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E. Coli Lysate Clarification – Scale up

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Abstract

This application note is showing the performance for cell debris retention and 75 kD GFP protein recovery using filters with different fluid paths and the same fiber (750 kD MWCO, 0.5 mm lumen ID). It demonstrates an equivalent scale-up performance between modules, a Re-use line Explorer 12-inch and a Re-use line Explorer 24-inch filter using the 3× concentration and 3 diavolumes (DV) at 9,000 sec⁻¹ shear.

A permeate flux rate of 20 LMH during concentration produced a desirable low and stable (flat) TMP profile between 2–3 psig when operating at a crossflow shear rate of 9,000 sec⁻¹, a membrane mass loading of 1.7–2.0 kg/m² and volume loading of ~30 L/m² both trials. The equivalence of the TMP profiles confirms a linear scale-up going from a 12-inch module to a 24-inch module.

Introduction

Clarification of E. coli lysate focused on separating cell debris of the E. coli homogenate to recover the 75 kD target protein in the permeate.

Materials

For this clarification process two Single-use line Explorer with a length of 12-inch and 24-inch and a molecular weight cut-off (MWCO) of 750 kD with 0.5 mm fiber ID were used. The filter area of each module is 0.0321 m² Like all our Hollow Fiber Modules the membrane consisted of modified Polyethersulfon (m-PES).

Details of used Hollow Fiber Module

Family	Re-use	Re-use
Product Size	Explorer	Explorer
MWCO Pore Size	750 kD	750 kD
Fiber ID	0.5 mm	0.5 mm
Length	12 inch	24 inch
Filter Area	0.0155 m ²	0.0321 m ²
No. of Fibers	36	36
Recommended batch volume per module	150 - 750 mL	250 - 1,500 mL
Diameter Module (cm)	1.3 cm	1.3 cm
Feed Retentate connectors	½-inch TC	½-inch TC
Permeate connector	¾-inch Hose Barb	¾-inch Hose Barb
Material	WA75005EXP12S0 (1-pack)	WA75005EXP24S0 (1-pack)

Methods

- Duplicate the performance for cell debris retention and 75k D GFP protein recovery using filters with different fluid paths and the same fiber (750k D MWCO, 0.5 mm lumen ID).
- Demonstrate equivalent scale-up performance between modules, a Re-use line Explorer 12-inch and a Re-use line Explorer 24-inch filter using the 3× concentration and 3 diavolumes (DV) at 9,000 sec⁻¹ shear.

Results

Homogenization Process	1 pass at 900 bar
Lysate cell density (LCD)	60 g/L
Initial Feed Turbidity	1650 NTU
Initial Feed Volume & Loading	34 L/m ²
Membrane Mass Loading	~2 kg/m ² Explorer 12-inch = 30 g/155 cm ² Explorer 24-inch = 60 g/321 cm ²
Process Flux Rate (constant)	20 LMH - Concentration
Process Flux Rate (variable)	20 - 30 LMH - Diafiltration

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

Element & Membrane:
Explorer 12, 750k D PES, 150 cm²,
0.5 mm ID

Cell Density & Lysis:
2.0 kg/m², 32 L/m²
(post centrifuge)

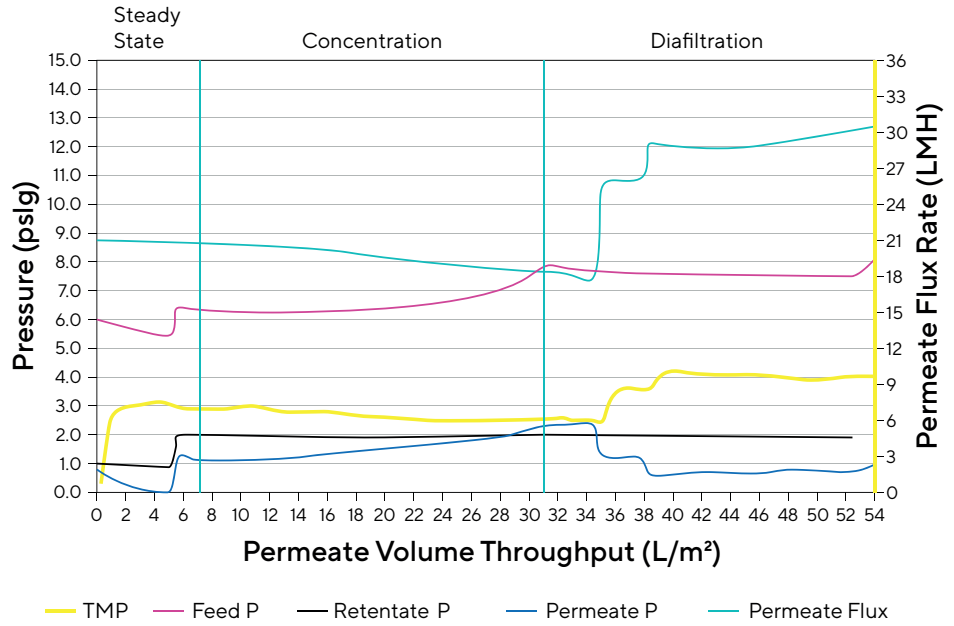


Figure 1: Pressure profiles and permeate flux during processing (concentration & diafiltration) with an Explorer 12-inch

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

Element & Membrane:
Explorer 24, 750k D PES, 310 cm²,
0.5 mm ID

Cell Density & Lysis:
1.7 kg/m², 28 L/m²
(post centrifuge)

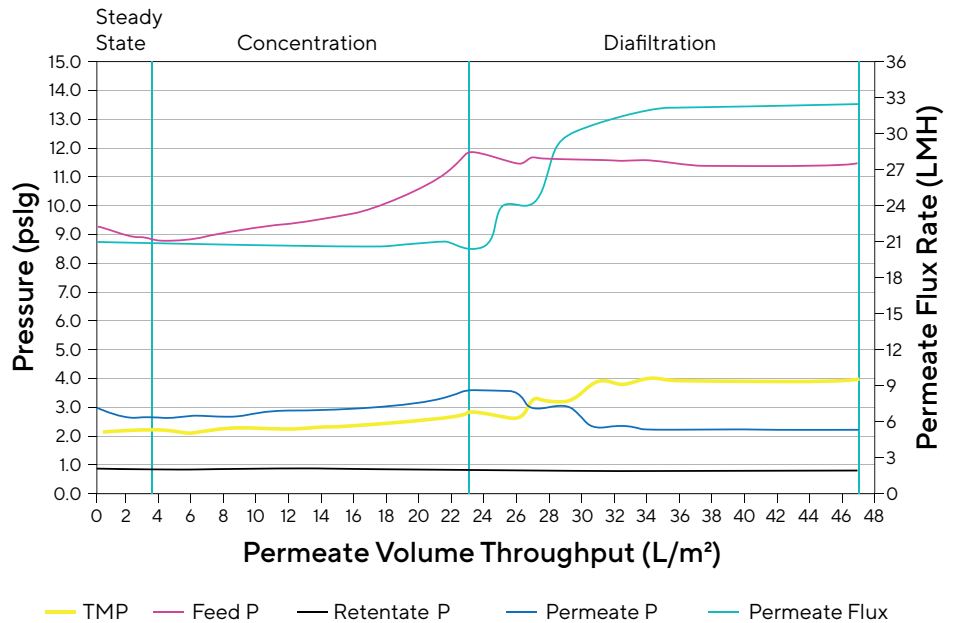


Figure 1: Pressure profiles and permeate flux during processing (concentration & diafiltration) with an Explorer 24-inch

Conclusion

A permeate flux rate of 20 LMH during concentration produced a desirable low and stable (flat) TMP profile between 2–3 psig when operating at a crossflow shear rate of $9,000 \text{ sec}^{-1}$, a membrane mass loading of $1.7 - 2.0 \text{ kg/m}^2$ and volume loading of $\sim 30 \text{ L/m}^2$ both trials.

The equivalence of the TMP profiles confirms a linear scale-up going from a 12-inch module to a 24-inch module. The fitting hollow fiber module for this E.Coli process would have a module length of 24 inches.

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