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Application Note

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Mammalian CHO Cell Clarification

Bengt Persson*
North America, Inc. 565 Johnson Avenue Bohemia, NY 11716 United States

* Correspondence
E-Mail: bengt.persson@sartorius.com

Abstract

This application note is focusing on mammalian cell clarification on the example of CHO cell clarification with a Green Line Explorer 12-inch (filter area: 155 cm^2), 0.45 µm, 1.0 mm fiber ID. It was shown that the target protein (140 kD) could be recovered nearly 100%.

Introduction

The goal of this trial was to determine optimum membrane pore size with maximum retention of cell and cell debris and operating conditions that will produce maximum transmission of target protein (140 kD). Maximum transmission would result in high product recovery in shortest time.

Materials

For this cell clarification process a Green Line Explorer with a length of 12-inch , a pore size of 0.45 µm and a 1.0 mm fiber ID were used. The filter area of each module is 0,0155 m^2 . Like all our Hollow Fiber Modules the membrane consisted of modified Polyethersulfon (m-PES).

Details of used Hollow Fiber Module

Family	Green
Product Size	Explorer
MWCO | Pore Size	0.45 µm
Fiber ID	1.0 mm
Length	12 inch
Filter Area	0.0155 m^2
No. of Fibers	18
Recommended batch volume per module	150 – 750 mL
Diameter Module (cm)	1.3 cm
Feed | Retentate connectors	½-inch TC
Permeate connector	⅜-inch Hose Barb
Material	SU94510EXP12S6 (6-pack)

Methods

In cell clarification of mammalian cell processes like this CHO cell clarification, pore sizes between 0.2 µm and 0.45 µm are recommended. Beside the recommended shear rate should be between 2,000 – 4,500 sec^{-1}.

Feed:
- Total Cell Density: 3.27E06 cells/mL
- Viability: 63%

Details of Trial

Membrane & Module	Green Line Explorer 12-inch, 0.45 µm, m-PES, 155 cm^2, 1.0 mm fiber
Initial Feed Volume	900 mL
Membrane Loading	900 mL / 155 cm^2 ≈ 60 Liters/m^2
Process Objective	12× concentration + 2 diavolumes (DV)
Process Flux	Constant Permeate Flux at 30 LMH (7.4 mL/min) @ ~4,400 sec^{-1}

Results

The cell clarification of the target molecule from CHO feed was performed with an Green Line Explorer 12-inch, (155 cm^2), 0.45 µm, 1.0 mm lumen hollow fiber cartridge. As shown in Figure 1, the transmembrane pressure profile at a constant permeate flux mode of 30 LMH was steady and under 1 psig up to 12× concentration. At a membrane loading of approximately 60 L/m^2, the overall product recovery was nearly 100%.

Process Step:
CHO Cell Clarification

Element:
Explorer 12

HF Membrane:
0.45 µm PES - 150 cm2 - 1.0 mm ID

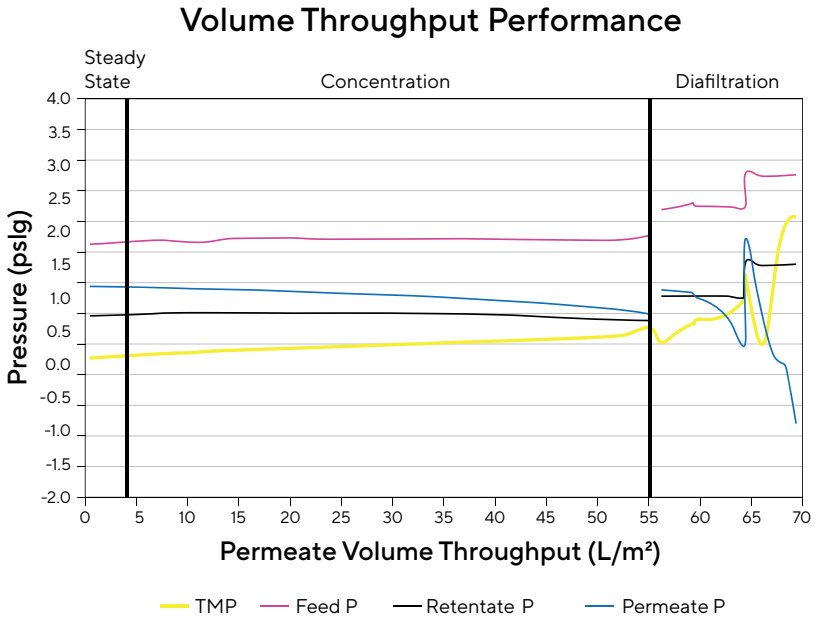

Figure 1: Pressure profiles during processing (concentration & diafiltration)

Process Step:
E. coli Lysate Clarification (60 g/L)

Element & Membrane:
Explorer 24, 750k D PES, 310 cm^2, 0.5 mm ID

Cell Density & Lysis:
1.7k g/m^2, 28 L/m^2
(post centrifuge)

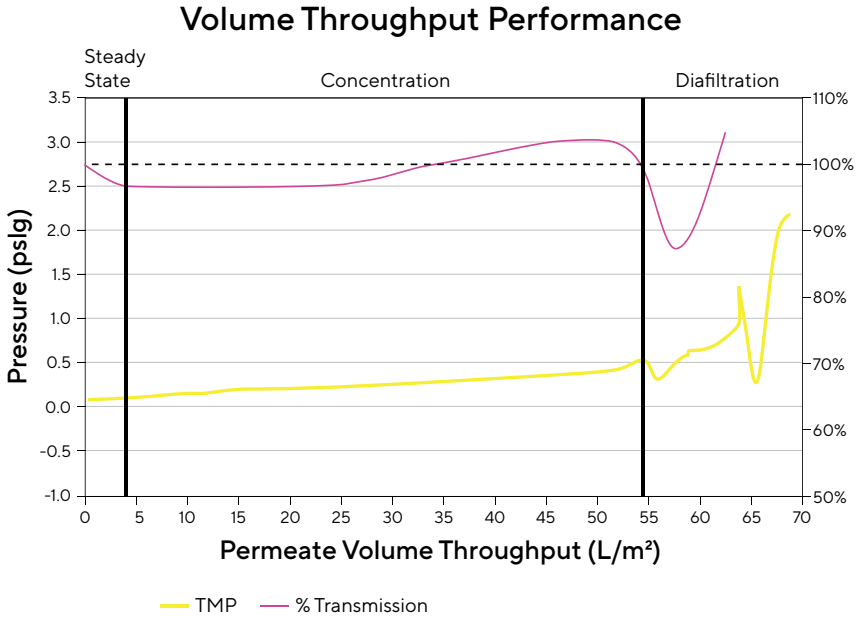

Figure 2: Volume throughput performance during processing

Conclusion

For this CHO cell clarification the Green line Explorer 12-inch Green (filter area: 155 cm^2), 0.45 µm, 1.0 mm fiber ID was the perfect choice to optimize process conditions.
At a membrane loading of approximately 60 L/m^2, the overall product recovery was nearly 100%.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com

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