Application Note

Optimization of ATF CHO Cell Culture with SepraPor® Transmembrane Pressure Control

Introduction

Alternating Tangential Flow (ATF) is a cell retention method for continuous culture using tangential flow filtration. A diaphragm pump alternates the flow direction inside a hollow fiber filter, retaining cells in the bioreactor while allowing product containing permeate to pass through the membrane. This back-and-forth motion improves mass transfer and reduces fouling to extend operational timelines. **Figure 1** depicts a standard ATF assembly.

Continuous culture technologies such as ATF are increasingly utilized in biomanufacturing for their ability to maintain stable environmental conditions and achieve superior volumetric productivity within smaller bioreactor volumes. This process intensification translates into shorter turnaround times, reduced facility footprints, and enhanced operational flexibility, which are key factors for next generation bioprocess manufacturing.

This study focuses on optimizing ATF parameters including hollow fiber filter selection, pressure, and recirculation interval for a CHO cell culture. The objective was to maintain a steady state viable cell density of 60 × 10^6 cells/mL over a 20-day period while minimizing membrane fouling, maximizing protein titer, and preserving filter performance.

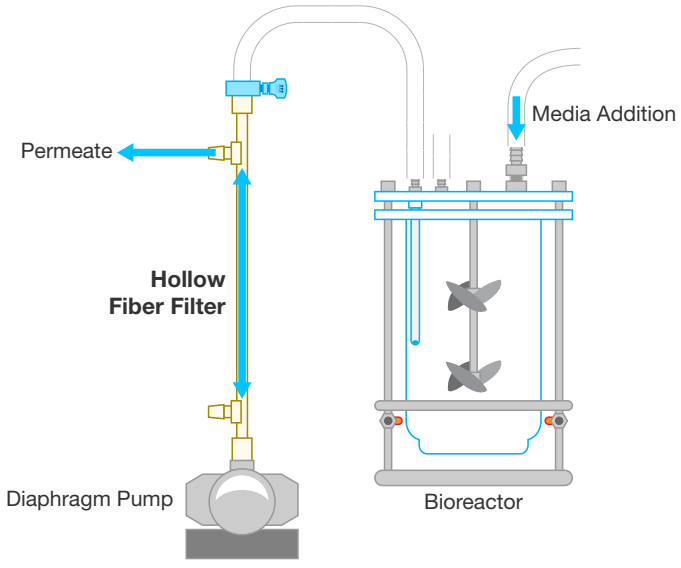

Figure 1: Standard ATF setup for cell culture.

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Case Study: CHO Cell ATF Process Optimization

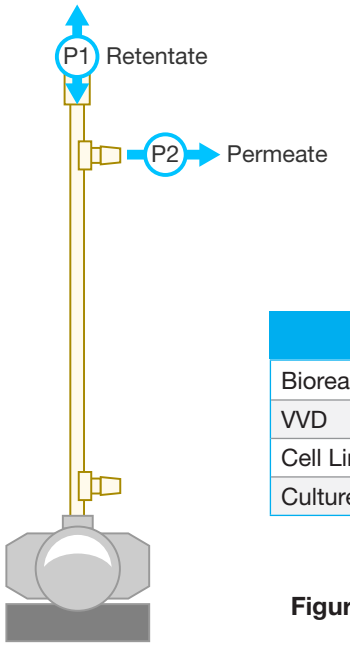
This study evaluated critical parameters of an ATF-based CHO cell perfusion process including filter selection, membrane pore size, ATF pressure, and recirculation interval. The objective was to minimize membrane fouling and maximize overall cell culture performance and productivity.

Previous perfusion studies conducted by FujiFilm Biosciences demonstrated that maintaining a steady-state viable cell density (VCD) between 60-80 × 10^6 cells/mL led to enhanced protein yields. Specifically, cell cultures held at a steady 60 × 10^6 cells/mL for extended durations achieved higher cumulative titers than those operated to peak at 90 × 10^6 cells/mL before viability decline. Building on these findings, this study focused on optimizing filter selection and ATF process parameters to reduce fouling and extend steady-state perfusion duration.

Methods & Materials

Perfusion studies were performed using 250 mL stirred-tank bioreactors inoculated with CHO DG44 cells at an initial density of 10 × 10^6 cells/mL. ATF operation was initiated on day 3 to establish steady perfusion conditions. Bioreactor controls maintained a pH of 7.0, 40% saturation dissolved oxygen, temperature of 37 °C, and agitation rate of 300 RPM. BalanCD CHO Perfusion Media was exchanged at a rate of 1 vessel volume per day (1 VVD).

Pressure sensors (Pendotech®) were installed at the retentate inlet/outlet and permeate outlet (**Figure 2**). ATF pressure, designated as P_1, represents the retentate line pressure, and P_2 represents the permeate line pressure.



ATF Setup	
Bioreactor Volume	250 mL
VVD	1
Cell Line	CHO DG44
Culture Media	BalanCD CHO Perfusion

Figure 2: ATF setup, cell line, culture media, and location of pressure sensors.

Two studies were performed to assess the effects of filter membrane structure, ATF operating pressure, membrane pore size, and recirculation interval.

Experiment 1

Cultures were maintained at a steady-state viable cell density (VCD) of 60 × 10^6 cells/mL, and filter performance was assessed at two ATF pressures: 30 kPa (4.4 psi), representing a standard operating condition, and 50 kPa (7.3 psi), representing a higher-pressure test condition. The filter specifications and experimental parameters are summarized in **Tables 1 and 2**.

Filter	Membrane Material	Pore Size	EFA	Path Length (Nominal)	Fiber Lumen (Nominal)
Meissner's SepraPor® (XFCM20C012-7711)	PS	0.2 µm	110 cm^2	30 cm	1 mm
Alternate Supplier's Filter	PES		88 cm^2	20 cm	

Table 1: Two 0.2 µm hollow fiber filters were compared under different ATF pressure conditions to assess robustness and performance.

Bioreactor	Filter Supplier	Pore Size	Target VCD (cells/mL)	ATF Pressure	Recirculation Rate
1	Alternate Supplier	0.2 µm	60 × 10^6	±30 kPa (4.4 psi)	8 s
2	Meissner				
3	Alternate Supplier			±50 kPa (7.3 psi)	
4	Meissner		60 » 80 × 10^6		

Table 2: Experimental parameters evaluating performance of two hollow fiber filter suppliers at two different ATF pressure conditions.

Experiment: 1 Results & Discussion

Figure 3 displays the viable cell density (VCD) and viability data collected in Experiment 1. Bioreactor 3, operated with the Alternate Supplier's filter at 50 kPa (7.3 psi), showed initial growth similar to Bioreactors 2 and 4 but lost membrane integrity by Day 5, resulting in cell leakage into the permeate and early termination. Under the same pressure, Bioreactor 4 with the SepraPor® filter maintained full integrity and stable performance, demonstrating superior pressure tolerance.

A temporary system pressure increase occurred on Day 9 during gas tank replacement, briefly affecting all bioreactors. Bioreactor 1, which had shown slower early growth and was approaching 60 × 10^6 cells/mL, experienced membrane failure immediately following the pressure event, ending the run. Bioreactors 2 and 4 with SepraPor® filters remained unaffected.

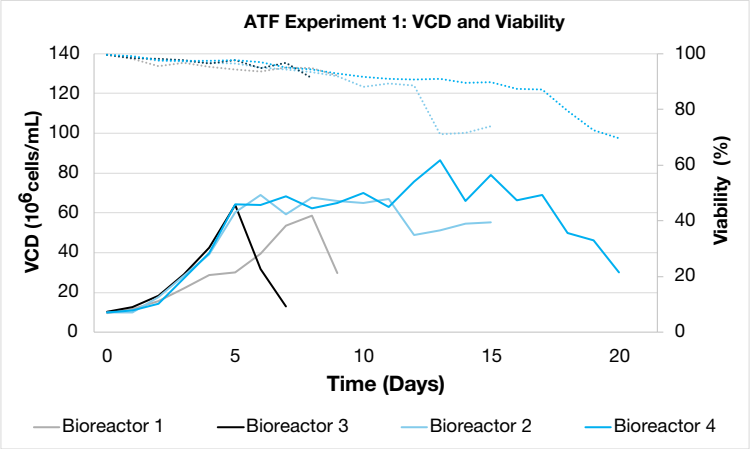


Figure 3. Viable cell density (VCD) and viability data of bioreactors operated with the Alternative Supplier filters (black) and SepraPor® filters (blue).

The SepraPor® membrane maintained integrity under high pressure conditions, while the alternate supplier's membrane failed, highlighting the robustness of the SepraPor® structure.

Bioreactor 2 reached the target VCD by Day 6 and remained stable until Day 11, when fouling caused increased TMP and reduced viability, ending the run. In comparison, Bioreactor 4, operated with the same SepraPor® filter at 50 kPa (7.3 psi), showed improved performance and the longest culture duration. The VCD remained near 60 × 10^6 cells/mL and was later increased to 80 × 10^6 cells/mL. Transmembrane pressure stayed stable throughout 20 days (**Figure 4**), indicating no fouling, while the decline in viability was likely due to metabolic factors rather than filter performance.

At 50 kPa, Bioreactor 4 maintained stable TMP and minimal fouling compared with Bioreactor 2, indicating that higher pressure improved filtration performance and extended process duration without compromising membrane integrity. Overall, the results show that a robust membrane and higher operating pressure improve TMP stability, reduce fouling, and extend culture duration.

SepraPor® membranes enable robust ATF operation under both standard and elevated pressure conditions, providing consistent performance and long-term reliability.

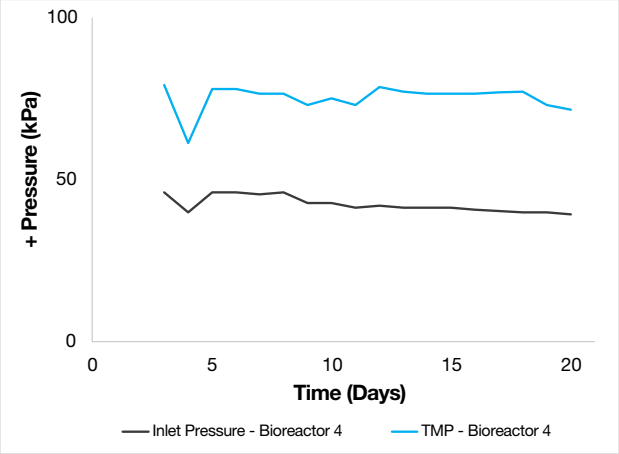


Figure 4: Positive inlet pressure and TMP of Bioreactor 4 is stable throughout the 20-day ATF cell culture.

Experiment 2

Given the improved ATF performance and membrane robustness observed under higher-pressure conditions in Experiment 1, SepraPor® filters (XFCMxxC012-7711) were utilized for all bioreactors in Experiment 2. In this follow-up study, the effects of membrane pore size and ATF recirculation interval on process performance and product yield were investigated. All bioreactors were maintained at a steady-state VCD of 60 × 10^6 cells/mL, and the experimental conditions are summarized in **Table 3.**

Bioreactor	Pore Size	Target VCD (cells/mL)	Recirculation Rate	ATF Pressure
A	0.2 µm	60 × 10^6	8 s	± 30 » 50 kPa (4.4 » 7.3 psi)
B	0.2 µm		6 s	± 30 kPa (4.4 psi)
C	0.4 µm		8 s	± 50 kPa (7.3 psi)

Table 3: Summary of ATF run parameters exploring the effect of membrane porosity and recirculation interval.

With Bioreactor A, the pressure was initially set at 30 kPa (4.4 psi); however fouling was observed around Day 10. To recover and prolong the run, the ATF pressure was increased to 50 kPa (7.3 psi).

Experiment 2: Results & Discussion

All bioreactors maintained a steady-state VCD near 60 × 10^6 cells/mL through Day 18. Bioreactor B began to decline after Day 18, and Bioreactor C concluded on Day 24 due to agitation challenges and volume fluctuations from cell bleeding. Transmembrane pressure remained stable across all runs after steady state VCD was established around Day 5.

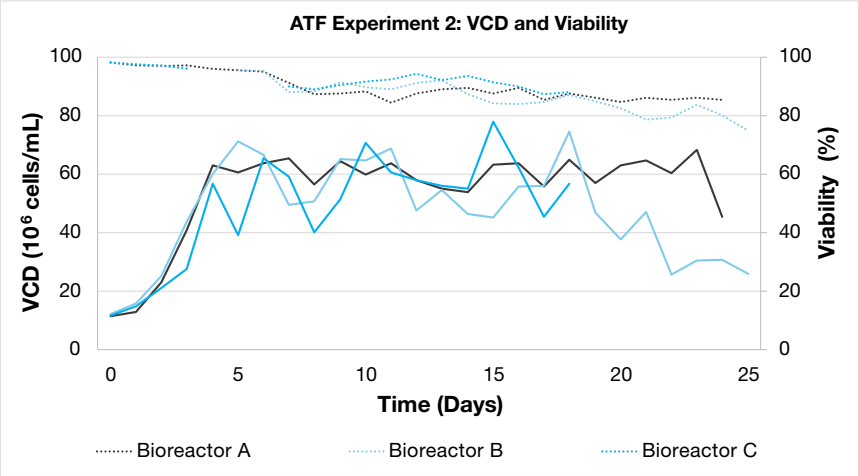
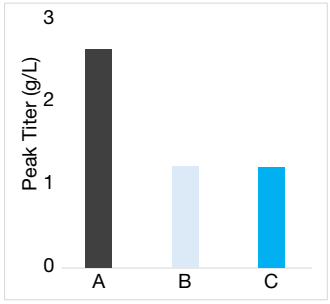
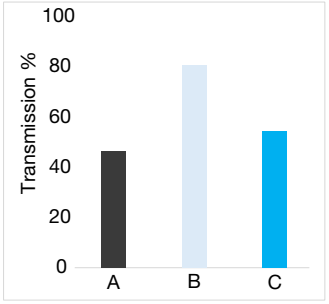


Figure 5. Viable cell density (VCD) and viability of ATF CHO cell cultures with SepraPor® hollow fiber filters.

Peak titer and protein transmission were measured to evaluate culture productivity (**Figure 6**). Bioreactor A achieved the highest peak titer but showed the lowest protein transmission into the permeate. When membrane fouling was observed on Day 10, ATF pressure was increased to extend the run, but protein transmission still fell from 83% to 46%. Although the pressure adjustment reduced further fouling and prolonged operation, passage efficiency did not fully recover.





Bioreactor A	
± 30 >> 50 kPa	
%T before	%T after
83%	46%

Figure 6. Peak titer and percent transmission of protein into the permeate during Experiment 2.

The shorter 6-second recirculation interval in Bioreactor B improved protein transmission efficiency, although the influence of membrane porosity in Bioreactor C remains inconclusive under the tested conditions.

Cell bleeding, calculated at 6%, 12%, and 15% for A, B, and C, likely contributed to reduced overall product recovery. Further optimization of bleed and feed control is expected to improve metabolic stability, transmission efficiency, and product yield. Under the tested conditions, 50 kPa pressure, an 8-second interval, and the SepraPor® 0.2 µm filter provided the most stable and productive performance, while the 6-second interval increased transmission, which warrants further evaluation.

Conclusion

- SepraPor® membranes maintained integrity and stable TMP under operational pressures up to 50 kPa, enabled by a macro-void free, mechanically robust membrane structure.
- High-pressure conditions (50 kPa) reduced fouling and extended culture duration, supporting long, high-density ATF operation and providing a basis for future process optimization.

Choose SepraPor® for your demanding applications

The SepraPor® product line is suitable for the most demanding tangential flow filtration applications and is easily scalable from 1 L to greater than 1,000 L bioreactor volumes. SepraPor® filters have a range of effective surface areas from 0.011 m^2 to 8.3 m^2 and are available in microporous ratings of 0.1, 0.2 and 0.4 µm. SepraPor® filters can be sterilized via gamma irradiation, autoclave, steam-in-place, and chemical sanitization methods.

Learn How SepraPor® Can Help You

We welcome customer collaborations to continuously develop the best products and membranes in the industry.

For more information about the SepraPor® filter used in this study (PN XFCMxxC012-7711) and other SepraPor® filters, please visit www.meissner.com/seprapor.

ATFSP-1.0A

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