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An Intensified N-1 Perfusion Process for High-Density Cultivation in the Biostat® RM With 4Cell® SmartCHO Cell Culture Medium

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Abstract

The Biostat® RM system is a versatile and scalable platform for advanced N-1 perfusion processes in biopharmaceutical production. This application note presents a case study demonstrating the implementation of an N-1 perfusion process using the Biostat® RM system. It outlines the development of a robust and scalable cell expansion strategy that enables the generation of high-density, healthy seed cultures, suitable for subsequent production bioreactors.

The study highlights key process parameters, including perfusion control, cell growth dynamics, and viability performance over multiple cultivation phases. By detailing the setup, operation, and results, this application note serves as a practical example of how the Biostat® RM can be leveraged to support intensified upstream processing and improve seed train efficiency.

Introduction

The Biostat® RM system stands at the forefront of innovative bioprocessing technology, offering a sophisticated yet straightforward solution for achieving high cell densities in N-1 perfusion mode. The system is designed to meet the demanding needs of modern biopharmaceutical production, where maximizing cell growth and productivity is crucial.

As an alternative to traditional stirred-tank reactors, the Biostat® RM provides a simple and flexible solution for process intensification or for seamlessly connecting a seed train for cell culture expansion. The system offers a low-shear environment delivering efficient oxygen transfer and mixing, supporting a wide range of cell lines for microbial fermentation, mammalian cell culture (CHO, HEK293, stem cells, and CAR-T cells), and insect cell culture. The Biostat® RM excels in amplifying the seed train step during the biomanufacturing process; using N-1 perfusion allows the batch to be steadily intensified to reach the target cell density required for large-scale reactors.

This application note demonstrates the performance of the Biostat® RM system in the cultivation of CHO cells. In combination with the 4Cell® SmartCHO Media System — formulated to support robust cell growth and maintain stable culture conditions — the Biostat® RM provides a reliable foundation for high-performance perfusion processes. The integration of an optimized cell-specific perfusion rate (CSPR) and BioPAT® Viamass-based feeding protocol further enhanced the system's capabilities. Leveraging Biobrain® Supervise, this protocol enabled precise control over nutrient delivery and waste removal, ensuring that cells received the required nutrients while efficiently removing metabolic byproducts. By maintaining a balanced environment, the Biostat® RM system promoted consistent cell growth and viability during high-density cultures, which are essential for efficient biopharmaceutical production.

Materials

Biostat® RM
The Biostat® RM control tower is equipped with Biobrain®, a powerful operating system for automation and bioprocess control. Biobrain® monitors and controls gassing, rocking, temperature, pH, and dissolved oxygen (DO). All data is viewed and stored in this historian solution for integrated process values, alarms, and process analytical technology (PAT). The automation recipe is also governed by Biobrain® Supervise. The Biostat® RM and process parameters used in this study are shown in Figure 1 and Table 1, respectively.

Complex processes like perfusion can be easily executed through recipes that use a harvest and feed protocol using the peristaltic pumps positioned on the tower. The tower also includes a BioPAT® Viamass sensor for online cell density measurements using capacitance technology — a critical element for maintaining accurate perfusion rates.

Table 1: N-1 perfusion process parameters and setpoints

Process parameter	Setpoint
Working volume [L]	1
Temperature [°C]	36.8
DO [%]	40
pH	7.1 starting, 6.95 shift
Rocking [rpm]	30 – 42
Rocking angle [°]	10
CSPR [pL/cell/day]	50
Vessel volumes per day (VVD)	Min 0.25 | max 5

Figure 1: Biostat® RM

2 L Flexsafe® RM Perfusion Bag

The 2 L Flexsafe® RM Perfusion Bag is equipped with single-use sensors such as DO, pH, and BioPAT® Viamass. The 1.2 µm PES perfusion membrane is positioned at the bottom of the bag, with one harvest line extending from the center. The bag's working volume ranges from 200 mL to 1 L. The rocking feature of the bag enables low-shear mixing that can support high cell density cultures.

Overlay gassing and rocking motion control the DO while preventing the accumulation of foam due to the absence of a sparger. Thus, no antifoam was required throughout the entire perfusion batch. For gas exhaust, the HEPA-rated hydrophobic filter, alongside the filter heater, allows for the aseptic transport of gas outside the bag while maintaining pressure. The operational bag pressure ranges from 6–30 mbar.

Media and cell line

The 4Cell® SmartCHO Media System, comprised of 4Cell® SmartCHO Production Medium, 4Cell® SmartCHO Feed Medium A, and 4Cell® SmartCHO Feed Medium B, was used as the main source of nutrient supplementation. The process used a well-characterized robust CHO DG44 cell line (Table 2).

Table 2: Cell line and media

Cell line	CHO DG44
Media platform	4Cell® SmartCHO Media System + 1 g/L Pluronic

Perfusion setup

The perfusion setup (Figures 2 and 3) includes the 4Cell® SmartCHO Media System stored in a 50 L Flexboy® 2D Bag. The permeate line was routed to the bag, which was positioned on a balance for gravimetric monitoring. Other feeds, such as 1 M sodium carbonate and 400 g/L glucose, were stored in a 250 mL Flexboy® 2D Bag connected to the Flexsafe® RM Perfusion Bag. All aseptic connections from the 2 L Flexsafe® RM Perfusion Bag to all feed and harvest Flexboy® bags were performed by the Biowelder® TC using ⅛" × ¼" Sartorius TPE tubing. The pump tubing sizes were reduced to 1/32" Bioprene® (Watson-Marlow). Control parameters are presented in Tables 3–7.

Figure 2. Perfusion rate calculation

$$y = \left(\left((\textbf{CSPR.}\ \text{Value} \times \textbf{BR\_VOL.}\ \text{Value} \times \textbf{BMASS.}\ \text{Value}) \times {}^{1}/_{24} \right) \right)$$

Note. CSPR = cell-specific perfusion rate, BR_VOL = bioreactor volume, BMASS = BioPAT® Viamass reading. The equation includes a conversion factor (× 1/24) to express the flow rate in mL/h. This represents the perfusion flow rate, i.e., the rate at which medium is pumped for harvesting permeate.

Table 3: PID parameters for temperature control

Parameter	Value
Setpoint ramp [s]	0
Setpoint range max. [°C]	40
Setpoint range min. [°C]	15
Controlled variable limit max. [°C]	40
Controlled variable limit min. [°C]	15
Controlled variable default [°C]	37
Controlled variable saturation	Limitation
Anti-windup	None

Figure 3: 2 L Biostat® RM perfusion setup

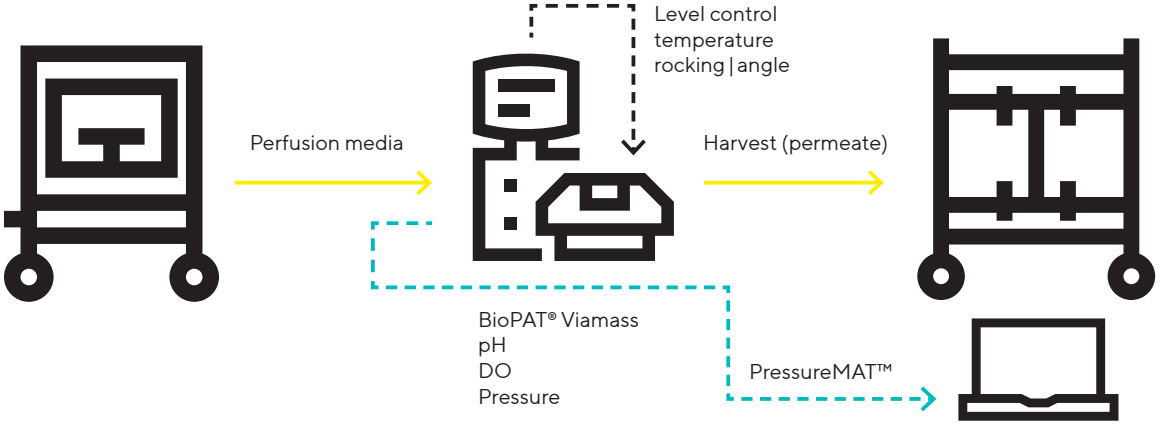


Table 4: PID parameters for pH control in both N-1 and perfusion mode

Parameter	N-1 batch	Perfusion
Gain	0.25	0.25
Ti [s]	999	999
Td [s]	0	0
pH setpoint ramp [s]	0	0
pH setpoint range max.	10	10
Setpoint range min.	4	4
Controlled variable limit max. [%]	50	1) 50 2) 70, when pH setpoint = 6.95
Controlled variable limit min. [%]	0	0
Anti-windup	None	1) None 2) Zero, when pH setpoint = 6.95
Dead band (±)	None	1) None 2) 0.05, when pH setpoint = 6.95

Transmembrane pressure (TMP)

The transmembrane pressure (TMP) was recorded using the PressureMAT™ (PendoTECH) in line with the permeate. With a sample rate of every 30 seconds, pressure measurements in PSI can capture the exact moment the perfusion membrane blocks.

Analytics

Key process data, such as viable cell density, cell viability, pH, osmolality, and metabolites, were captured by the BioProfile® FLEX2 (Nova Biomedical). The titer measurements were performed on the Octet® R8 Protein Analysis System. A BioPAT® Viamass single-use probe was used to estimate live cell counts based on the capacitance signal. This signal was then used by Biobrain® Supervise to calculate the live perfusion rate.

Table 5: DO polygon | cascade

Controller	Control output [%]					
	0	20	40	60	80	100
N_2 overlay [LMP]	0.03	0	0.03	0	0	0
Air overlay [LMP]	0	0.09	0.06	0.03	0	0
O_2 overlay [LMP]	0	0.015	0.03	0.06	0.09	0.12
Rocking [rpm]	30	30	30	34	38	42

Table 6: PID parameters for DO control

Parameter	Value
Gain	1.25
Ti [s]	999
Td [s]	0
Setpoint ramp [s]	0
Setpoint range max. [%]	250
Setpoint range min. [%]	0
Controlled variable limit max. [%]	100
Controlled variable limit min. [%]	0
Controlled variable default [%]	20
Controlled variable saturation	20
Anti-windup	None

Table 7: PID parameters for level control

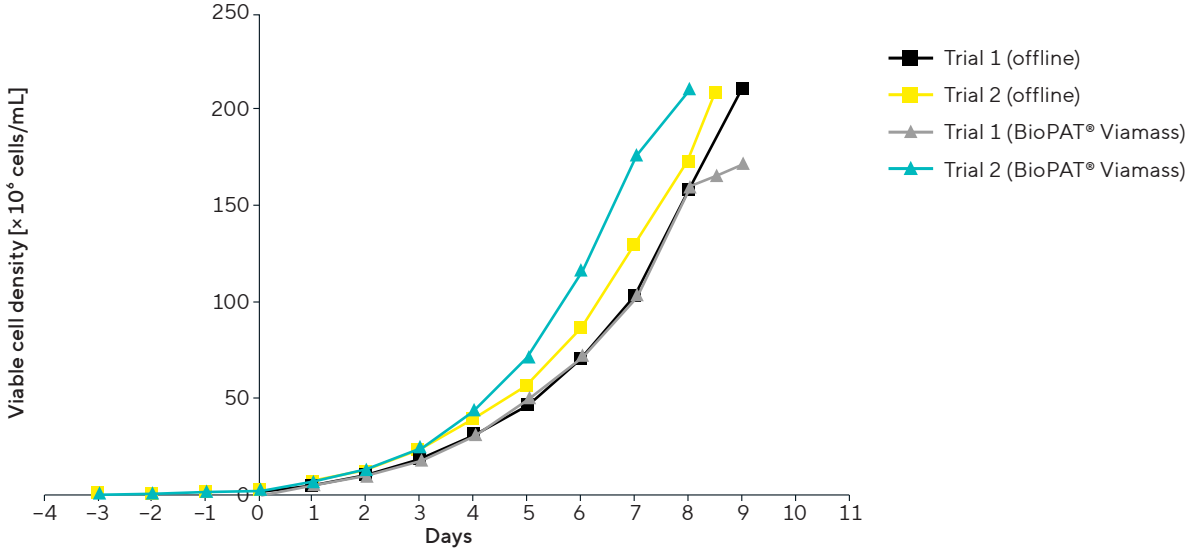
Parameter	Value
Gain	500
TI [s]	500
TD [s]	0
Setpoint ramp [s]	0
Setpoint range max. [kg]	5
Setpoint range min. [kg]	0
Controlled variable limit max. [%]	70
Controlled variable limit min. [%]	0
Controlled variable default [%]	0
Controlled variable saturation	Limitation
Anti-windup	None

Results

Cell growth, viability, and process performance
Two independent trials were performed in 2 L Flexsafe® RM Perfusion bags to evaluate the system's ability to support exponential cell growth, maintain cell viability, and sustain process control under high-density conditions. Peak viable cell densities exceeded 200 × 10^6 cells/mL, with viability above 97% throughout both trials (Figures 5 and 6).

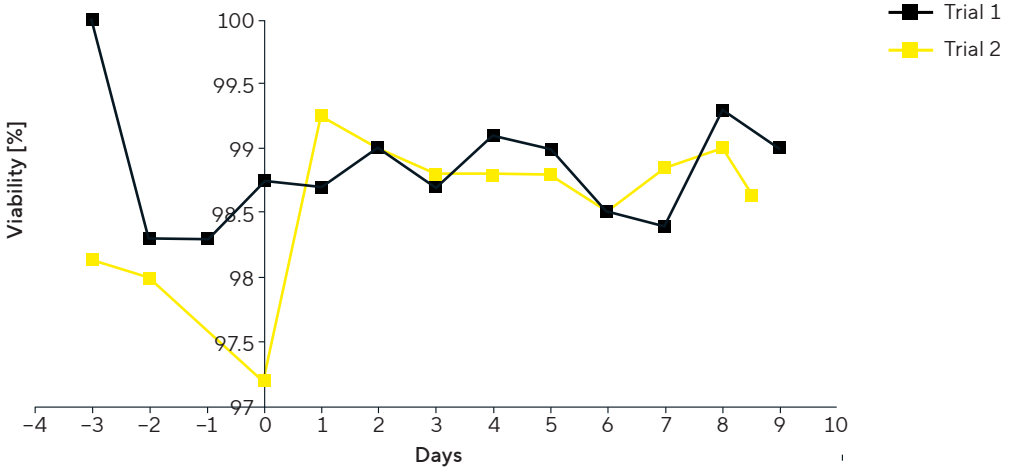
Minor variations in growth rate were observed between the two trials. The second trial exhibited faster growth, reaching the high-density limit one day earlier than the first trial. This variation is typical for this CHO DG44 cell line, and falls within the expected deviation.

Figure 5: Online (BioPAT® Viamass) and offline viable cell density measurements



Note. Maximum capacitance is 400 pF/cm

Figure 6: Offline cell viability measurements



The CSPR-controlled feed strategy, guided by real-time BioPAT® Viamass capacitance measurements, prevented nutrient limitation or overfeeding. Exponential growth was observed in both trials, as indicated by offline integral viable cell concentration (IVCC) and BioPAT® Viamass readings.

Cell viability remained consistently high (Figure 6), with no late-stage decline even at peak densities. This indicates that the system's low-shear rocking motion and controlled environment effectively minimized cellular stress. Minimal to no foam formation was observed during the entire perfusion process, demonstrating the effectiveness of overlay gassing and low-shear mixing in supporting cell health.

Overall, both trials demonstrated reproducible high-density perfusion performance, indicating that the Biostat® RM system can consistently deliver reliable outcomes across independent runs. These results confirm the platform's suitability for high-density perfusion and highlight its potential for upstream process intensification.

Process control: DO and pH

DO and pH were tightly controlled throughout both trials. Figure 7 shows that during the batch growth phase for Trial 1, DO was maintained at 60% and was intentionally lowered to 40% once perfusion was initiated to optimize oxygen availability for high-density cultures and minimize oxidative stress (Figure 7). Offline pH measurements confirmed stable control within the target range of 7.1 to 6.95, with the natural pH drift supporting improved lactate metabolism and minimizing the need for base addition (Figure 8).

The optical sensors demonstrated reliable performance even under high cell density and dynamic perfusion rates, confirming the robustness of the control strategy.

Figure 7: Online pH, DO, and rocking measurements for Trial 2

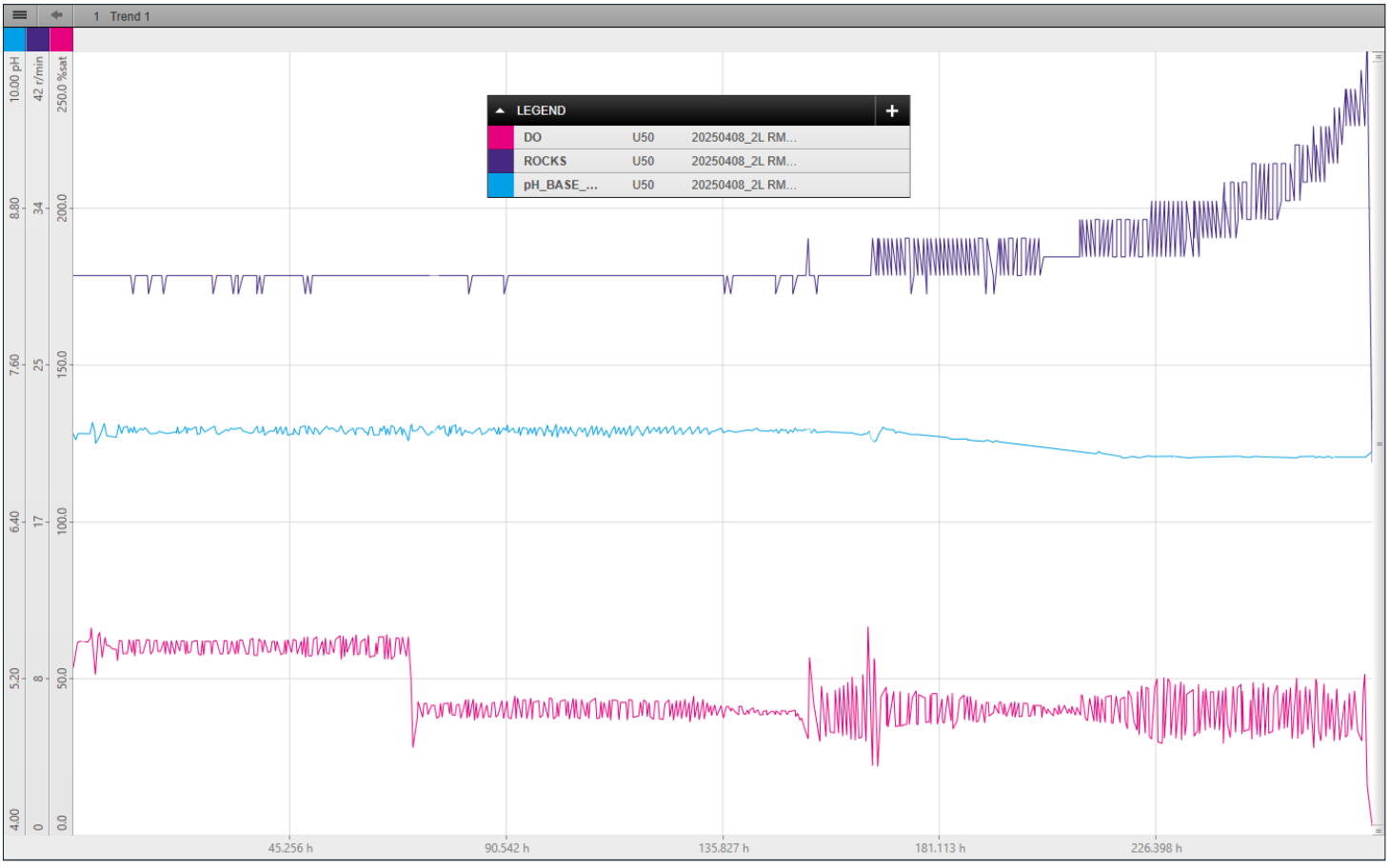
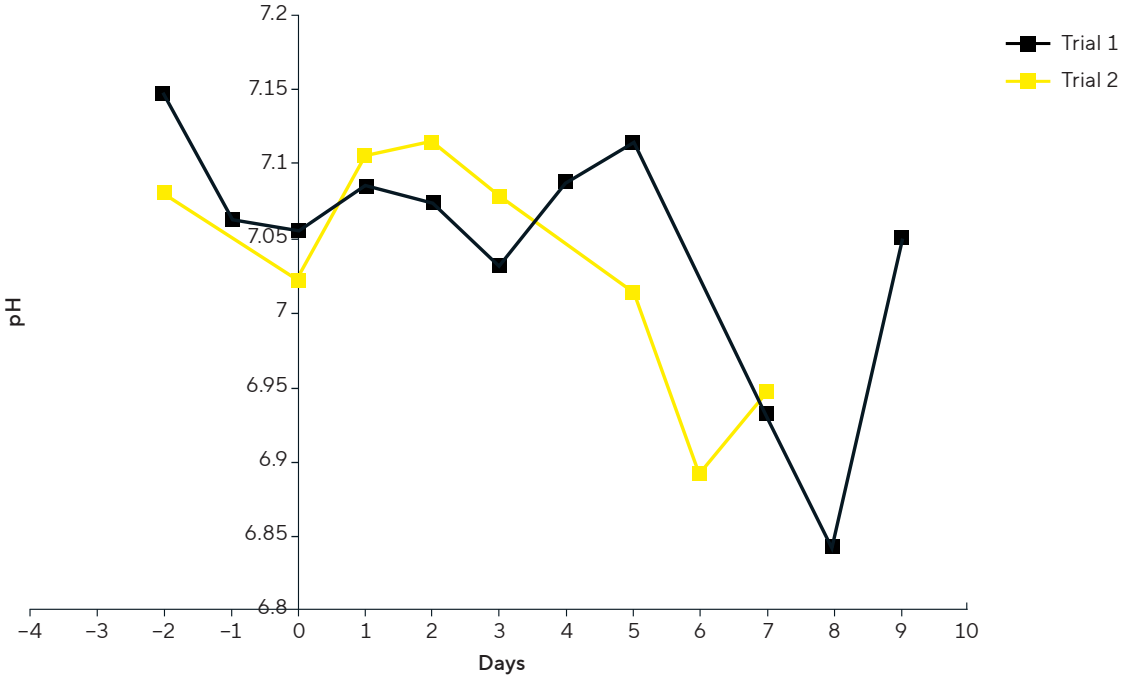


Figure 8: Offline pH measurements



Membrane performance and perfusion dynamics

Membrane performance was evaluated by monitoring permeate throughput and TMP. Total permeate volumes increased steadily until TMP reached approximately 0.83 bar, reflecting the gradual accumulation of biomass at the membrane surface (Figures 9 and 10). The second trial exhibited a one-day shift in TMP behavior corresponding to its faster growth rate, but the overall endpoint for both trials was comparable.

Figure 9: Total permeate throughput

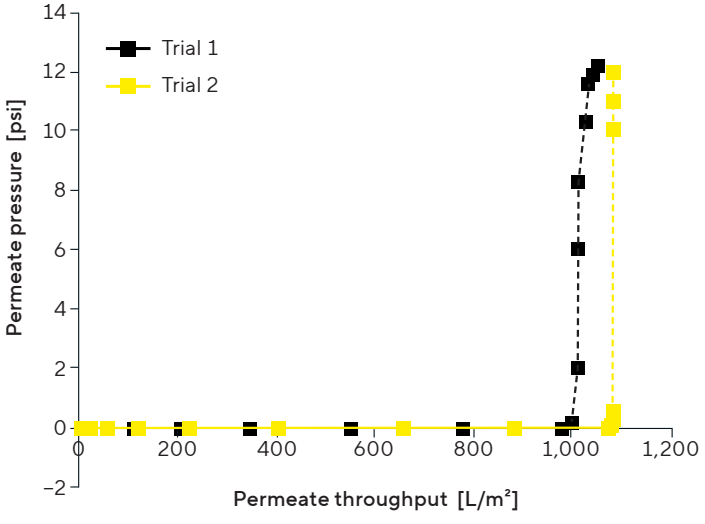
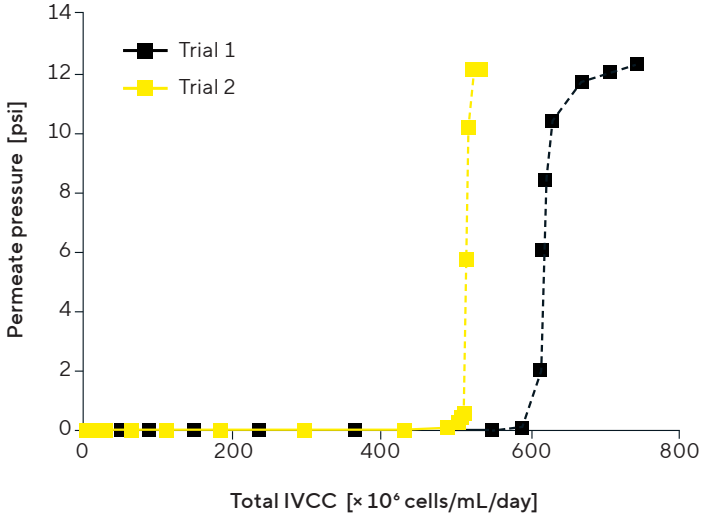


Figure 10: Total integral viable cell concentration and permeate pressure (health of membrane)

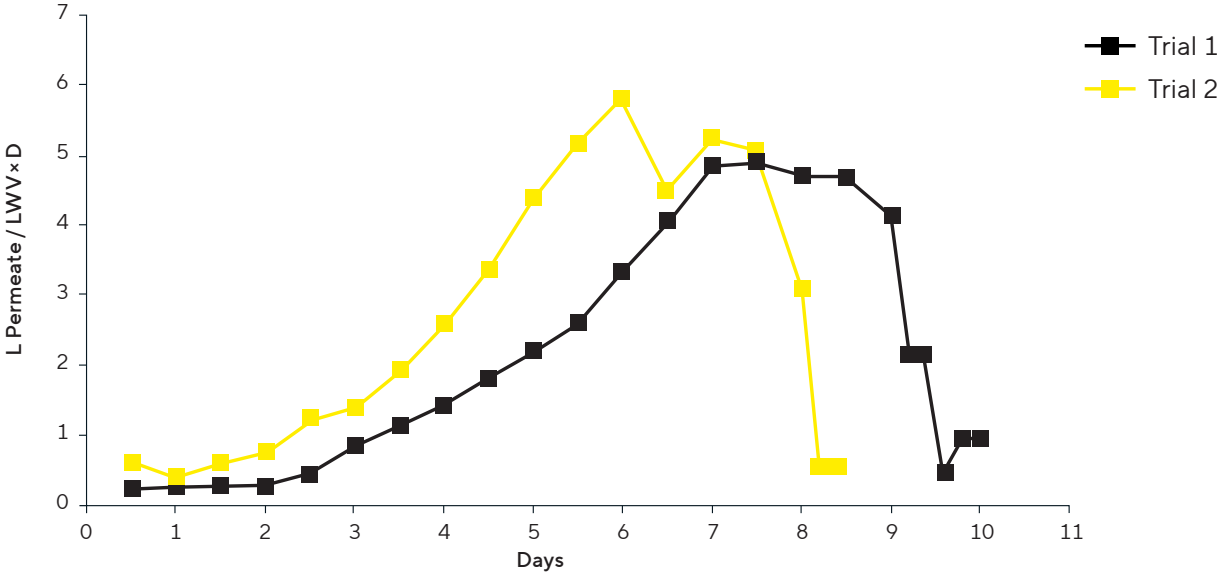


Note. Dotted lines indicate linearly interpolated values

Gravimetric monitoring of the permeate confirmed that perfusion rates followed the programmed CSPR strategy effectively (Figure 11). The maximum volumetric exchange per day was controlled to prevent overfeeding, which can negatively impact cell growth due to increased osmolality. These results confirm that the system's load cell-based level control, combined with automated CSPR adjustments, can maintain consistent perfusion while minimizing cellular stress.

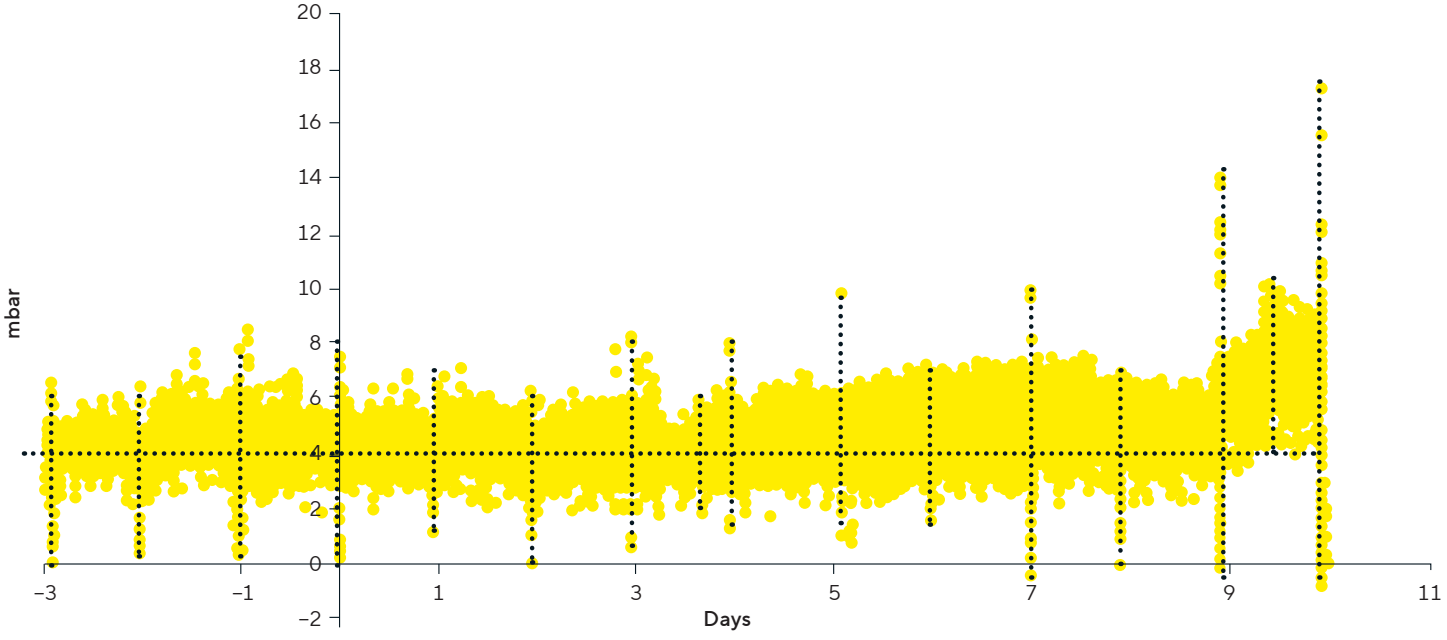
Slight backpressure spikes were observed during manual sampling in Trial 1, but did not affect the overall process performance (Figure 12). The cumulative permeate throughput exceeding 1,000 L/m² demonstrates the membrane's robustness, indicating its suitability for scaling to larger-volume bioreactors.

Figure 11: Daily perfusion rate



Note. Liters were converted from the kilogram weight of the permeate bag. The y-axis is shows the amount of permeate collected over the course of 1 day (L/D) normalized to the working volume (L).

Figure 12: Bag pressure (Trial 1)



Product titer
Titer concentrations were measured in both the bioreactor and permeate lines using the Octet® R8 Protein Analysis System. Results showed close agreement between the two sample sets for each trial (Figure 13), confirming efficient sieving through the perfusion membrane and consistency of product collection.

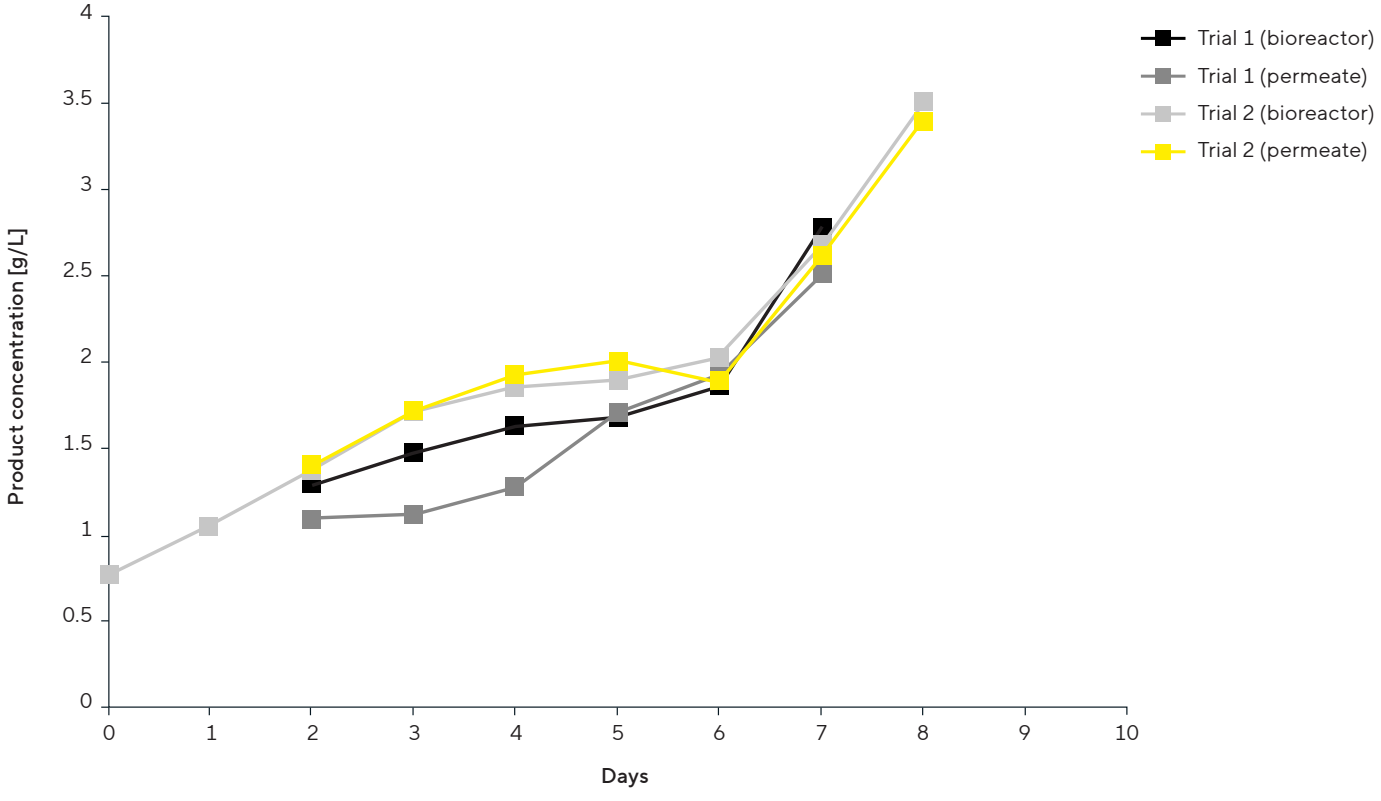
The combination of high cell density, stable perfusion, and optimized nutrient supply resulted in harvest volumes of approximately 25 L per trial, with product concentrations reaching up to 3.5 g/L. These outcomes highlight the ability of the Biostat® RM system to support efficient upstream intensification while maintaining product integrity, demonstrating suitability for scalable biopharmaceutical applications.

Comparison of independent trials
Despite minor differences in growth rate, both trials exhibited similar endpoint performance across key parameters, including viable cell density, viability, TMP, permeate throughput, and titer concentration.

The observed differences in growth rate were consistent with the expected variation for this CHO cell line. TMP and perfusion dynamics mirrored these growth trends, with the earlier TMP spike in the second trial reflecting its faster approach to high-density conditions. Overall, the trials confirm reproducibility of the system and robustness of the N-1 perfusion strategy.

In summary, the integrated Biostat® RM perfusion system — comprising the control tower, BioPAT® Viamass, 2 L Flexsafe® RM bag, and 4Cell® SmartCHO Media System — demonstrated reliable high-density performance, precise environmental control, robust membrane operation, and consistent product titers. Both trials validated the reproducibility of the N-1 perfusion strategy and its suitability for upstream process intensification. Together, these results show that the Biostat® RM platform can efficiently generate high-density cultures for larger-scale bioreactors while maintaining cell viability and product quality, establishing a strong foundation for scalable perfusion-based manufacturing.

Figure 13: Product titers



Discussion

In two independent trials, the 2 L Flexsafe® RM Perfusion bag provided a robust platform for intensifying cell culture processes. The evaluation of the process performance, bag integrity, and cell productivity underscores this bioreactor as a critical enabler of upstream scale-up efficiency.

N-1 perfusion strategy and Biobrain® Supervise (SCADA) recipe

With the growing demand for rapid and cost-effective biologics manufacturing, N-1 perfusion has emerged as a promising upstream intensification strategy, particularly in single-use systems. Its adoption is driven by the need for high productivity while maintaining flexibility in biomanufacturing pipelines.

N-1 perfusion is typically used to intensify processes by achieving high cell density in seed trains. This allows for scalable production by transferring cell culture to larger production reactors. Unlike traditional fed-batch seed trains, which rely on fixed feeding schedules and often experience limitations in cell density and viability, the perfusion approach sustains an optimal environment for exponential growth through continuous media exchange and waste removal. By improving cell growth conditions, the N-1 process can lead to improved quality and yield.[1]

In this study, the N-1 perfusion strategy successfully supported high-density cultivation and consistent process control. For the first three days (N-1 phase) after inoculation from the shake flask to the Biostat® RM, the cells were allowed to grow until 2.5×10^6 cells/mL, at which point perfusion was initiated. The DO setpoint was then lowered to enable finer oxygen control at higher cell densities, minimizing oxidative stress. The natural pH drift downward to 6.95 supported lactate metabolism reversal and improved metabolic efficiency, eliminating the need for excessive base addition.

The perfusion strategy employed a CSPR-based feeding approach, supported by online biomass readings using a BioPAT® Viamass sensor (Figure 2). A CSPR of 50 pL/cell × day was chosen as the optimal feeding regime for this cell line using 4Cell® SmartCHO media. This strategy enabled the viable cell density to exceed 200 million cells/mL, with viability greater than 97%. At this density, a 1 L working volume can effectively inoculate a 500 L stirred-tank bioreactor at a starting density of 0.3×10^6 cells/mL, illustrating the efficiency of the system for seed train scale-up.

The working volume was maintained using level control via the rocker's load cells. As the perfusion rate increased, a total media exchange of up to 5 L was allowed (within the programmed range of 0.25 – 5 L VVD). The product was gradually filtered through the perfusion membrane, and media was replenished by the recipe-controlled peristaltic pump on the tower (Figure 5). The lower CSPR of 0.25 VVD was designed to both reduce media consumption and prevent overfeeding. Excessive feeding can shock cells due to elevated osmolality and glucose concentration, leading to lower peak cell densities, reduced titers, and increased cellular stress resulting in unwanted byproducts.

The permeate included an in-line pressure sensor; as the membrane approached blockage, the pressure increased until approximately 0.827 bar, which indicates full membrane obstruction (Figure 11). Comparison of the IVCC with TMP revealed the expected relationship between increasing cell biomass and membrane fouling: as cell density increased, more biomass accumulated on the membrane surface, increasing resistance and causing a gradual rise in pressure and reduced membrane performance.

By applying the N-1 perfusion strategy with the 4Cell® SmartCHO Media System, overall manufacturing costs can be significantly reduced through optimized nutrient supply and efficient waste removal. The tailored 4Cell® SmartCHO formulation enabled efficient nutrient delivery with minimal supplementation, reducing the overall cost of goods. Combined with the low medium-to-product ratio of perfusion-based processes, this approach supports a more sustainable and economically viable production process.

The harvest material (~25 L) was collected in a 50 L Flexboy® bag and yielded up to 3.5 g/L of product as measured by the Octet® R8 system (Figure 14). Comparable concentrations between bioreactor and permeate samples indicated adequate product sieving and consistent harvest quality.

Cumulated permeate throughput exceeded 1,000 L/m², demonstrating membrane robustness and scalability of the system to larger-volume reactors with proportional membrane area. This performance is typical for small-scale perfusion and highlights the strong membrane capacity and overall efficiency of the Biostat® RM system.

Conclusion

In conclusion, the Biostat® RM perfusion platform highlights the potential of the Biostat® RM as a strong player in scale-up manufacturing for many therapeutics, including advanced therapies that require a low-shear environment. Data from two trials confirm the reproducibility of achieving cell densities above 200×10^6 cells/mL (via online BioPAT® Viamass measurements), underscoring both the robustness of the N-1 perfusion strategy and the strength of the 4Cell® SmartCHO media platform.

The data supports the Biostat® RM platform as a key player in scalable processes that can be integrated into GMP manufacturing with minimal adjustments, offering flexibility across different clones and product types.

References

1. Rittershaus, E. S. C., Rehmann, M. S., Xu, J., He, Q., Hill, C., Swanberg, J., Borys, M. C., Li, Z.-J., & Khetan, A. (2022). N-1 perfusion platform development using a capacitance probe for biomanufacturing. *Bioengineering,* 9(4), 128. https://doi.org/10.3390/bioengineering9040128

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