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Application Note

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Mammalian Vero Cell Clarification

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Abstract

This application note is focusing on the cell clarification of VERO cells after detachment from microcarriers.
The objective was to determine an optimum membrane with maximum retention of cells and operating conditions to maintain cell viability ≥90%. Using permeate flow control, the transmembrane pressure profile was stable at <1 psig and the permeate flux was constant 60 LMH during the process (12× concentration and 4 DVs).

The total process time was 45 min with approximately 30 minutes for the concentration and approximately 15 minutes for the diafiltration. The cell viability was 92.7% and the cell mass recovery was nearly 93% which achieved the process objective of ≥90% cell viability for the process.

Introduction

This application note is focusing on the cell clarification of VERO cells after detachment from microcarriers. In the following you will see the determination of the optimum membrane with maximum retention of cells and operating conditions to maintain cell viability ≥90%.

Materials

For this clarification of VERO cells, a Single-use Line Explorer Hollow Fiber Module was used. With a length of 24-inch and a pore size of 0.2 µm and 1.0 mm fiber ID. Like all our Hollow Fiber Modules the membrane consisted of modified Polyethersulfon (m-PES). The Explorer Hollow Fiber Module has a diameter of 1.3 cm and a corresponding filter area of 0.032 m².

Details of used Hollow Fiber Module

Family	Single-use
Product Size	Explorer
MWCO | Pore Size	0.2 µm
Fiber ID	1.0 mm
Length	24 inch
Filter Area	0.0321 m²
No. of Fibers	18
Recommended batch volume per module	250 – 1,500 mL
Diameter Module (cm)	1.30 cm
Feed | Retentate connectors	½-inch TC
Permeate connector	⅜-inch Hose Barb
Material	SU92010EXP24S6 (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. In cell clarification of mammalian cell processes like this VERO cell clarification, pore sizes between 0.2 µm and 0.45 µm are recommended. Besides, the recommended shear rate should be ideally between 2,000 - 4,000 sec^{-1}.

Feed:
- Total Cell Density: 5E06 cells/mL
- Viability: 97.5%

Process Conditions

Membrane & Module	Single-use Line Explorer 24-inch, 0.2 µm, 320 cm², 1.0 mm fiber diameter
Initial Feed Volume	1 L
Membrane Loading	1 L / 320 cm² ≈ 30 Liters/m²
Process Flux	Constant Permeate Flux at 60 LMH @ ~2,200/sec^{-1}
Process Objective	12× concentration + 4DVs; ≥ 90% viability at end
Cell Mass	>90% Recovery
Cell Viability	at start: 97.5%; at end of run: 92.7%
Cell Mass Recovery	92.7%

Results

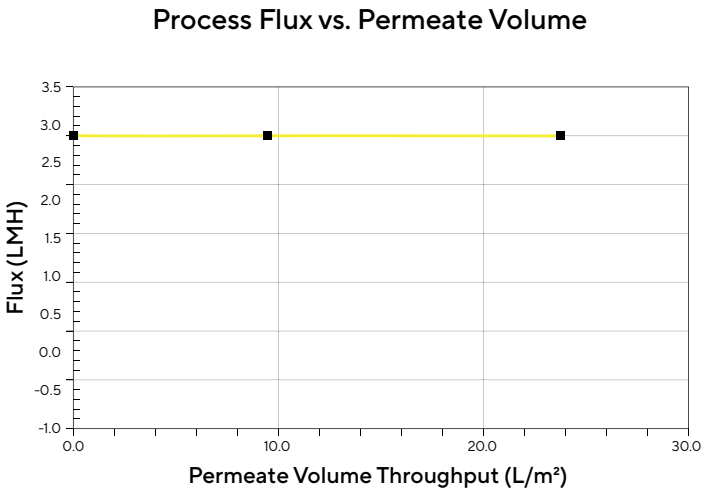

Figure 1: Process flux vs. permeate throughput for Single-use Line Explorer 24-inch 0.2 µm cartridge

Conclusion

Using permeate flow control, the transmembrane pressure profile was stable at <1 psig and the permeate flux was constant 60 LMH during the process (12× concentration and 4 DVs). The total process time was 45 min with approximately 30 minutes for the concentration and approximately 15 minutes for the diafiltration.

The cell viability was 92.7% and the cell mass recovery was nearly 93% which achieved the process objective of ≥90% cell viability for the process.

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