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Perfusion Cultivations with ExpiCHO-S Cells Using the Minifors 2 Bioreactor

Authors: Jan Ott[1], Regine Eibl[1], Dieter Eibl[1], Kulwant Kandra[2], Simon Stöckli[3], Antony Sibilia[3], Jürg Burkart[3]
Affiliations: [1] ZHAW Zurich University of Applied Sciences, Section for Cell Cultivation Techniques [2] INFORS HT [3] Levitronix GmbH

Abstract
This application note demonstrates the suitability of the Minifors 2 benchtop bioreactor (INFORS HT) for ultra-high cell density perfusion cultivation of IgG-producing ExpiCHO-S cells. A tangential flow filtration (TFF) loop, comprising a single-use centrifugal pump (PuraLev-i30SU, Levitronix GmbH) and a hollow fibre module (HFM) (Repligen), was integrated and operated within the eve® process control platform via OPC UA. Within 8 days, peak viable cell densities (VCDs) exceeding 100 × 10^6 cells mL^{-1}, alongside IgG titres of up to 901 mg L^{-1} were achieved. These results confirm that the Minifors 2, in combination with the eve® process control platform, is a capable and accessible development platform for intensified and continuous biomanufacturing processes, delivering ultra-high cell density performance in a compact benchtop footprint.

Keywords
Perfusion, Minifors 2, ExpiCHO-S, monoclonal antibody, tangential flow filtration, TFF, PuraLev, ultra-high cell density, continuous bioprocessing, eve®

Objective
This study aims to demonstrate the capability of the Minifors 2 bioreactor, controlled via eve®, to support ultra-high cell density perfusion processes in combination with a single-use TFF system.

Specific objectives:
- Achieve VCDs exceeding 100 × 10^6 cells mL^{-1}
- Integrate and control the PuraLev-i30SU TFF loop via the eve® platform
- Validate process control performance for pH, DO, perfusion rate, and nutrient concentration within a single control environment
- Confirm feasibility for N-1 seed culture and continuous manufacturing development

Introduction
Monoclonal antibodies (mAbs) constitute the largest and economically most significant class of biopharmaceutical products. Conventionally, mAbs are manufactured in stirred reusable or single-use bioreactors operated in fed-batch mode, from laboratory to cubic metre scale. Growing interest in process intensification and continuous bioprocessing has brought perfusion technology into focus as a strategy for improving volumetric productivity and product quality.
In perfusion operation, fresh medium is continuously supplied to the bioreactor while an equivalent volume of cell-free harvest is withdrawn continuously, enabling the maintenance of ultra-high viable cell densities over extended cultivation periods. This mode of operation requires robust cell retention systems and precise, coordinated process control across multiple loops at the same time — a key capability to validate at laboratory scale before transfer to manufacturing.

This application note demonstrates that the Minifors 2 benchtop bioreactor (INFORS HT) is well-suited for ultra-high cell density perfusion processes. A TFF loop comprising a single-use centrifugal pump (PuraLev-i30SU, Levitronix GmbH) and a hollow fibre module (HFM) (S02-E65U-07-S, Repligen) was integrated and operated via the eve® process control platform, which managed loop flow, pH, and dissolved oxygen within a single control environment. The experiments were conducted at ZHAW in collaboration with INFORS HT and Levitronix GmbH.

Methods and materials

Bioreactor and TFF Configuration

Perfusion cultivations were carried out in the Minifors 2 equipped with a 3 L culture vessel at a working volume of 1.5 L. The PuraLev Console System PCS-i30SU.1 (Levitronix GmbH) was fully integrated into the eve® bioprocess platform (INFORS HT) via OPC UA.

eve® served as the single control and data acquisition environment for all process loops (pH, dissolved oxygen, temperature, perfusion rate, and TFF loop flow) eliminating the need for separate control hardware and providing a consolidated process record throughout both cultivations. Loop flow was regulated via the PuraLev-i30SU pump head and a LeviFlow Clamp-on flow sensor.

Transmembrane pressure was monitored using single-use pressure sensors (PREPS-N-38, PendoTECH). Gravimetric weighing controllers (Maxxis 5, Sartorius) were used to regulate the perfusion rate. Glucose concentration was maintained at approximately 3 g L^{-1} by continuous addition of a concentrated glucose solution (200 g L^{-1}) via an Ismatec peristaltic pump (tubing inner diameter: 0.25 mm), enabling feed rates in the µL min^{-1} range.

A complete overview of materials and process parameters is provided in Table 1.

Table 1. Technical specifications, process parameters, and materials.

Parameter / Material	Specification
Bioreactor	Minifors 2 (3 L culture vessel, cell culture version, INFORS HT)
Working volume	1.5 L
Process control software	eve® (INFORS HT), connected via OPC UA
Cell line	ExpiCHO-S (Gibco)
Medium	High-Intensity Perfusion CHO Medium (Gibco) + 0.2 % Anti-Clumping Agent + 4 mM L-glutamine + 5 ppm Antifoam C
Starting viable cell density	1.0×10^6 cells mL^{-1}
Stirrer	3-blade segment impeller, Ø 65 mm
Stirrer speed	200 rpm
Tip speed (w_tip)	≈ 0.7 m s^{-1}
Volumetric power input (P/V)	≈ 60 W m^{-3}
Mixing time (t_M)	≈ 2.5 s
Temperature	37 °C
pH setpoint	≤ 7.2 (controlled via CO_2 addition)
Dissolved oxygen (DO)	≥ 60 % saturation (controlled via O_2 addition)
Perfusion rate (D)	Max. 3.5 / 3.1 vvd; gravimetric control (Maxxis 5, Sartorius)
Cell-specific perfusion rate (CSPR)	Min. 55 pL $cell^{-1}$ d^{-1} until reaching the max. perfusion rate
Hollow fibre module (HFM)	mPES, 0.65 µm pore diameter, 520 cm^2 area, 0.75 mm fibre ID, 110 fibres, 20 cm length (Repligen)
Perfusion pump	PuraLev-i30SU (Levitronix GmbH)
Loop flow rate	300–700 mL min^{-1}
Loop length	150 cm (including HFM, excluding dip tubes)
Loop volume	65 mL (including HFM, excluding dip tubes)

Loop Design and Integration

To accommodate the TFF loop, custom stainless steel dip tubes were designed for the 10 mm ports of the Minifors 2. The loop inlet tube was dimensioned to position its opening near the vessel bottom (distance to lid: 29 cm), minimising the risk of gas bubble aspiration into the pump loop. The return tube was designed shorter (distance to lid: 20 cm) to prevent short-circuit flow. A sampling port was integrated into the loop between the HFM and the bioreactor return; this port also served to prime the loop prior to inoculation, as the centrifugal pump is not self-priming.

To minimise shear stress and pressure drop, all dip tubes and silicone tubing were manufactured with an inner diameter of 6 mm. An exception was made for the segment between the pump and HFM, where 1/4" × 3/8" tubing was required to accommodate the clamp-on flow sensor. Loop volume was minimised to limit the risk of oxygen depletion in the recirculating cell suspension. Dissolved oxygen in the loop was monitored using an optical flow-through sensor (FTC-PSt3-YOP-3/8", PreSens) positioned close to the bioreactor return. The process scheme and experimental setup are shown in Figure 1.

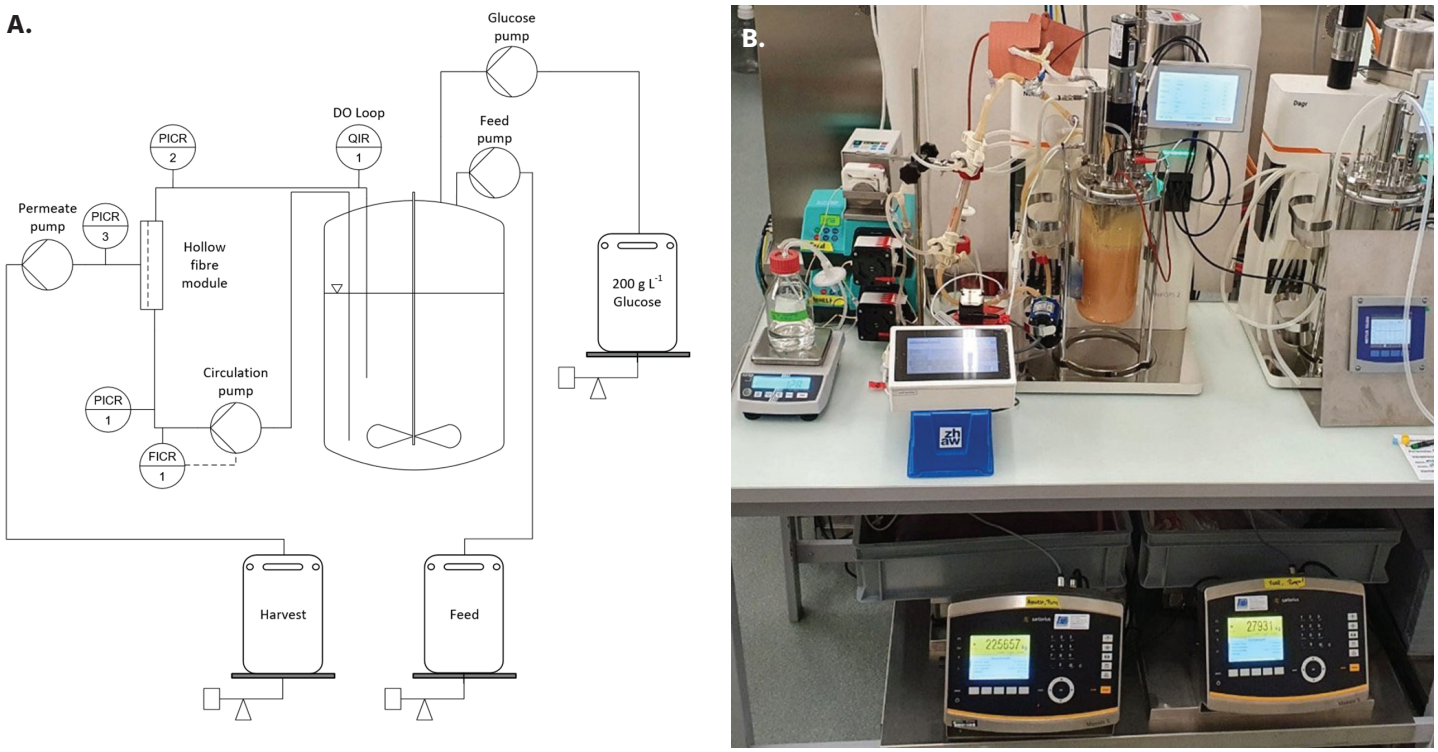

Figure 1. Simplified process scheme (A) and experimental setup (B) of the CHO perfusion cultivations.

Results

Cell Growth and Viability

Two independent cultivations were performed with a target of VCD > 100 × 10^6 cells mL^{-1}. Both bioreactors, inoculated at 1 × 10^6 cells mL^{-1}, achieved peak viable cell densities of 121 × 10^6 cells mL^{-1} (cultivation 1) and 110 × 10^6 cells mL^{-1} (cultivation 2) within 8 days. Cell viability remained above 95% until day 5, followed by a reduced viability down to 85% due to limited perfusion rate of 3 to 3.5 vvd. (Figure 2).

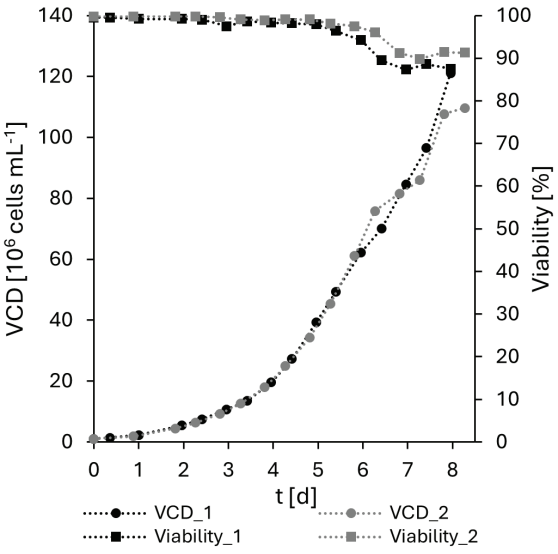

Figure 2. Shows the viable cell density (VCD) in mL^{-1} and viability profiles in % over the course of both perfusion cultivations. From day 5, a reduced viability can be observed due to limited perfusion rate of 3 to 3.5 vvd settings.

Perfusion Rate and Cell-Specific Perfusion Rate

The perfusion rate was increased incrementally twice daily in proportion to the rising VCD, reaching a maximum of 3.5 vvd (cultivation 1) and 3.1 vvd (cultivation 2). The CSPR was maintained above the target minimum of 55 pL cell^{-1} d^{-1}. Once the predefined maximum perfusion rate was reached, it was held constant for the remainder of the cultivation, resulting in a progressive decline in CSPR as VCD continued to rise (Figure 3).

IgG Titre

IgG titres in the harvest stream reached maxima of 901 mg L^{-1} (cultivation 1) and 838 mg L^{-1} (cultivation 2) after 8 days. The cell-specific IgG production rate was estimated at 10–15 pg cell^{-1} d^{-1} (Figure 4).

Nutrient and Metabolite Control

Glucose was supplied via the perfusion medium at a basal concentration of 6 g L^{-1} and supplemented by continuous glucose addition to maintain a target bioreactor concentration of approximately 3 g L^{-1}. In cultivation 1, a brief pump interruption at day 2.4 led to a temporary rise in glucose above 11 g L^{-1}, which resolved promptly; glucose control performed without interruption in cultivation 2. The transient accumulation did not affect cell growth or viability outcomes, as evidenced by the continued rise in VCD in both cultivations and maintained viability. Lactate remained below 2 g L^{-1} throughout both cultivations, indicating efficient glucose metabolism without overflow (Figure 5). The medium was supplemented with 4 mM L-glutamine as the primary nitrogen source. Glutamine was depleted during the first two days and stabilised at approximately 0.5 mM thereafter. Ammonium remained below 5 mM throughout both cultivations, indicating no significant metabolic stress from nitrogen catabolism (Figure 6).

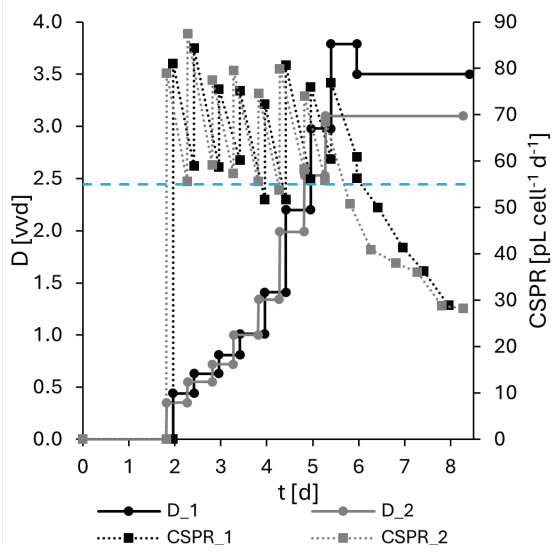

Figure 3. Perfusion rate (D) and cell-specific perfusion rate (CSPR) during both cultivations. Once the predefined maximum perfusion rate was reached, it was held constant for the remainder of the cultivation, resulting in a progressive decline in CSPR as VCD continued to rise.

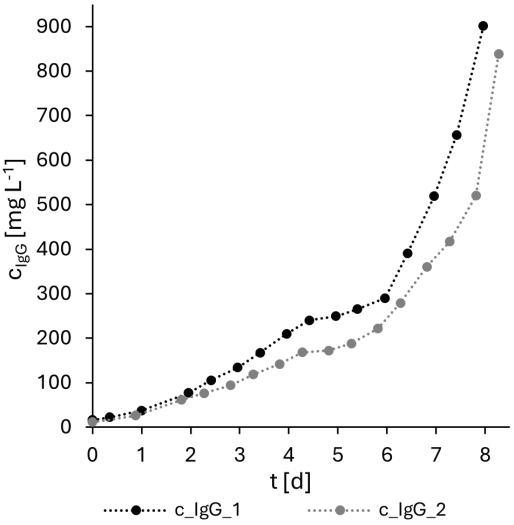

Figure 4. Shows the IgG titre in the harvest streams with maximum reached titres of), 901 mg L^{-1} (cultivation 1) respectively 838 mg L^{-1} (cultivation 2).

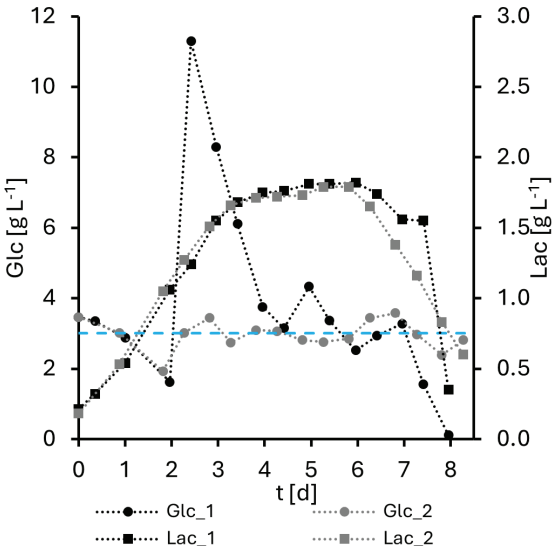

Figure 5. Glucose (Glc) and lactate (Lac) concentrations during both perfusion cultivations. Glucose was maintained close to the target concentration of 3 g L^{-1} throughout both cultivations. A brief pump interruption at day 2.4 in cultivation 1 caused a temporary glucose excursion above 11 g L^{-1} , which resolved promptly with no lasting effect on culture performance; glucose control in cultivation 2 performed without interruption.

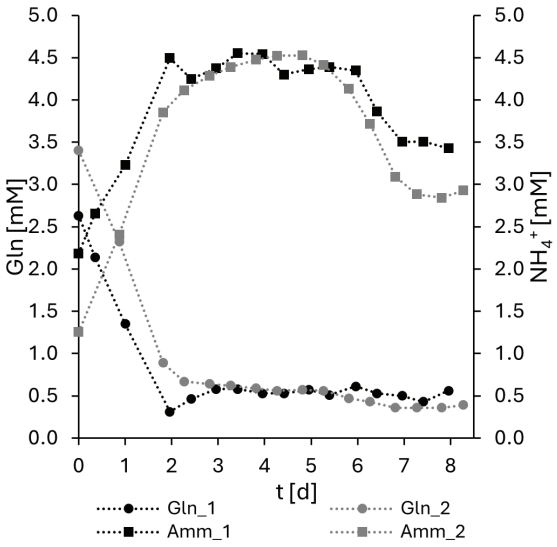

Figure 6. Shows the Glutamine (Gln) and Amm concentrations during both performed cultivations. The metabolites show standard perfusion trends.

Online Process Control via eve®

The Minifors 2 connected to eve® provided a unified interface for monitoring and controlling all process parameters throughout both cultivations. pH was regulated to ≤ 7.2 by CO_2 addition and remained above 7.0 at all times. Bioreactor DO was controlled at 60 % saturation; as VCD increased, the cellular oxygen demand increased proportionally, requiring an oxygen supply of up to 900 mL min^{-1} at the end of cultivation. Loop DO closely followed bioreactor DO for the first four days, thereafter declining to approximately 20 % by the end of cultivation 2 (Figure 7).

PuraLev Console System PCS-i30SU.1 parameters (pump speed, loop flow rate, and line pressures) were recorded continuously in eve®, confirming stable pump operation over the full cultivation duration (Figure 8). The consolidated data record generated by eve® across all control loops provides full process traceability and facilitates straightforward process analysis and transfer.

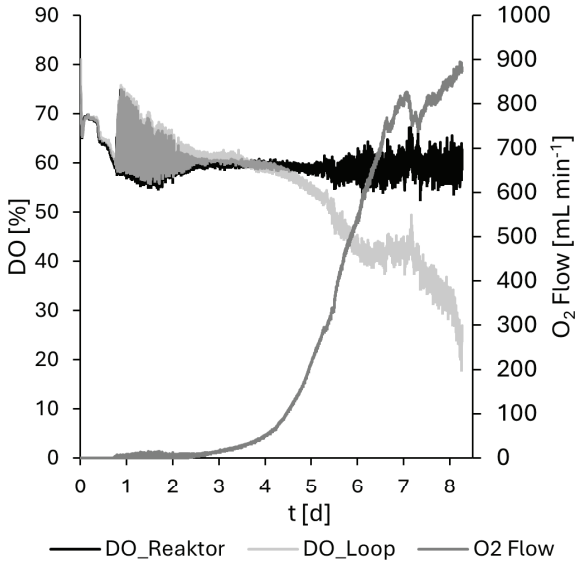

Figure 7. Dissolved oxygen (DO) in the bioreactor, recirculation loop, and O_2 flow rate during cultivation 2.

Figure 8. PuraLev Console System PCS-i30SU.1 parameters recorded in eve® during cultivation 1 (pump speed, flow rate, and pressures).

Conclusion

The data presented in this application note confirms that the Minifors 2 benchtop bioreactor is fully capable of supporting ultra-high cell density perfusion processes in a compact laboratory footprint. The system's native OPC UA connectivity enabled seamless integration of the PuraLev Console System PCS-i30SU.1 into the eve® bioprocess platform, which managed all critical process loops (pH, DO, temperature, perfusion rate, nutrient dosing, and TFF loop flow) within a single, consolidated control environment.

This integration was central to the process success: consistent setpoint control across all parameters throughout 8-day cultivations at VCDs exceeding 100 × 10^6 cells mL^{-1} would not have been practically possible without coordinated, software-driven process management.

Peak viable cell densities of 121 × 10^6 cells mL^{-1} with sustained viability above 95 % (until day 5) and IgG titres of up to 901 mg L^{-1} demonstrate that the Minifors 2 is well-suited for continuous antibody production when combined with a cell bleed strategy. Very high viability until ≈60 × 10^6 cells mL^{-1} makes the bioreactor a suitable choice for N-1 seed culture production in fed-batch workflows targeting bioreactors up to 500 L scale.

The combination of compact benchtop format, intuitive process control via eve®, and demonstrated perfusion capability positions the Minifors 2 as an effective and accessible development platform for intensified and continuous bioprocessing, delivering production-relevant performance without the footprint or complexity of larger systems.

Learn more at
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