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# E. Coli Whole Cell Clarification

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## Abstract

This application note is focused on clarification of whole E. coli cells from the fermenter to concentrate them 4x from the media and wash them with 2 diavolumes (DV) volumes for homogenization. Therefore a 750 kD ultrafiltration and a 0.1  $\mu$ m microfiltration hollow fiber were compared. It shows that the operating pressures were much lower using the 750 kD membrane and thus it performed better than the 0.1  $\mu$ m membrane for clarifying the whole cell E. coli.

# Introduction

The goal of cell clarification is to separate a from a cell culture medium. The cells are filtered and remain in a feed | retentate loop, while the permeate contains the product of interest. This application note focused on the clarification of whole E. coli cells. The goal was to compare the performance of a 750 kD MWCO membrane to a 0.1 µm pore hollow fiber module in lab scale. Single-use (Green Line) Discover 41-inch Hollow Fiber module is used. For the comparison a Green Line Discover Module with the length of 41-inch in 750 kD and 0.1 µm was used. Both had a Fiber ID of 1.0 mm and a corresponding filter area of 0.193 m<sup>2</sup>. Like all our Hollow Fiber Modules the membrane consisted of modified Polyethersulfon (m-PES).

# Materials

For the comparison a Green Line Discover Module with the length of 41-inch in 750 kD and 0.1 µm was used. Both had a Fiber ID of 1.0 mm and a corresponding filter area of 0.193 m<sup>2</sup>. Like all our Hollow Fiber Modules the membrane consisted of modified Polyethersulfon (m-PES).

## Details of used Hollow Fiber Module

	Green	Green
Family	Green	Green
Product Size	Discover	Discover
MWCO   Pore Size	0.1 µm	750 kD
Fiber ID	1.0 mm	1.0 mm
Length	41 inch	41 inch
Filter Area	0.0193 m <sup>2</sup>	0.0193 m <sup>2</sup>
No. of Fibers	6	6
Recommended batch volume per module	80 - 850 mL	80 - 850 mL
Diameter Module (cm)	0.95 cm	0.95 cm
Feed   Retentate connectors	Luer Lock	Luer Lock
Permeate connector	Luer Lock	Luer Lock
Material	SU91010DIS41L6 (6-pack)	SU75010DIS41L6 (6-pack)

# Methods

Separating a protein from a cell culture medium is accomplished by cell clarification. Cells are filtered and remain in a feed | retentate loop, while the permeate contains the product of interest.

E. coli have been used for many years for the expression of a wide range of recombinant proteins, vaccines, and enzymes. High circulation flows with 12,000 to 16,000 sec<sup>-1</sup> shear rates provide better transmission of the target protein and also more stable flux rates. Insufficient shear rate or excessive TMP will cause the formation of a gel layer on the membrane surface that acts as an additional filtration layer. Unlike fragile mammalian cells, bacterial cells in our case E. coli, can withstand significant shear forces without destruction.

# Results

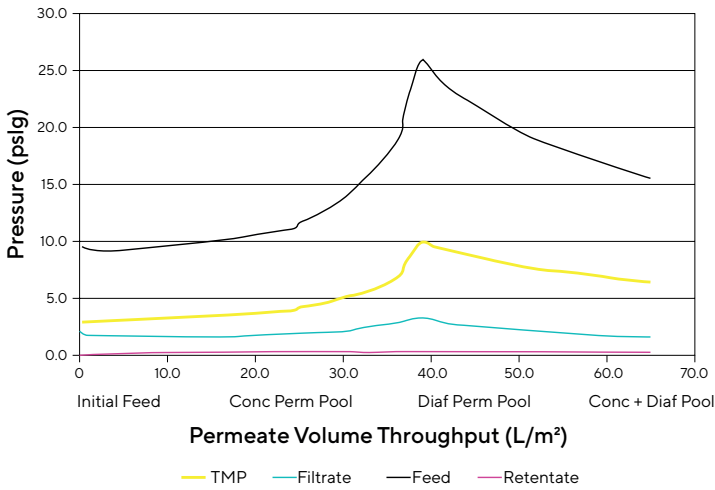
Cell Clarification & wash conditions: (4× concentration + 2 diavolumes (DV's))	Module #1: Green Line Discover 41-inch, 750 kD, 1.0 mm fiber ID	Module #2: Green Line Discover 41-inch, 0.1 µm, 1.0 mm fiber ID
Harvest Volume		1L
Membrane Loading		50 L/m <sup>2</sup>
Shear rate	5,300/sec <sup>-1</sup>	5,100/sec <sup>-1</sup>
Flux Rate		20 LMH
Wash volume		250 mL/DV
Process time	3hr 40 min	3hr 50 min

During concentration with the 750 kD membrane, a permeate flux rate of 20 LMH produced a desirable TMP profile and maintained a TMP ≤ 10 psig at the operating conditions. During concentration with the 0.1 µm membrane, a permeate flux rate of 20 LMH produced TMP >20 psig at the operating conditions. The process times were similar at 3 hours 40 minutes for 750 kD fiber compared to 3hr 50 mins for the 0.1 µm fiber.

When comparing the TMP profiles, the maximum pressure of 23.2 psig for the 0.1 µm membrane was significantly higher than the maximum pressure of 10 psig for the 750 kD membrane.

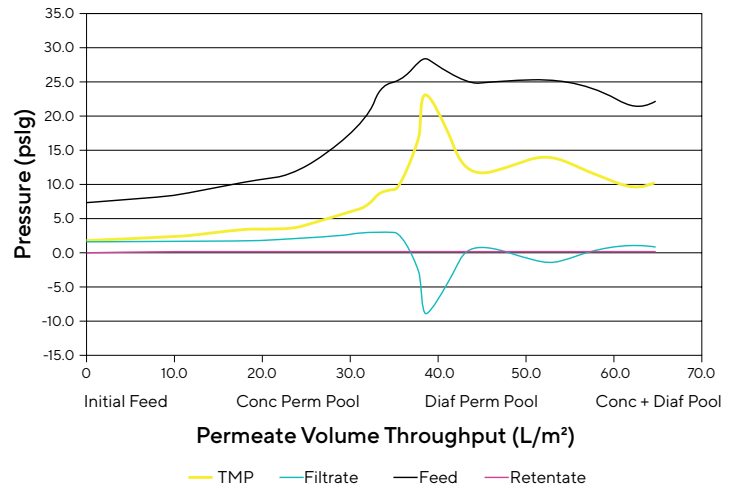
Because the operating pressures were much lower using the 750 kD membrane, the 750 kD membrane performed better than the 0.1µm membrane for this step.

**Pressure Profiles vs. Permeate Throughput**



**Figure 1:** Step 1 Pressure profiles vs. permeate throughput for Module 1 (Green Line Discover 41-inch 750 kD)

**Pressure Profiles vs. Permeate Throughput**



**Figure 1:** Step 1 Pressure profiles vs. permeate throughput for Module 2 (Green Line Discover 41-inch 0.1 µm)

## Conclusion

Overall, the 750 kD membrane performed better than the 0.1 µm membrane for clarifying the whole cell E. coli.

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