Application Note

Cell Culture Perfusion Using SepraPor® Hollow Fiber Filters

Introduction

Cell culture perfusion, a continuous upstream manufacturing process, is becoming a standard practice in drug manufacturing due to its process flexibility, cost efficiency, and optimized facility utilization. Fresh cell media is continuously fed into a bioreactor while waste products are filtered out of the cell suspension at the same rate using tangential flow filtration (TFF).

Perfusion using TFF hollow fiber filters has been implemented in both production bioreactors and N-1 seed train intensification processes, where cell cultures maintain consistent vessel volumes, media concentrations, and environments. These conditions allow for higher cell densities, longer run time, and more consistent product quality than traditional fed-batch processes in production bioreactors. In seed train intensification processes, perfusion allows for higher cell densities for shorter seed train timelines and/or for seeding a production bioreactor with a smaller inoculum volume.

Implementation of perfusion processing has been facilitated by improved control systems, optimized culture media and cell lines, and improved single-use systems.

A diagram of a typical perfusion setup is presented in **Figure 1,** demonstrating a large-scale schematic that is conceptually equivalent for small-scale operations. Here we present a small scale perfusion experiment carried out using our SepraPor® TFF product.

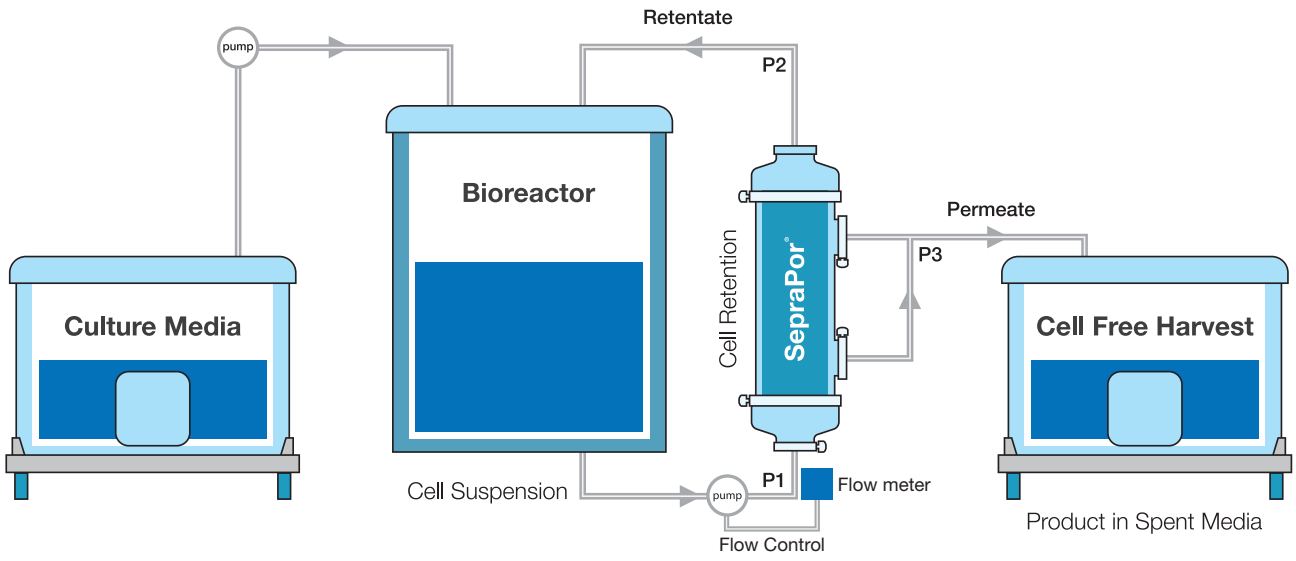

Figure 1: Typical perfusion setup with SepraPor® TFF used as a cell retention device. Locations of pressure sensors are indicated by P1 (feed), P2 (retentate), and P3 (permeate).

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Membrane Technology

SepraPor® hollow fiber membranes are ideally suited for perfusion processes in which consistent filter performance is desired for reproducible cell culture results. SepraPor® hollow fibers have consistent membrane thickness, fiber dimensions, and porosity. Their macrovoid-free structure and highly symmetric cross-section provide robust filtration performance, as shown in **Figure 2**. This hydrophilic polysulfone membrane design has a higher strength, provides stable performance, and is more resilient to pressure variations and thermal cycling.

The porosities of SepraPor® filter membranes were established using standard biopharma industry porosity rating techniques based on bacterial challenge studies. Our well-defined, narrow bubble point ranges and repeatable lot-to-lot clean water permeabilities ensure consistent performance in our customers' processes and overall easier process control strategies.

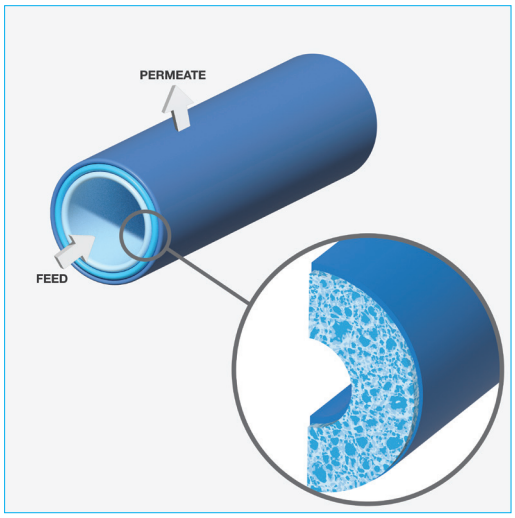

Figure 2: SepraPor® hollow fiber cross section illustration.

Case Study: CHO Cell Perfusion using SepraPor® Filters

In the perfusion process presented here, a 0.2 µm pore size, 220 cm^2 SepraPor® hollow fiber filter with 60 cm flow path length (P/N XFCM20C024-771) was used as the cell retention device. The filter specifications are defined in **Table 1**. An Applikon 3 L STR glass benchtop stirred-tank bioreactor with ez-Control DCU was used as the cell culture vessel. The bioreactor was inoculated with Agarabi CHO cells (ATCC® CRL-3440™) to an initial concentration of 0.43 million cells/mL. The bioreactor setpoints were controlled at pH 7.0, 30% DO, 37 °C, and agitated at 140 RPM with a pitch-blade elephant ear impeller.IS CHO-CD P21.1 medium was provided by FUJIFILM Irvine Scientific and utilized in these continuous perfusion bioreactor experiments. IS CHO-CD P21.1 is a chemically defined, animal component free growth medium for CHO perfusion cultures and is part of FUJIFILM Irvine Scientific's CHO Perfusion Media Survey Panel (P-MSP).

Perfusion Run Parameters	
Process Duration	21 Days
Vessel Volume	2 L: Days 1 - 9 1 L: Days 10 - 21
Max VCD	Day 9: 32 · 10^6 cells/mL Day 21: 54 · 10^6 cells/mL
Average Cell Viability	> 90%
VVD	1

Table 1

The SepraPor® filter was operated in a TFF loop driven by a Levitronix® Puralev®-i30SU centrifugal pump and a clamp-on flow meter was used as part of the control loop. Feed pressure, retentate pressure, and permeate pressure were monitored continuously with PendoTECH™ PREPS-N-5-5 pressure sensors connected directly to the Levitronix® LCO-i100 console with the values displayed and recorded on the console.

Monitoring pressure, particularly TMP, throughout the process provides insight to the performance of the filter. An increase in TMP while maintaining constant operating parameters indicates that the filter membranes are fouling. Filter fouling eventually will develop over time and pore plugging by cell debris typically will lead to a rapidly increasing TMP toward the end of the fillter life. Stable TMP values that increase slowly during the process allow for long operational runs while providing consistent production and cell viability.

A Vi-CELL™ XR Cell Viability Analyzer (Beckman Coulter) was used daily to measure the viability, total and viable cell count, and average diameter and circularity of the cells. Additionally, critical metabolic indicators were monitored with a Cedex Bio Analyzer (Roche 06395554001) including glutamine, glutamate, glucose, lactate, ammonia, sodium, magnesium, calcium, and phosphate. A 400 g/L glucose solution was fed daily to maintain concentration above 2 g/L.

The filter feed flow rate was 400 mL/min, and 1 bioreactor volume was exchanged each day, i.e. 1 vessel volume per day (VVD). The process parameters are defined in **Table 2.** During the 18-day perfusion process, the maximum viable cell density (VCD) reached approximately 54 million cells/mL, and viability was > 90% with average cell diameter of 13.5 µm.

The SepraPor® filter performed consistently throughout the perfusion process, which started on Day 3 post-inoculation, with minimal increase in TMP over the course of the process **(Figure 3)**. The TMP began at about 1 psig and, on Day 21, was approximately 2.5 psig. Feed pressure increased from 3 psig to 4 psig throughout the run. The Levitronix® pump automatically adjusted the RPM to maintain a 400 mL/min crossflow throughout.

TFF Filter Parameters	
Membrane Surface Area	220 cm^2
Flow Path Length	60 cm
Porosity	0.2 µm
Lumen Size	1 mm
Membrane Material	Polysulfone (hydrophilic)

Table 2

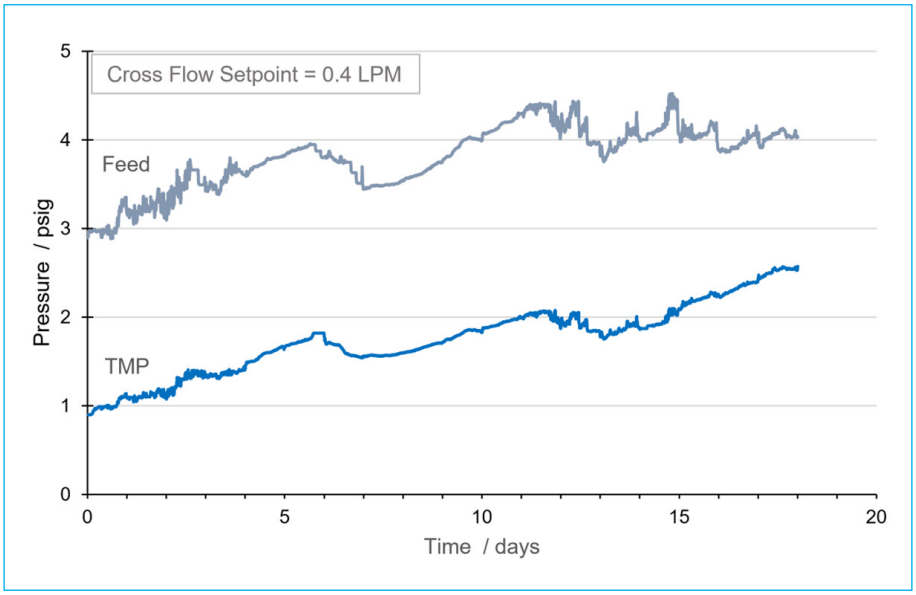

Figure 3: The SepraPor® filter performed consistently with minimal increase in TMP or feed pressure during the perfusion process, which started on Day 3.

Conclusion

The 0.2 µm SepraPor® hollow fiber filter provided consistent filtration performance with minimal increase in TMP during the 18-day perfusion process. The cell culture achieved a maximum VCD of 54 million cells/mL with > 90% viability.

Consistent filtration performance, high culture VCD and viability, well-characterized bubble point ranges, and reliable minimum flux specifications make SepraPor® an excellent option for cell retention in high-density perfusion processes.

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 (P/N XFCM20C024-771) and other SepraPor® filters, please visit www.meissner.com/seprapor.