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Perfusion Cultivations with NIST CHO Cells Using the Multifors 3 Bioreactor

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Abstract

This application note demonstrates the suitability of the Multifors 3 bench-top bioreactor (INFORS HT) for long-term perfusion cultivation of a NIST CHO cell line.

All bioreactor control was performed by Multifors 3. A tangential flow filtration (TFF) loop, equipped with a centrifugal pump (Levitronix GmbH) and a hollow fiber module (Asahi Kasei Bioprocess), was used for the perfusion filtration process. Over a 23-day cultivation, the system maintained a steady-state capacitance of 85 pF cm^{-1}, corresponding to a viable cell density (VCD) of 23–30 × 10^6 cells mL^{-1} at a perfusion rate of 2 vvd, with viability consistently above 95%.

These results demonstrate that Multifors 3 is suitable to sustain a continuous and stable perfusion process over this period, including the control of all bioreactor-side variables-gravimetric volume control, feed rate, pH, dissolved oxygen and biomass - handled by the integrated control system.

Keywords
Perfusion cultivation, process intensification, NIST CHO cells, mammalian cell culture bioreactor, tangential flow filtration (TFF), cell-specific perfusion rate (CSPR), continuous bioprocessing, Multifors 3, eve®, capacitance-based biomass control, gravimetric volume control, viable cell density (VCD)

Objective

This study aims at demonstrating the capability of the Multifors 3 bioreactor, controlled via eve®, to support long-term perfusion processes in combination with a Levitronix TFF system.

Specific objectives:

- Demonstrate reliable gravimetric volume and feed control using the integrated Multifors 3 pumps and balance interfaces
- Validate process control performance for pH, DO, perfusion rate and biomass control.

Introduction

The rapid growth of the biopharmaceutical industry presents significant challenges for biomanufacturing. Cross-disciplinary expertise across biotechnology, chemistry, engineering, control theory, and digitalization is essential to improve product yield and purity in mammalian cell culture. Increasingly, the industry is adopting process intensification strategies including N-1 perfusion for high density inoculation of fed-batch processes or main stage continuous perfusion operations.

However, perfusion bioprocessing introduces substantial operational complexity by requiring the simultaneous management of multiple subsystems. Therefore, operating such system demands competencies beyond cell culture, extending into IT integration and process control. Beyond standard process parameters, operators must maintain tight bioreactor volume control and stable cell density, as well as additional crucial parameters, such as pH and dissolved oxygen (DO). The Multifors 3 bench-top bioreactor addresses this challenge by handling the bioreactor-side fluid path on board: integrated peristaltic pumps for medium feed, permeate, bleed and correction agents, together with direct balance interfaces for gravimetric volume and feed control.

By utilizing integrated pumps and supporting direct connection of external balances, the setup eliminates complex third-party hardware integration while maintaining open interfaces for specialized perfusion peripherals.

This application note presents the results of one perfusion run performed on the Multifors 3, demonstrating its suitability for continuous bioprocess applications. The experiments were conducted at the FHNW Bioprocess Technology Laboratory in collaboration with INFORS HT.

Methods and materials

Cell line and media

Perfusion cultivation was carried out using the NIST CHO cell line, a publicly available CHO reference cell line expressing the NISTmAb monoclonal antibody. Cells were cultivated in Cellvento® XCHO medium (Merck), both during pre-inoculum and bench-top cultures.

Bioreactor setup and process intrumentation

Cultivations were performed in an INFORS HT Multifors 3 bench-top bioreactor with a 2.5 L glass vessel (3.75 L total volume, 0.83–2.5 L working-volume range), operated at 1.7 L working volume and controlled via the eve® bioprocess platform software. The vessel was equipped with a dissolved oxygen (DO) sensor (Mettler Toledo) as well as a pH, pCO_2 and biocapacitance probe (Hamilton). All four inline probes were operated in parallel on the 1.7 L working volume using the vessel's Pg13.5 sensor ports and the digital sensor interfaces of the controller.

Core bioreactor parameters (pH, DO, temperature, biomass) and gravimetric volume control were directly managed by the integrated Multifors 3 control system. A comprehensive overview of all materials, consumables, and analytical equipment is summarized in Table 1.

Process automation and control strategy

Advanced process control loops and soft sensors were configured in eve® and executed on the Multifors 3 controller, so that control continued independently of network availability:

- Gravimetric Volume Control: A precision balance (Kern) positioned under the bioreactor vessel continuously monitored weight, triggering the integrated harvest/ permeate pump to maintain a constant working volume.
- Feed Rate Control: A second balance beneath the feed reservoir precisely controlled daily feeding rates.
- Automated cell bleeding was regulated via inline biocapacitance. The setpoint was dynamically adjusted during the run to 85 pF cm^{-1} to stabilize the culture, maintaining glucose concentrations above the critical threshold.
- Perfusion Rate (vvd) Adjustment: Perfusion rate was stepped up in increments of 0.5 vvd whenever BioProfile® FLEX2 measurements indicated glucose concentration below 2 g L^{-1}.

Table 1. Materials, consumables, and analytical equipment

Parameter / Material	Specification
Bioreactor	Multifors 3 bench-top bioreactor, 3.75 L total volume (INFORS HT)
Working volume	1.7 L
Cultivation duration	23 days
Process control software	eve® bioprocess platform software (INFORS HT)
Cell line	NIST CHO
Medium	Cellvento® XCHO (Merck)
Starting viable cell density	0.7 × 10^6 cells/mL
Biomass control (bleed) setpoint	85 pF/cm (inline biocapacitance)
Steady-state viable cell density	23-30 × 10^6 cells/mL
Stirrer	Rushton impeller
Stirrer speed	270 rpm
Temperature	36.5 °C
pH setpoint	7.17 (controlled via CO_2 sparging and 1 M $NaHCO_2$ addition)
Dissolved oxygen (DO)	50% saturation
Perfusion rate (dilution rate, D)	2 vvd, stepped in 0.5 vvd increments
Cell-specific perfusion rate (CSPR)	approx. 67–87 pL $cell^{-1}$ d^{-1} (2 vvd at 23–30 × 10^6 cells mL^{-1})
Hollow fiber module (HFM)	Asahi Kasei Bioprocess; 0.2 µm pore diameter, 0.22 m^2 area, fiber inner diameter 1.4 mm, module length 299 mm
Perfusion pump	PuraLev® i30SU single-use centrifugal pump with LCO-i100 control unit and LEVIFLOW® LFSC-iX flow sensor, Levitronix GmbH
Pressure sensors	Three single-use sensors: two retentate (up-/downstream of TFF), one permeate (PendoTECH)
Loop flow rate	1200 mL min^{-1}
Loop volume	100 mL
DO sensor	Mettler Toledo
pH, pCO_2 and biocapacitance sensors	Hamilton
Gravimetric control	Precision balances under vessel and feed reservoir (Kern)
Offline analytics	BioProfile® FLEX2 analyzer (Nova Biomedical)

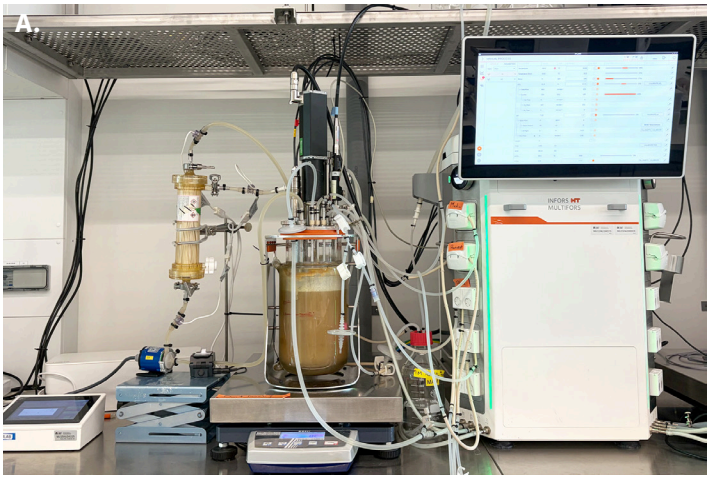
TFF perfusion loop

The perfusion retention loop operated via a TFF system interfaced with Levitronix automation (shown in Figure 1A and schematically in Figure 1B):

- Recirculation & Flow Control: A Levitronix centrifugal pump system was equipped with an inline flow sensor positioned upstream of the pump head, enabling flow control by the Levitronix system.
- Pressure Monitoring: Three pressure sensors (PendoTECH) were integrated into the loop: two on the retentate line (upstream and downstream of the hollow fiber module) and one on the permeate line.

Offline analytics

Reference samples were analyzed using a BioProfile® FLEX2 analyzer (Nova Biomedical). These measurements provided VCD, cell viability, and key metabolic analytes to monitor the cell growth and nutrient consumption throughout the run.



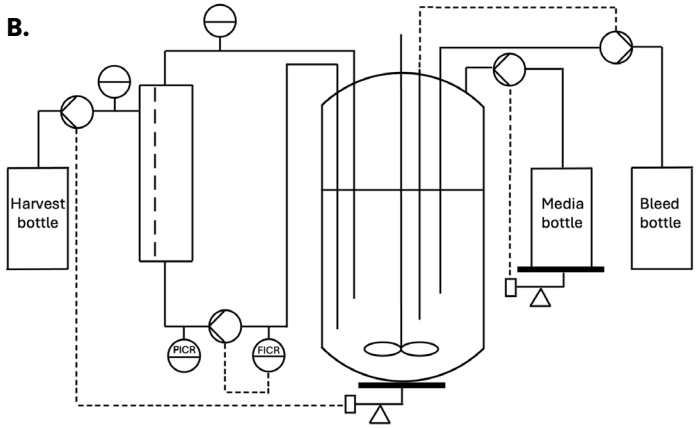


Figure 1. Perfusion setup on the Multifors 3. (A) Bioreactor vessel with the Levitronix TFF loop (feed, harvest, and bleed bottles with auxiliary balances are located underneath the bench). (B) Process scheme of the perfusion loop and control strategy.

Results

Cell growth and viability

The cultivation successfully maintained a capacitance of 85 pF cm^{-1}, corresponding to a VCD of 23–30 × 10^6 cells mL^{-1}, with 15 days of steady-state operation across the 23-day run. As the primary objective was to demonstrate the Multifors 3's capability to sustain continuous operation, CSPR optimization was not pursued. With cell bleeding controlled at a capacitance setpoint of 85 pF cm^{-1}, offline Nova analytics consistently showed a residual glucose concentration between 0.2 and 0.7 g L^{-1}, confirming that a dilution rate of 2 vvd was appropriate under these operating conditions. Viability remained above 95% throughout the steady-state phase (Figure 2).

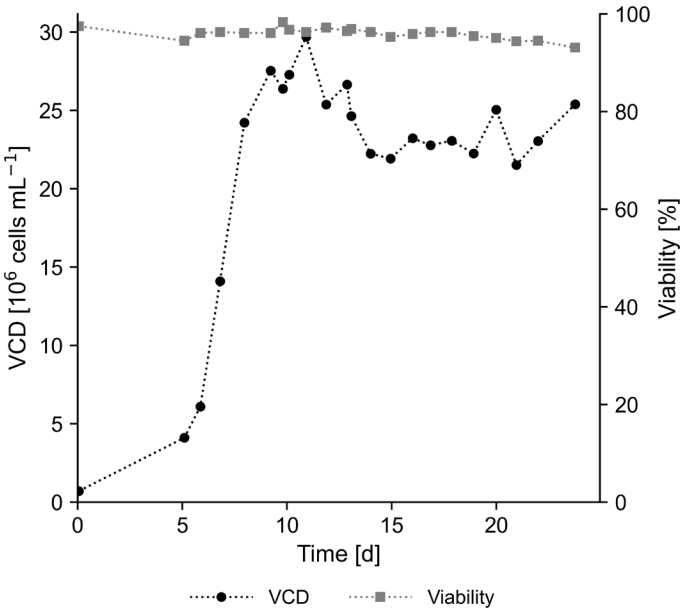


Figure 2. Offline VCD (filled circles) and cell viability (grey squares) measured via BioProfile FLEX2 across the 23-day process.

A gradual decoupling between inline biocapacitance and offline VCD was observed over the steady-state phase (Figure 3). While the capacitance signal remained on its 85 pF cm^{-1} setpoint and the bleed control acted accordingly, the offline VCD declined progressively over the same period. This behavior is explained by an increase in mean cell diameter, which rose from 16 µm at the beginning of the steady state to 18.5 µm towards the end of the run, corresponding to an approximately 55% larger mean cell volume. Since biocapacitance responds to the total polarizable membrane volume rather than to cell number, a lower number of larger cells produced an unchanged capacitance signal. The permittivity setpoint therefore remained a robust control handle for total biomass, but the fixed capacitance-to-VCD correlation established at the start of the cultivation progressively overestimated the actual cell count.

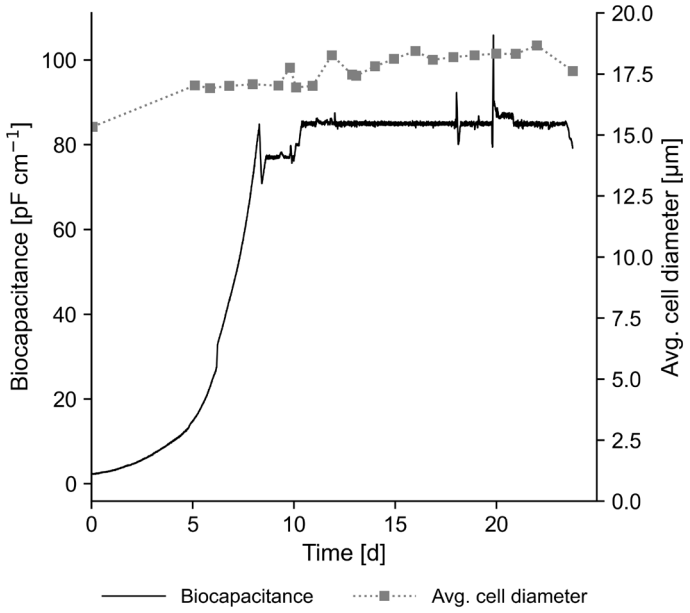


Figure 3. Inline biocapacitance monitoring and mean cell diameter over time. The capacitance signal was maintained at the 85 pF cm^{-1} setpoint via automated bleed control, while the offline mean live cell diameter increased from 16 to 18.5 µm during the steady-state phase.

The stepwise adjustment of the perfusion rate during the growth phase is shown in Figure 4. Starting from inoculation, the dilution rate was increased in 0.5 vvd increments until 2 vvd, reached on day 8, marking the onset of steady-state operation. The CSPR remained stable while cell number increased and rose after day 13 as the offline VCD declined at constant biomass setpoint.

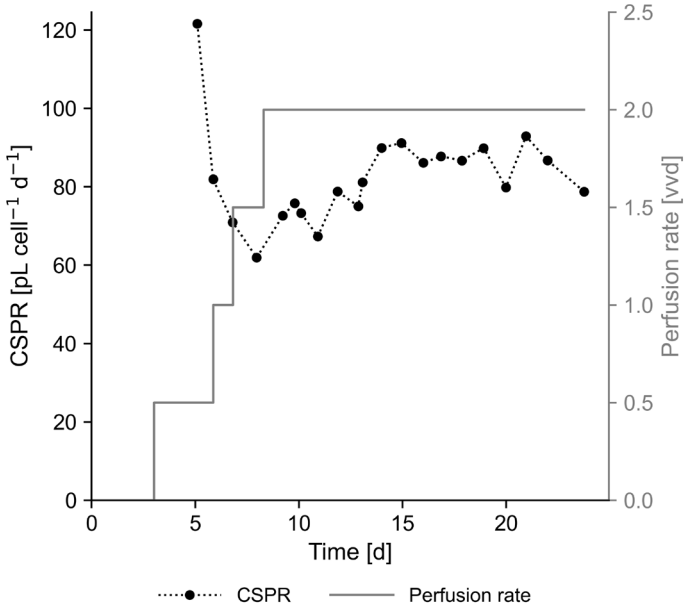


Figure 4. The CSPR and perfusion rate over the cultivation. The perfusion rate was stepped up in 0.5 vvd increments to 2 vvd by day 8. The CSPR increase after day 13 reflects the decreasing cell number at constant biomass setpoint.

Titer measurement

Offline titer measurements are shown in Figure 5. At a constant dilution rate of 2 vvd, volumetric productivity remained stable at approximately 0.4 g L^{-1} d^{-1} throughout the steady-state phase, confirming that the process sustained a constant output while biomass was held at its setpoint.

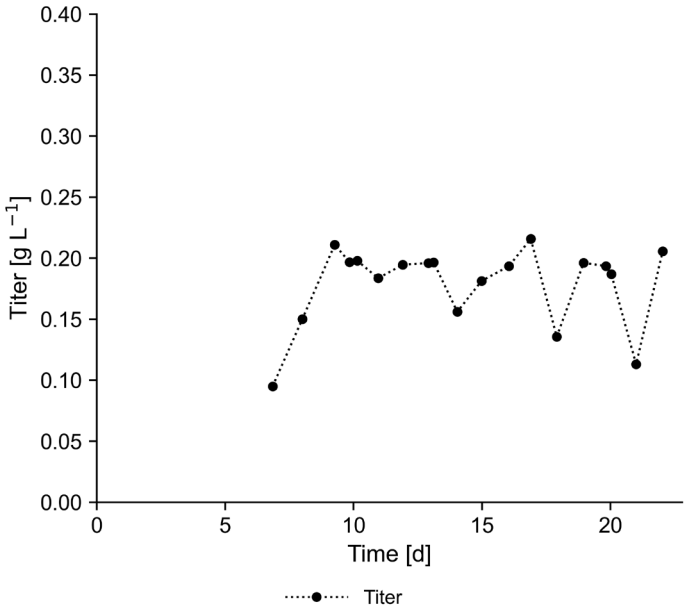


Figure 5. Offline titer measurement over the cultivation. Titer was quantified by Protein A HPLC.

Metabolite profiles and perfusion rate

Metabolite profiles followed the trend expected for a perfusion process at fixed dilution rate (Figure 6). Glucose decreased from 7.1 g L^{-1} at inoculation to below 1 g L^{-1} from day 8 onward and remained between 0.2 and 0.7 g L^{-1}, while lactate peaked at approximately 3.2 g L^{-1} during exponential growth (days 5–6) and then stabilized between 1.9 and 2.5 g L^{-1}.

On the final day the medium feed was interrupted, and residual glucose was depleted within hours, while lactate began to decrease from its stable 1.9–2.5 g L^{-1} level. This inverse behavior indicates a shift from net lactate production to net lactate consumption, with lactate converted to pyruvate and re-entering the TCA cycle as the cells switched to oxidative metabolism. The shift is also visible in the oxygen profile (Figure 7), where the higher specific oxygen uptake rate prompted the DO controller to increase the gas flow and the oxygen fraction in the gas mix.

Dissolved oxygen and pH control

Dissolved oxygen was maintained at 50% saturation throughout the perfusion run, as reported in Figure 7. The rising oxygen demand, occurred during the exponential phase of cells, was firstly compensated by ramping the total gas flow, followed by a further raising the oxygen fraction in the gas mix, which reached 100% from day 5.5 onward. At steady state the total gas flow stabilized at 400 mL min^{-1}. Though, a minor internal leak was subsequently identified as contributing to the increase gas requirement and thus was readily resolved.

pH was held at the setpoint of 7.17 within ±0.02 units for most of the 23-day cultivation (Figure 8). Two excursions occurred on day 9 and day 22, with pH dropping below 7. In both cases base consumption was higher than anticipated and the $NaHCO_3$ reservoir was emptied before it was replaced; the excursions therefore originated from reagent supply rather than from the control loop, which returned pH to setpoint once the reservoir was refilled. The metabolic shift from glucose consumption to lactate consumption is also visible in the pH trace: once the cells stopped producing lactate, the pH began to rise, and as it moved above the setpoint of 7.17 the controller responded by sparging CO_2 to counteract this change.

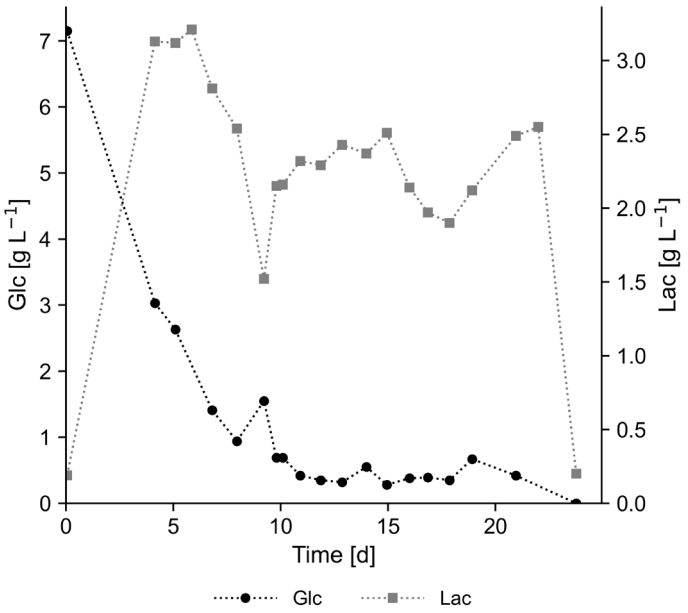


Figure 6. Offline glucose (black circles) and lactate (grey squares) concentration profiles. On the final day the medium feed was interrupted, resulting in glucose depletion and the onset of net lactate consumption.

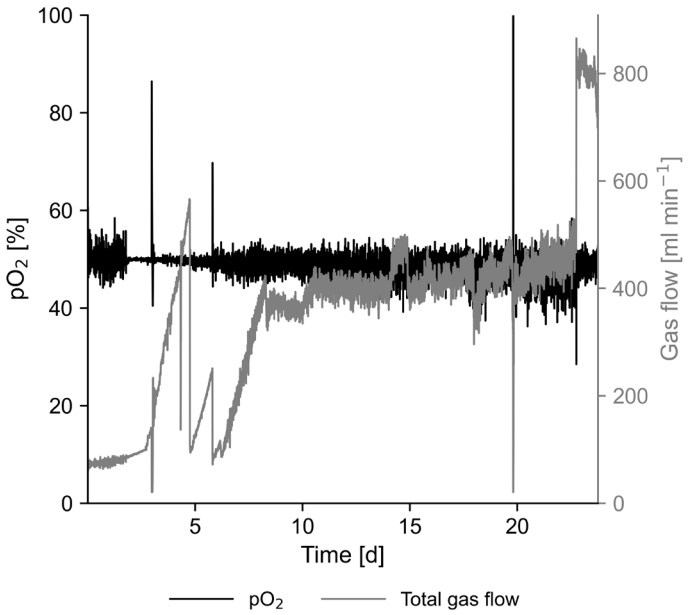


Figure 7. Dissolved oxygen control over the cultivation. Measured pO_2 and setpoint (50% saturation) and total gas flow.

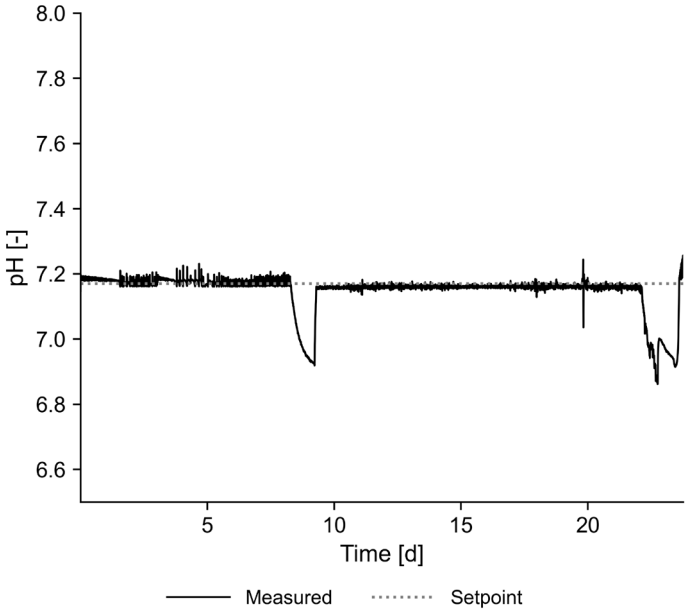


Figure 8. pH control over cultivation. Measured pH and setpoint (7.17), controlled via CO_2 sparging and 1 M $NaHCO_3$ addition. On day 9 and day 22 the base reservoir was depleted, as base consumption was higher than anticipated.

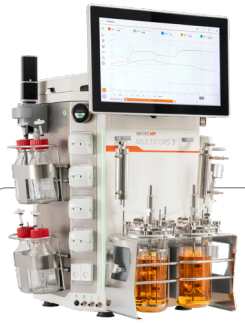
Conclusion

The perfusion cultivation presented here demonstrates the robustness of the Multifors 3, operated through the eve® bioprocess platform software, able to sustain a 23-day perfusion process. A constant capacitance of 85 pF cm^{-1} was maintained at 2, while cell viability remained consistently above 95%. Both specific objectives were met: on one hand, the effective gravimetric control maintained a stable culture volume over the cultivation while feeding the correct amount of fresh medium. On the other hand, pH, dissolved oxygen and biomass volume were all held at their setpoints, through the cascade controls and built-in peristaltic pumps.

Beyond the process result, the run exercised the Multifors 3 capabilities that matter specifically for perfusion. All bioreactor-side fluid handling - medium feed, permeate/ harvest, bleed and base addition - was carried out by integrated peristaltic pumps addressed directly by the controller, whose flow range covers from the low bleed rate to the high permeate rate required simultaneously in this process; the balances used for gravimetric volume and feed control were connected natively, without intermediate software. Four inline probes were operated in parallel on a 1.7 L working volume, supported by the sensor port and digital interface density of the vessel. The oxygen demand at high cell density was met by the cell-culture gassing configuration, with the mass flow controllers covering the full range between inoculation and steady state.

The gravimetric, bleed and perfusion-rate strategies were configured as soft-sensors and control loops in eve® but executed on the controller itself, thus process execution continues independently of network availability - a prerequisite for uninterrupted operation over a run of this length. The same controller and vessel configuration is used for batch and fed-batch operation, avoiding reconfiguration to switch between different process configurations.

Overall, this cultivation corresponds to a starting point. The unplanned events encountered during the run were useful and informative in showing how the control system responds to real world disturbances. Eventually, further work will address the optimization of the CSPR and the operation at higher cell densities, to further challenge the system and assess the reliability of Multifors 3 under more demanding conditions.



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