Application Note

Technical Application Publication

The effectiveness of PROPOR TFF filters can be demonstrated in microfiltration and ultrafiltration applications.

Summary

Tangential flow filtration operations are used extensively throughout biopharmaceutical manufacturing processes for both microfiltration and ultrafiltration applications.

Here, we examine the performance and scale-up of PROPOR TFF, Parker domnick hunter's new range of hollow fibre filters in an *E.coli* lysate clarification, a mammalian cell culture clarification and a concentration of a fusion protein.

PROPOR TFF hollow fibre filters are designed specifically to increase productivity, maximize yield and achieve reproducible product quality in biopharmaceutical microfiltration and ultrafiltration TFF operations.

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Key Filtration System Requirements:

- **Increased productivity**
  Low fouling properties of mPES ensure high flux rates can be achieved
- **Maximized yields**
  Low protein binding membrane prevents product loss during procesing
- **Reproducible performance**
  High quality and consistent membrane ensures stringent specifications can be achieved that reduce process variability, improve scaleability and reduce the risk of product loss
- **Minimized filtration costs**
  A PROPOR TFF product used in an ultra-filtration application will typically be 4-6 times less expensive that the equivalent membrane area in cassette format

ENGINEERING YOUR SUCCESS.

Introduction

PROPOR TFF filters, from Parker domnick hunter, are hollow fibre filters specially designed for use in biopharmaceutical applications.

The mPES membrane used in Parker domnick hunter's PROPOR TFF filters give higher flow rates, permeability and lower binding leading to better product transmission and the highest yields. The propriety manufacturing process used to produce the membrane delivers a membrane with a unique void-free structure). Traditionally hollow fibre membranes have contained finger-voids which lead to a weaker structure that is more likely to lead to fibre breakages and the loss of valuable product.

PROPOR TFF filters can be used in both microfiltration and ultrafiltration applications.

Microfiltration application examples

Hollow fibre filters are the ideal format for performing clarification or harvesting operations within bioprocesses. The open flow path construction of the hollow fibres prevents fouling when challenged with high solids loading and turbid feed streams. This construction makes cleaning and sanitizing easier and more effective when using reusable cartridges.

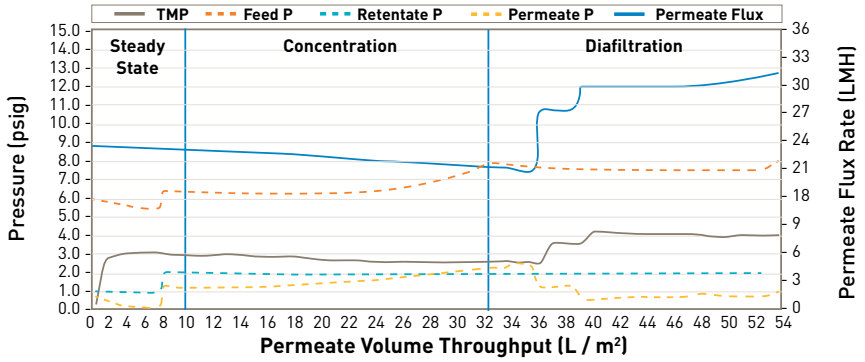
(a) *E.coli* lysate clarification

A green-fluorescent protein (GFP) with a molecular weight of 73 KDa was to be recovered from an *E.coli* expression system. The harvested *E.coli* cells were homogenized by a single-pass at 900 bar giving a lysate cell density of 60 g/L.

A trial was performed with a PROPOR TFF LabMax 12 with a membrane area of 150 cm^2 and a molecular weight cut-off of 750 kDa. The hollow fibre lumen diameter was 0.5 mm. A 3-fold reduction in the starting volume was performed followed by a buffer flush with 3 diavolumes of buffer at a shear rate of 9000 sec^{-1}. The pressure and permeate flux profiles are provided in Figure 1. The permeate flux was controlled at approximately 20 LMH by modulating the TMP.

Operating at a permeate flux rate of 20 LMH during concentration produced a desirable low and stable TMP profile between 2-3 psi (0.14-0.2 bar) when operating at a cross flow shear rate of 11000 sec^{-1}.

Figure 1: Pressure and flux profile for *E.coli* lysate clarification performance on PROPOR TFF LabMax 12

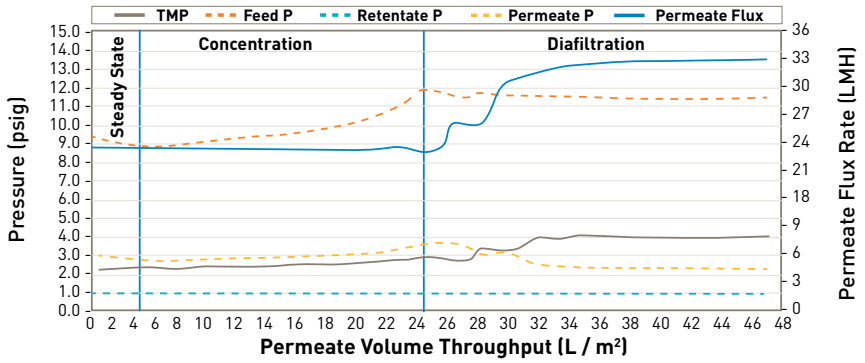


A final process configuration utilizing 60 cm modules was required. The experiment was, therefore, repeated using a LabMax 24 which has a filtration area of 310 cm^2. In both experiments an attempt was made to maintain a consistent lysate loading of approximately 2.2 kg/m^2. The pressure and permeate flux profiles are provided in Figure 2.

The pressure and flux profile shown in Figure 2 demonstrates equivalent performance between the LabMax 24 and the LabMax 12. This successful two-fold scale-up has been achieved by doubling the path length. Further scale-up would be performed by maintaining the path length and increasing the number of hollow fibres in the cartridge.

In conclusion, it has been demonstrated PROPOR TFF hollow fibre filters can be used to clarify *E.coli* lystes and recover recombinant proteins. The process was successfully scaled-up by a factor of two by doubling the filter path length.

Figure 2: Pressure and flux profile for *E.coli* lysate clarification performance on PROPOR TFF LabMax 24



(b) Mammalian cell culture clarification

A recombinant protein with a molecular weight of between 100 kDa and 140 kDa was being expressed using a CHO cell expression system. The total cell concentration was 3.3 x 10^6 cells/mL and the cell viability was 63%. A aim of the study was to demonstrate that PROPOR TFF hollow fibre filters could be used to clarify the mammalian cell culture and maximize the recovery of the protein. A PROPOR TFF LabMax 12 with a 1 mm lumen diameter and a 0.45 micron rating was used to concentrate the mammalian cell culture broth 12-fold. Two diavolumes of buffer were used as a flush to maximize product recovery. The results are presented in Figure 3a & 3b.

A full product mass balance showed that nearly 100% recovery of the recombinant protein could be achieved. At a constant permeate flux of 30 LMH the TMP was below 1 psig up to a twelve-fold concentration.

Figure 3a: Pressure and flux profile for mammalian cell culture clarification

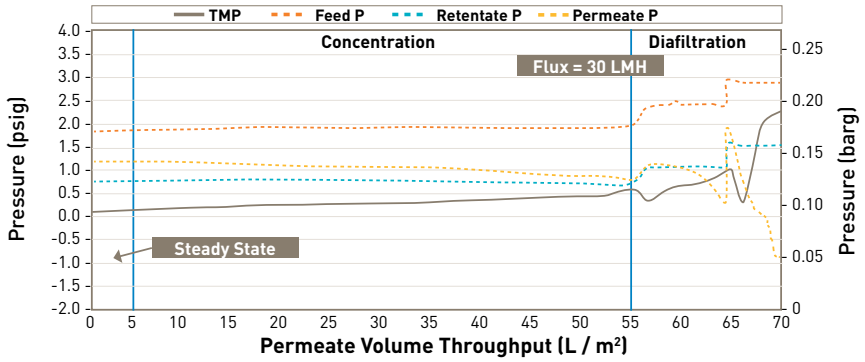
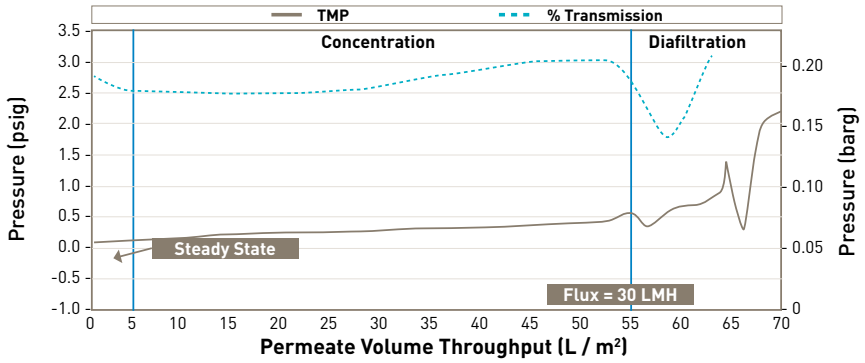


Figure 3b: Product transmission determined by comparing the permeate product concentration to the retentate product concentrate



Ultrafiltration application examples

Hollow fibre filters can be used in ultrafiltration applications such as the concentration and diafiltration of recombinant proteins and monoclonal antibodies. Although historically cassette formats have been used for ultrafiltration, a 5 m^2 cassette suitable for manufacturing can cost approximately 4 to 6 times more than a hollow fibre module of equivalent area. Additionally the need for expensive hardware required to operate cassettes is significantly reduced.

A process was developed to concentrate and diafilter a fusion protein with a molecular weight of 150 kDa. A PROPOR TFF LabMax 12 with a 30 kDa membrane cut-off and a 1 mm diameter lumen and membrane area of 150 cm^2 was used during the process development. Comparability of performance was demonstrated with a PROPOR TFF PilotPlus 12 cartridge with membrane area of 1300 cm^2.

The objective of the laboratory experiments was to develop a process that delivered a 24-fold concentration of the fusion protein followed by a diafiltration with 6 diavolumes of buffer.

A flow rate excursion curve showing the increase in flux rates with increasing TMP is provided in Figure 4. Results from both the LabMax 12 and the PilotPlus 12 are overlaid and show complete scaleability from one filter to the other.

Figure 4: Relationship between TMP and flux rate for PROPOR TFF LabMax 12 and PilotPlus 12 when filtering a solution containing a fusion protein with initial concentration of 2 g/L

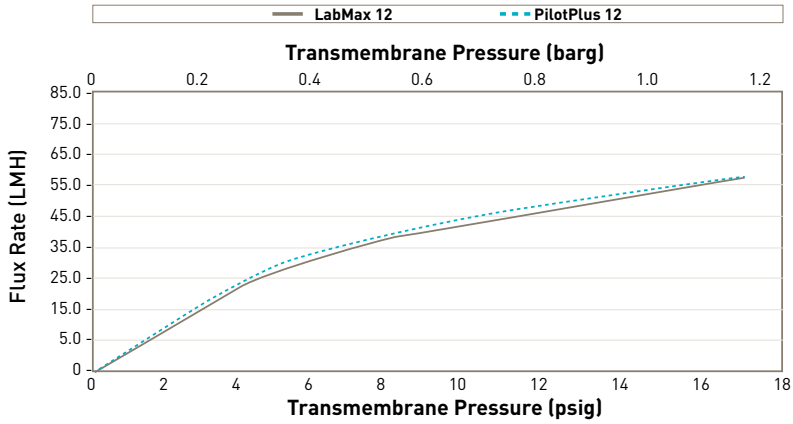
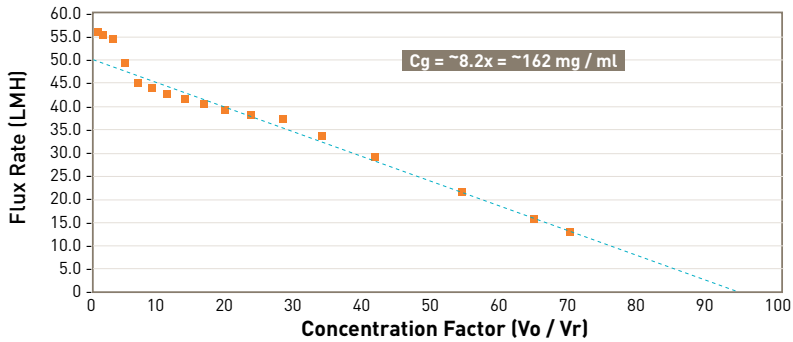


Figure 5: The decline in flux that occurred as the fusion protein concentration increased. The experiment was performed with LabMax 12



Conclusion

PROPOR TFF filters are hollow fibre filters designed for the biopharmaceutical industry. Their open channel structure **minimizes fouling in microfiltration** applications with feed streams that contain a high level of particulates. In ultrafiltration applications, PROPOR TFF filter filters are **15-25% of the cost of cassettes with the same filtration area**. In either microfiltration or ultrafiltration applications PROPOR TFF filters scale-up well either by increasing the length of the filter or preferably by maintaining the fibre path length and increasing the number of fibres.

Products

Hollow Fibre Cross Flow Filters

PROPOR TFF

Filtration

Modified Glycerine-Free Polyethersulphone	3K to 750K NMWC* 0.1 to 0.45 micron

- PROPOR TFF is a brand new range of microfiltration and ultrafiltration cross flow filters from Parker domnick hunter.
- Available in reusable, single-use and autoclaveable formats.
- Unique modified PES membrane minimizes fouling to give superior flux and throughput performance reducing processing costs and maximizing yields.

**NMWC - Nominal molecular weight cut-off*

Tangential Flow Filtration (TFF) System

SciLog PureTec®

Intelligent Lab Systems

- Ideal for lab-scale UF/MF/DF – concentration and diafiltration.
- Controls and monitors TMP (transmembrane pressure) and feed rate.
- Set end points and alarm for walk-away operation.

Cell Culture Maintenance System

SciLog MabTec®

Intelligent Lab Systems

- Promote high-density cell culture though automated seed, feed and perfusion strategies.
- Methods and alarms to protect your entire cell culture processes.
- Modular compact design enhances current setups.
- Seamless integration to any bioreactor to enhance performance.

Semi-Automated BioProcessing System

SciLog SciFlex®

BioProcessing Systems

- TFF, NFF, media transfer, media prep and bag filling models available.
- Mobile station for a multitude of bioprocessing applications.
- Ideal for batch operations in development and contract manufacturing environments.
- Off the shelf for multiple applications with SciLog single-use sensors manifolds.
- Works with any manufacturers' filters.

Automated BioProcessing System

SciLog SciPure®

BioProcessing Systems

- GMP – out of the box mobile processing platforms.
- Off the shelf for multiple applications with SciLog single-use sensors manifolds.
- Options available for fully automated, semi and manual operation.
- Works with any manufacturers' filters.
- Available in single-use, extended-use and stainless.
- TFF & NFF models available.
- Optimize filter throughput with patented Rate-Pressure controlled feed.

Sensor Products

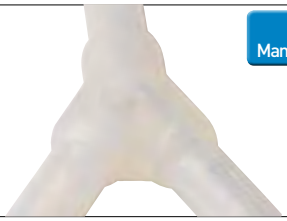
SciPres®, SciCon®, SciTemp®

Sensor Products

- Pre-calibrated sensors for single or extended-use.
- Each sensor is pre-calibrated with a unique identifier and is lot traceable.
- Factory calibration ensures the highest level of accuracy and is stored on each sensor's memory chip for out of the box, plug and play use.
- Custom calibrations available.
- Available in 5 connection variants.

Fluid Transfer

Molded Silicone Manifolds



Fluid Management

- Single-use systems tailored to your application.
- Proprietary technology allows any configuration of Ys, Ts, crosses, reducers, and elbows, with virtually unlimited lengths between molds.
- Molded junctions are stronger, eliminate entrapment, and offer smooth, unrestricted flow.
- Contact us to discuss integrating bags, tubing, sensors and connectors to suit your needs. Can also be made with zip-ties.

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