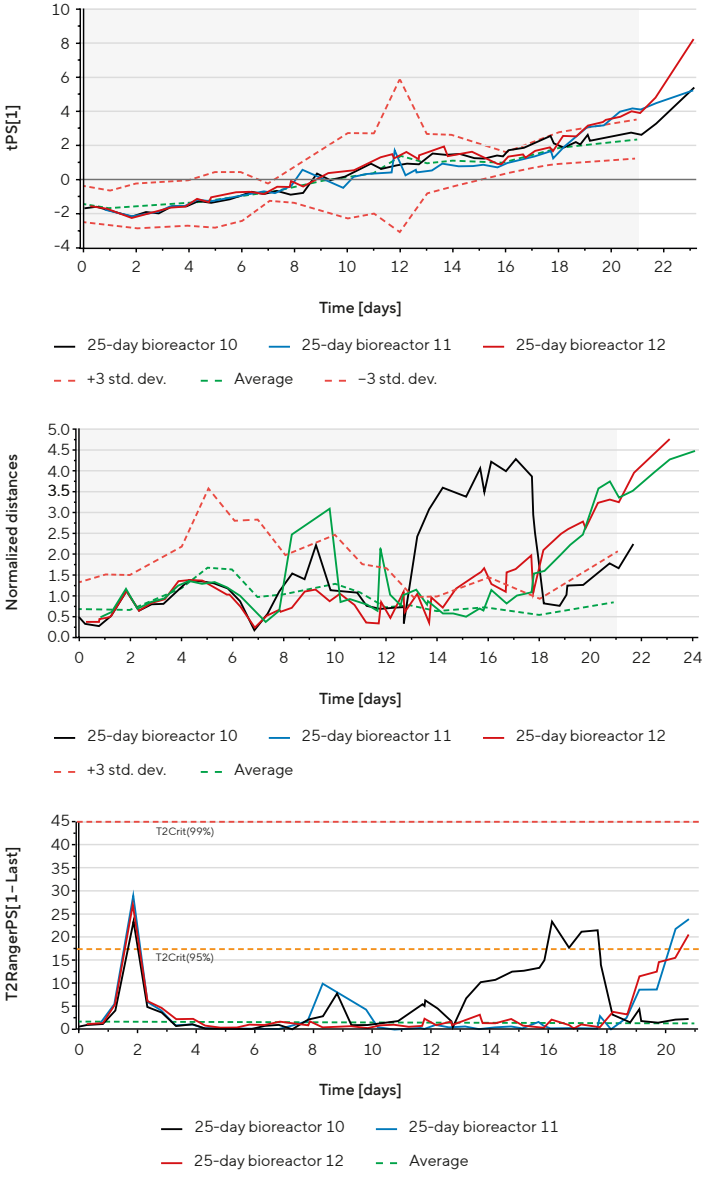
Data from each experiment were used as a prediction set for the BEM, and deviations from the expected trajectory were evaluated. Deviations were assessed from the predicted score as well as DModX, a measure for covariance not captured by the model, and Hotelling's T2, a measure for distance from the model fit. A DModX outlier would represent variables changing in ways not observed in the training set, whereas Hotelling's T2 would represent variables whose magnitude was outside the training set.

Figure 16: Score trajectory for batch evolution model (top) and contributions of individual variables (glucose, lactate, osmolality, viability, VCD) to model score (bottom)



For Experiment 1, the score trajectory matched the expected values well (Figure 17). An outlier flagged by DModX was observed between days 13 and 18 for bioreactor 10, which could be evaluated at the univariate level using the drill-down feature in SIMCA®. Univariate contributions are shown in Figure 18, where orange denotes a significant difference from the expected value. The bars can be drilled into further to show univariate trends. The univariate trends show a spike in glucose at this time, leading to a simultaneous spike in osmolality. This deviation was a result of pump programming on the Ambr® 250 High Throughput system and not process variability itself, and was not present in the other two batches.

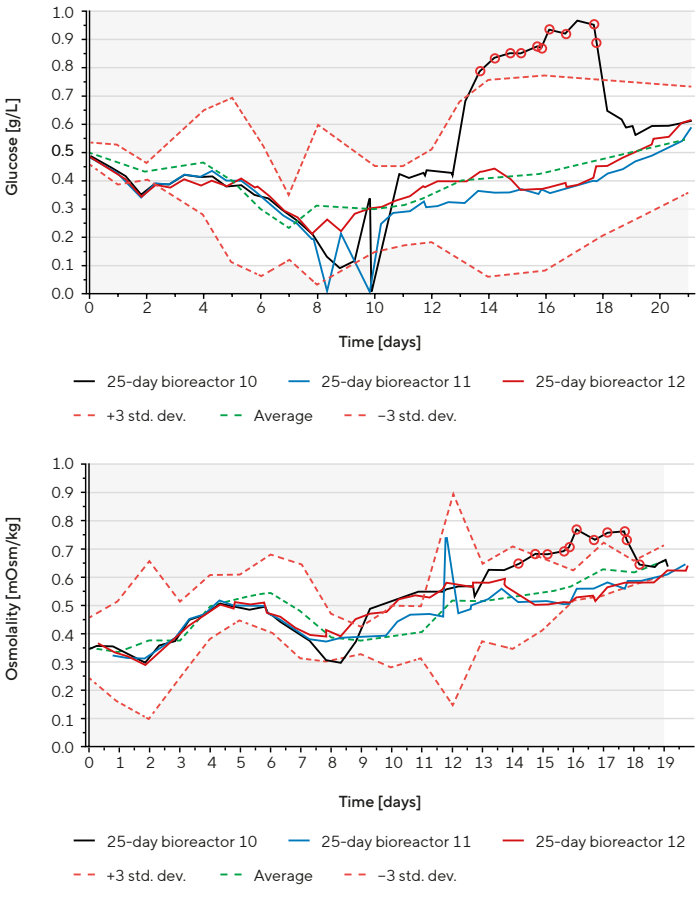
Figure 17: Experiment 1 as a prediction set. Score trajectory (top), DModX (middle), and Hotelling's T2 (bottom)

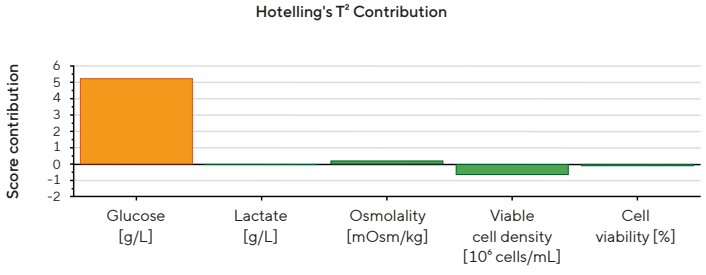


The same analysis can be performed for Experiments 2 and 3. For Experiment 2, a deviation was observed on Day 2 at the 95% criticality level (Figure 19). Deviations can be evaluated at the univariate level using drill-down functionality in SIMCA®. Univariate contributions are shown in Figure 20, where orange denotes a significant difference from the expected value. The bars can be drilled into further to show univariate trends. The observed deviation is attributed to a slightly higher VCD than expected on Day 2, resulting in lower glucose and higher lactate levels compared to the training set.

This deviation from the process in the training data is relatively low risk, and the respective values return to the expected levels by the following day. A similar outlier can also be observed in Experiment 1. For Experiment 3, each bioreactor stayed within the 3 standard deviation limit for the score trend, and no major outliers were observed (Figure 21).

Figure 18: Univariate contributions to Hotelling's T^2 (below) and DModX (bottom) for outliers observed between days 13 and 18. Univariate trends for glucose (top right) and osmolality (bottom right) to demonstrate magnitude (Hotelling's T^2) and covariance





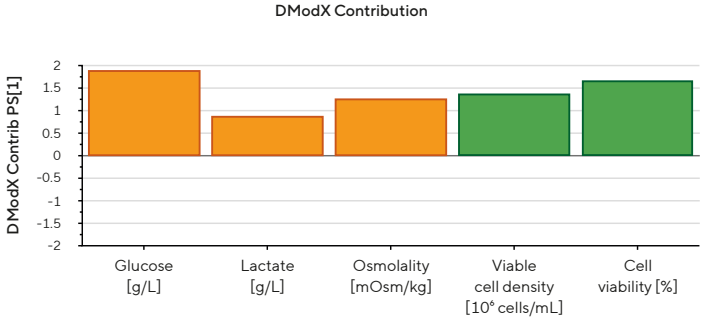


Figure 19: Experiment 2 as a prediction set. Score trajectory (top), DModX (middle), and Hotelling's T^2 (bottom)

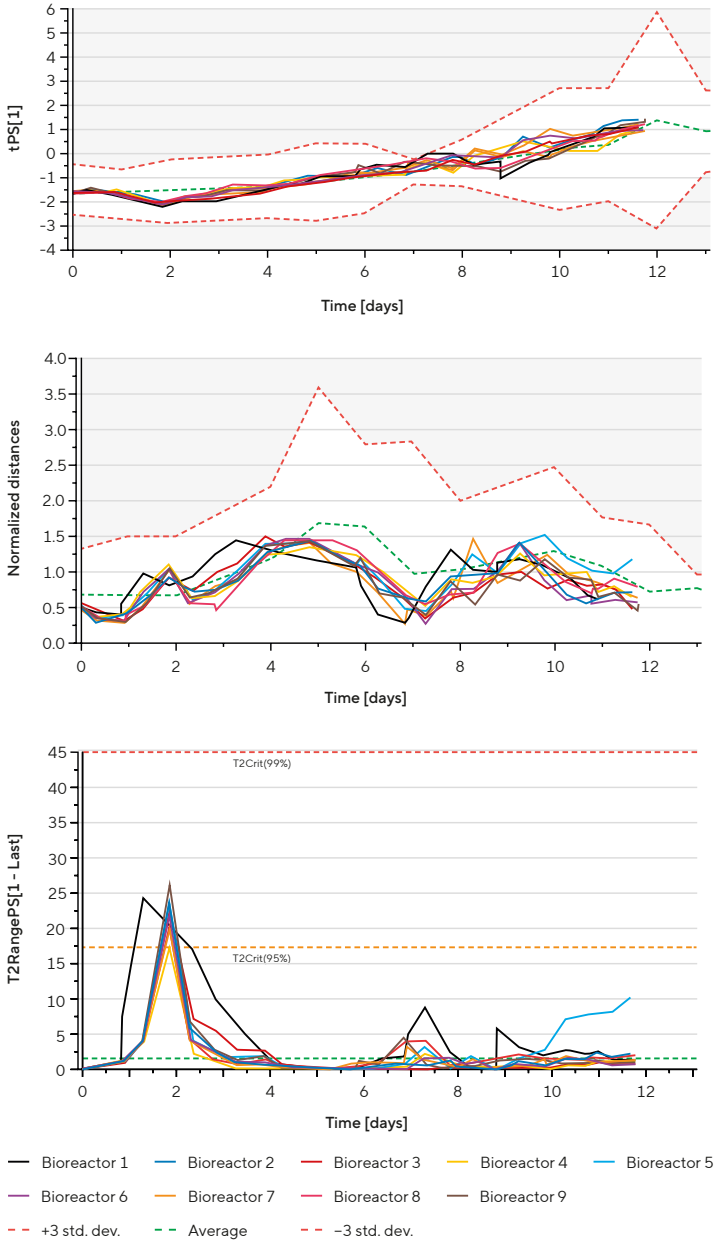


Figure 20: Hotelling's T^2 contribution (top) and univariate trends (second and third and fourth) of significant variables for the Hotelling's T^2 score on Day 2, shown in orange on the bar graph for Experiment 2

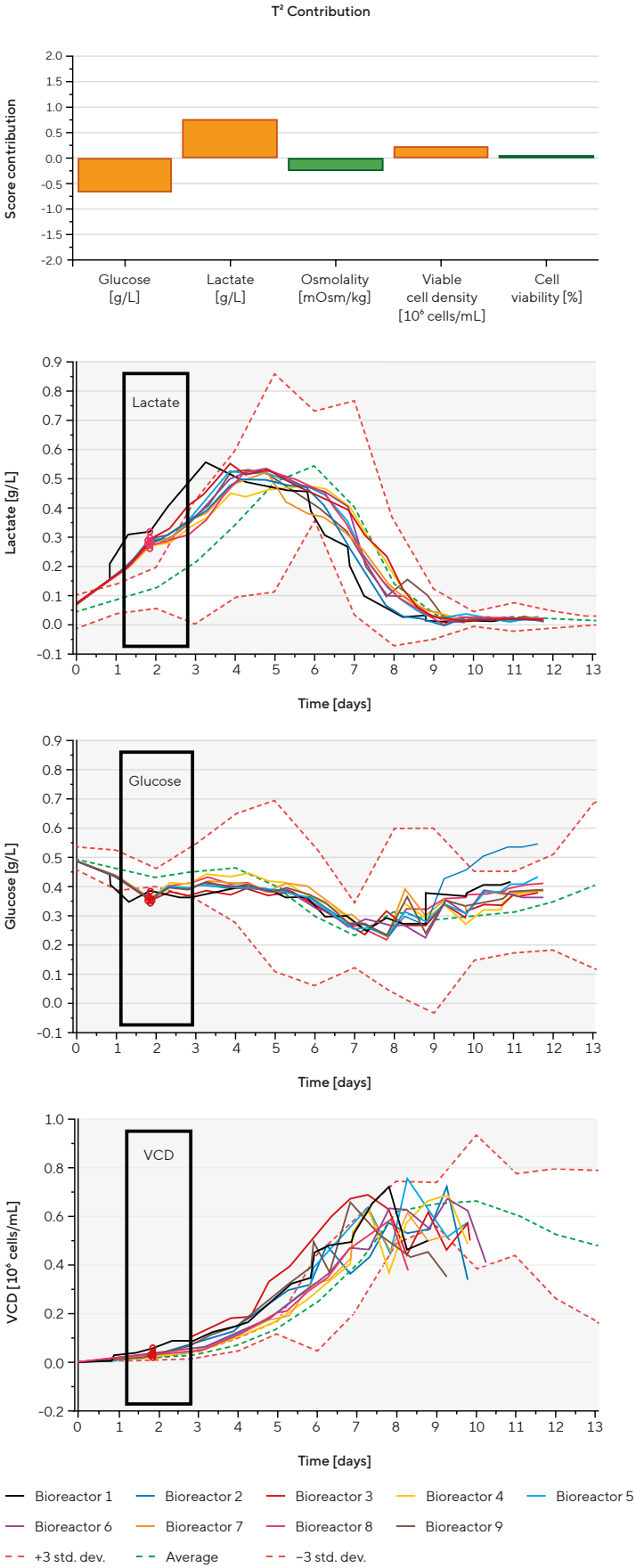
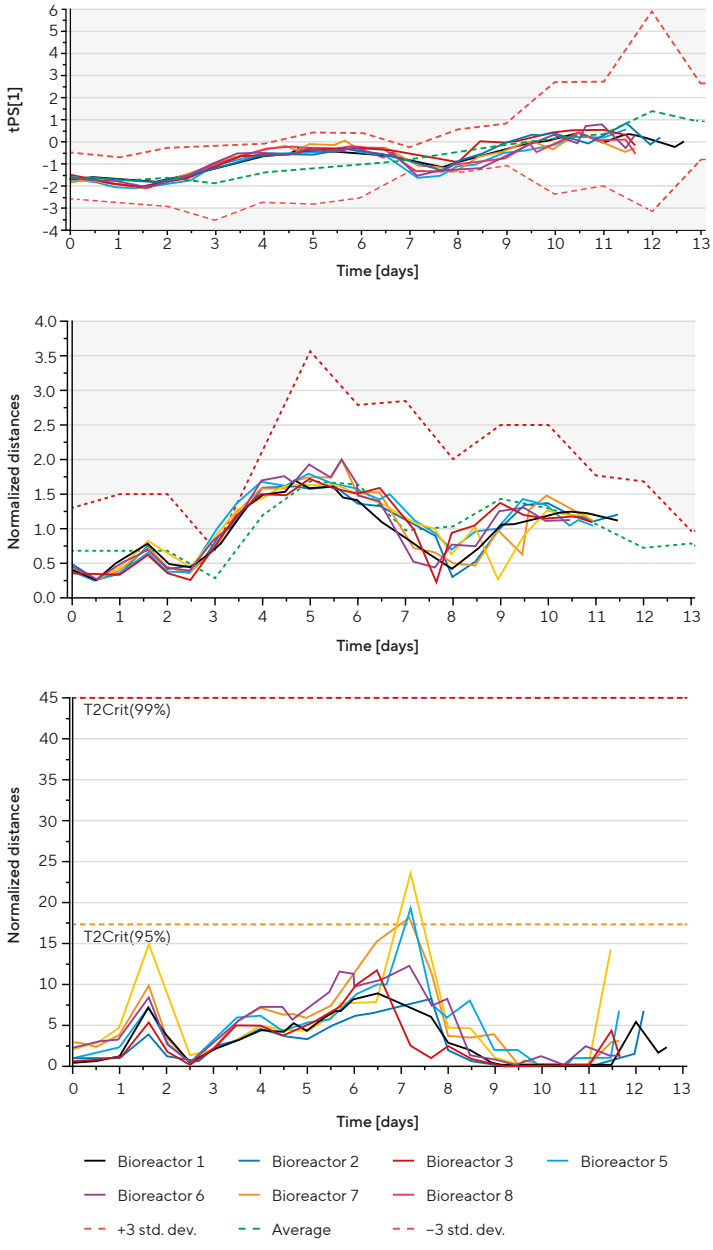


Figure 21: Experiment 3 as a prediction set. Score trajectory (top), DModX (middle), and Hotelling's T^2 (bottom)



These results demonstrate that the batches run on the Ambr® 250 system accurately reproduce the process trajectory of historical data provided by Just – Evotec Biologics. The BEM was trained on historical data at multiple scales, and the results of the three Ambr® 250 system experiments match these trends well. Deviations from historical data were analyzed, and root causes were identified to mitigate the risk of the process transfer to the Ambr® 250 system. The score trends demonstrate minimal impact of experimental conditions (e.g., agitation, crossflow rate, or clone) on process performance, suggesting a robust model of the Just – Evotec Biologics process at small scale.

Conclusion

These experiments demonstrated the ability of the Ambr® 250 High Throughput system to effectively utilize perfusion mode, replicate the performance of larger-scale processes, and facilitate the adaptation of top-performing clones from historical data to the smaller-scale systems. The experiments achieved the same results as the historical, larger-scale processes, but with a footprint reduced by threefold, and using sevenfold less media. These findings emphasize the potential of the Ambr® 250 High Throughput system to revolutionize bioprocessing efficiency and scalability, supporting future developments in the industry.

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