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Application Note

October 2025

Keywords or phrases:
Perfusion bioprocessing, bioreactor system, Biobrain® Supervise software, high cell density, titer production, gravimetric and volumetric perfusion strategies, 4Cell® SmartCHO media platform, automated bleed strategies, BioPAT® Viamass, scalability, process optimization, real-time monitoring, cell growth dynamics, resource efficiency, operational excellence, Univessel® SU, Biostat® B-DCU

Advancing Perfusion Bioprocessing With the Biostat® B-DCU

Clint Kukla[1], Anastasia Bizios[1], Peter Tomasello[1], Juan Bastidas[1], Rayan Mattamir[2]
[1]Sartorius Stedim North America Inc., 565 Johnson Avenue, Bohemia, NY 11716, USA
[2]Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany

Contact: rayan.mattamir@sartorius.com

Abstract

Perfusion bioprocessing enables high cell densities, consistent product quality, and scalable production for biopharmaceutical manufacturing. This application note highlights how the Biostat® B-DCU, integrated with Biobrain® Supervise software, supports advanced perfusion strategies through precise monitoring and automated control of critical parameters.

Using CHO DG44 cell lines at 2 L and 10 L scales, the system was evaluated with gravimetric and volumetric perfusion methods in combination with 4Cell® SmartCHO media and automated bleed strategies. The Biostat® B-DCU demonstrated robust control of cell growth and viability, reduced risk of filter fouling, and consistent performance across scales.

These findings establish the Biostat® B-DCU as a reliable and flexible platform for optimizing perfusion processes, enabling bioprocess engineers to improve productivity, efficiency, and scalability in biopharmaceutical production.

Introduction

Optimizing perfusion strategies is crucial for enhancing cell growth dynamics and ensuring consistent product quality in biopharmaceutical production. We conducted a comprehensive series of experiments to assess various perfusion methods, scales, and media platforms, providing valuable insights into their effects on cell density, viability, and overall process efficiency.

These experiments utilized both gravimetric and volumetric perfusion strategies at both 2 L and 10 L scales, employing the CHO DG44 cell line. The Biobrain® Supervise software, along with the Biostat® B-DCU, was employed for integrated monitoring and control, enabling precise adjustments to critical process parameters such as pH, dissolved oxygen (DO), temperature, and perfusion rates.

Key findings from the study include the successful implementation of bleed strategies, the adaptability of perfusion methods across different scales, and the robustness of the 4Cell® SmartCHO media platform in maintaining high cell densities without alternating tangential flow (ATF) filter fouling. The results underscore the versatility and scalability of the perfusion process, demonstrating its effectiveness in sustaining consistent cell growth and viability under varying conditions. The data provide a detailed view of cell density trends, titer levels, and system control over process variables, offering valuable data for optimizing perfusion strategies in biopharmaceutical production.

The insights gained from these experiments emphasize the importance of precise control and monitoring in achieving efficient and scalable bioprocesses.

Materials and Methods

The experiments assessed gravimetric and volumetric perfusion strategies across 2 and 10 L bioreactor scales, utilizing the Biostat® B-DCU's advanced automation capabilities. The 4Cell® SmartCHO media platform was tested for its ability to support high cell density without ATF filter fouling. Bleed strategies were implemented to regulate viable cell density (VCD), with Biobrain® Supervise enabling real-time control of process variables. Comprehensive data illustrate cell density trends, titer levels, and system performance, offering actionable insights for bioprocess optimization.

Table 1: *Overview of the Performed Runs and Their Parameters*

	Run 0	Run 1	Run 2	Run 3	Run 4	Run 5
	Media 1	**Continuous**	**N-1 (2 L)**	**N-1 (2 L)**	**N-1 (2 L)**	**10 L**
Gravimetric vs volumetric	Gravimetric	Gravimetric	Gravimetric	Gravimetric	Volumetric	Volumetric
Perfusion strategy (CSPR pL/cell/day)	50	50	50	50	40	40
Perfusion mode (N-1 vs. continuous; with or without bleed)	Continuous with bleed	Continuous with bleed	N-1 (bleed to 45×10^6, then let go)	N-1 (bleed to 45×10^6, then let go)	N-1 without bleed	N-1 without bleed
Bioreactor scale [L]	2	2	2	2	2	10
LMH (flux; max/target)	2.2	2.2	2.6	2.6	2.6	11.5
Multi-use (glass) vs. single-use (with multi-use vs. single-use sensors)	Glass	Glass	Glass	Glass	Glass	Single-use
Media used	Media 1	SmartCHO Perfusion media	SmartCHO Perfusion media	SmartCHO Perfusion media	SmartCHO Perfusion media	SmartCHO Perfusion media

Note. CSPR = Cell-specific perfusion rate, LMH = liter/m^2/h

Results

VCD and viability were tracked from the start of perfusion to completion. Figure 1 demonstrates the Biostat® B-DCU's ability to maintain efficient and stable cell growth across varied perfusion strategies and scales.

Figure 1: *VCD and Viability Trends (Runs 2, 3, 4, and 5) Across the Perfusion Process*

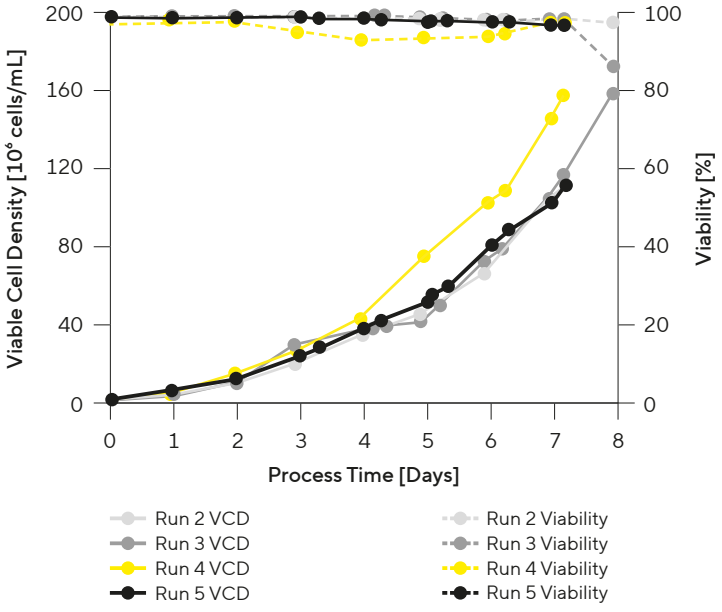
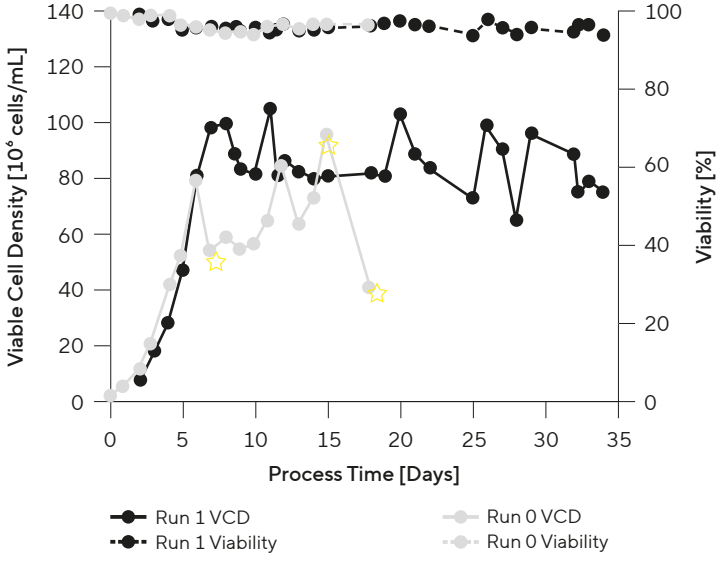


Figure 2 highlights the impact of media selection on ATF fouling. Run 0 experienced ATF fouling at three points, compromising cell density and viability (Figure 2, yellow stars). In contrast, Run 1 — using 4Cell® SmartCHO media and the Biostat® B-DCU's precise gravimetric control — sustained a VCD of 80 × 10⁶ cells/mL for 34 days without fouling, highlighting the media performance.

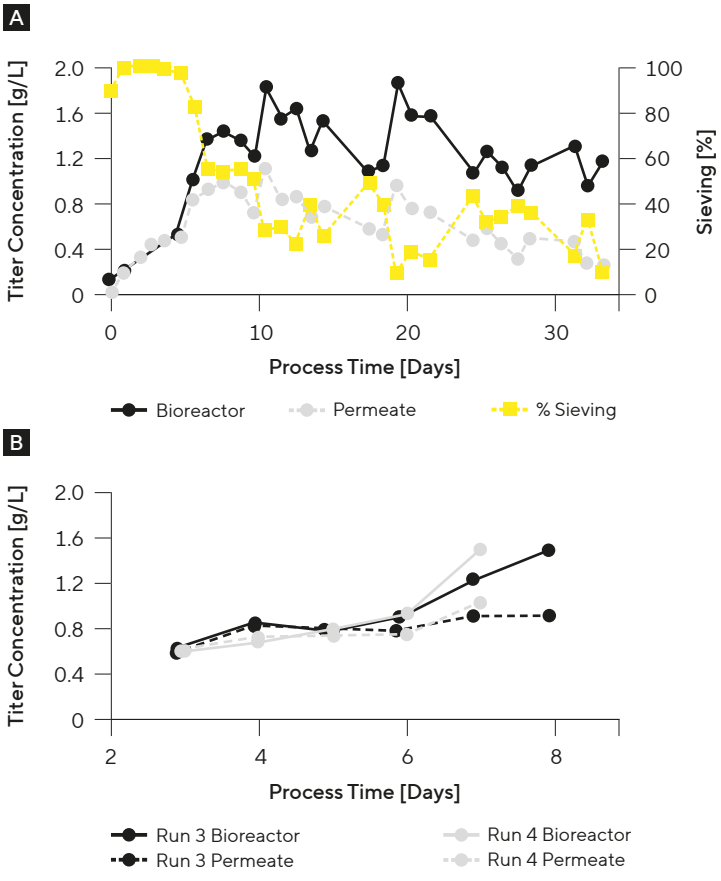
Figure 2: *The impact of media selection on ATF fouling (Runs 0 and 1) Across the Perfusion Process*



Note. Yellow stars indicate points where the ATF fouled.

Figure 3A provides a comprehensive view of titer levels and sieving efficiency throughout the perfusion process, revealing a gradual decline in permeate titer due to increased ATF pressure late in the run. Titer trends are compared between two runs in Figure 3B, with Run 4 lacking data from Day 8 onward due to analytical constraints. The Biostat® B-DCU's consistent performance across runs underscores its robustness.

Figure 3: ***(A)*** *Bioreactor Titers, Permeate Titers, and Sieving Analysis for Run 1 Across the Entire Perfusion Process* ***(B)*** *Bioreactor and Permeate Titers for Runs 3 and 4*



Note. **(B)** shows bioreactor and permeate titers for Runs 3 and 4, starting from a few days after perfusion initiation. For Run 4, titer results from Day 8 are unavailable.

Process efficiency was measured by analyzing the cell-specific permeate rate (CSPR) and permeate weight during Run 4 (Figure 4A). The results illustrate a reduced cell-specific perfusion rate with lower media consumption while maintaining titer levels comparable to Run 3 (Figure 3B), highlighting the Biostat® B-DCU's resource efficiency. We also compared results from online BioPAT® Viamass readings and offline VCD counts (Figure 4B). The strong agreement between results from the two methods validates the system's real-time monitoring accuracy despite a data gap on the final day due to an offline analyzer issue.

Figure 4: ***(A)*** *Process Efficiency (CSPR and Permeate Weight) for Run 4 and* ***(B)*** *Comparison of BioPAT® Viamass Readings With Offline Cell Counts for VCD Measurement*

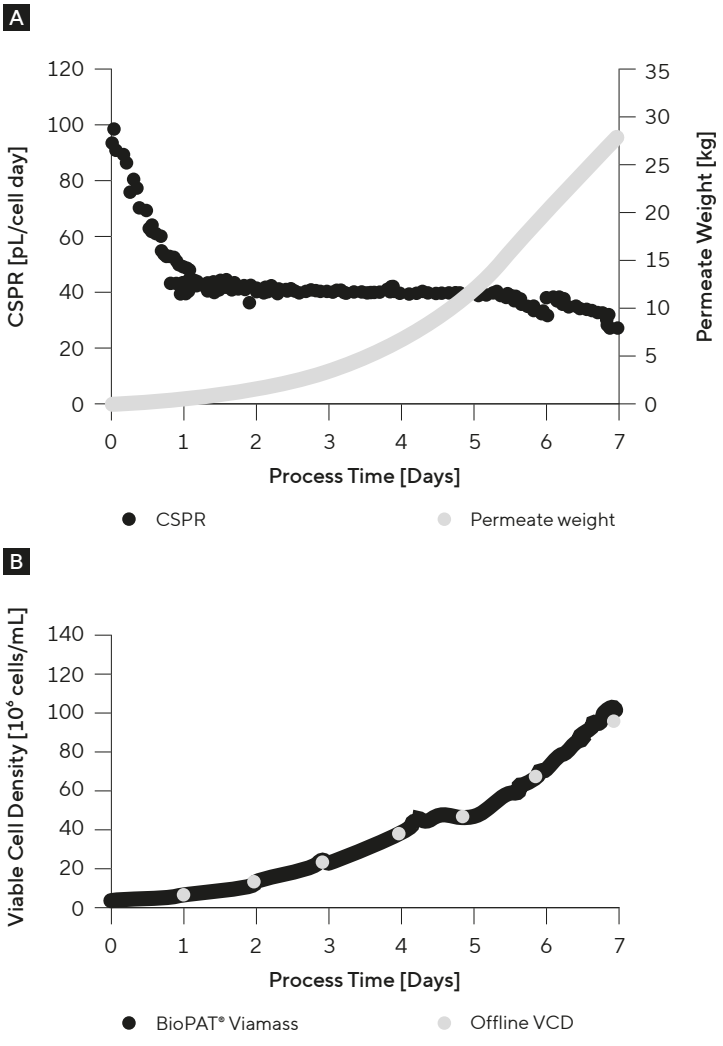
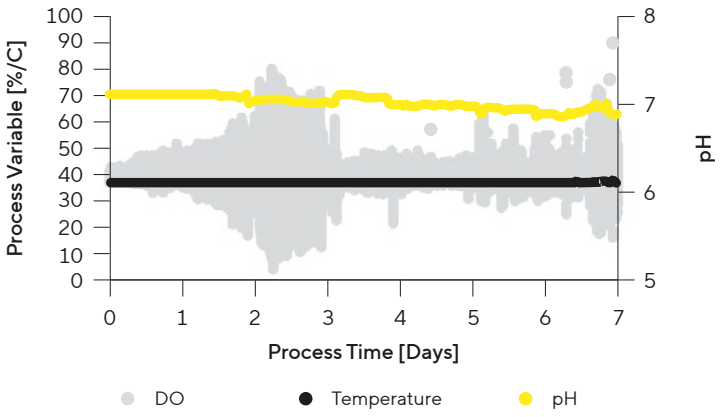


Figure 5: *Dissolved Oxygen (DO) Percentage, PH, and Temperature Trends for Run 4*



To test the control of critical parameters, DO, pH, and temperature were tracked for Run 4 across seven days (Figure 5). The results demonstrate the Biostat® B-DCU's ability to maintain stable conditions throughout perfusion, ensuring optimal cell growth.

Discussion

Biobrain® Supervise: Precision Automation
The Biostat® B-DCU is seamlessly integrated with the Biobrain® Supervise software, enhancing the monitoring, automation, and control of bioprocesses. This advanced software continuously records critical process parameters, including pH, DO, temperature, agitation, feed rates, and gas flows, among others. The data presented here shows that — by leveraging these parameters — Biobrain® Supervise facilitates the design of customizable processes.

Each of the five experiments used distinct perfusion strategies, guided by unique recipes that incorporated bleed strategies or were managed through gravimetric or volumetric control, with varying CSPRs as detailed in Table 1.

Bleed Strategy Automation
Runs 1, 2, and 3 employed automated bleed strategies. In Run 1, a gravimetric approach maintained a VCD of 80×10^6 cells/mL using BioPAT® Viamass capacitance measurements and a fixed gain variable to adjust flow rates. Managed by Biobrain® Supervise, this strategy extended the run to 34 days, showcasing the Biostat® B-DCU's ability to automate complex processes. Runs 2 and 3 supported secondary reactor inoculations, demonstrating the system's versatility for multi-purpose workflows.

Perfusion Strategy Flexibility
The Biostat® B-DCU supports both gravimetric (Runs 1 – 3) and volumetric (Runs 4 and 5) perfusion methods, accommodating user preferences for lab space or pump calibration. All runs utilized the same XCell® ATF 2 system, with Runs 4 and 5 targeting lower CSPRs to optimize media usage and reduce costs (Figure 4A). BioPAT® Viamass delivered accurate real-time VCD data, minimizing manual sampling risks and enabling dynamic perfusion rate adjustments for precise nutrient delivery.

Perfusion Performance
Run 4 demonstrated robust control of DO, pH, and temperature (Figure 5), with the capacity to meet increased oxygen demand. The single-use DO and pH patches of Univessel® SU, paired with a heating jacket in Run 5, ensured stable conditions. Flexible ATF connection options (side port in Run 5, bottom drain in Runs 1 – 4) enhanced vessel configuration adaptability, streamlining process setup.

Hollow Fiber Filter Performance
Run 1 validated the 4Cell® SmartCHO media platform, sustaining 80×10^6 cells/mL for 34 days without ATF fouling, unlike Run 0, where media fouling disrupted the run. Runs 4 and 5, conducted at 2 L and 10 L scales, respectively, achieved comparable growth dynamics using the same ATF system, demonstrating the Biostat® B-DCU's scalability and consistent performance across reactor sizes.

Cell Growth Dynamics
The experiments revealed consistent cell growth and viability across Runs 2, 3, and 5, despite varying CSPRs and perfusion methods. Table 1 shows comparable flux across scales, confirming the process's scalability. Run 5 (10 L) exhibited higher flux and a lower peak VCD, providing insights for large-scale optimization. Predictable ATF fouling events enabled proactive interventions, ensuring process reliability.

Conclusion

The Biostat® B-DCU sets a new standard for perfusion bioprocessing, delivering precision, scalability, and efficiency for biopharmaceutical production. These experiments highlight its ability to sustain high cell density and achieve multifold titer increases compared to fed-batch processes, driven by advanced automation and real-time monitoring via Biobrain® Supervise and BioPAT® Viamass. By offering flexible perfusion strategies and robust control, the Biostat® B-DCU enables bioprocess engineers to optimize productivity and reduce costs.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
sartorius.com

Last modified: 11 | 2025