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Bacillus Whole Cell Clarification

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Abstract

This application note is focused on the clarification of bacillus whole cell feed directly from a fermenter. In order to maximize the recovery of the 83 kD target protein, Sartorius Hollow Fiber Modules were used. In total a product recovery of 91% was achieved.

Introduction

The goal of this process optimization was to determine the optimum membrane with maximum retention of cell and cell debris, and the operating conditions that will produce maximum transmission of target protein. Besides, the maximum transmission would result in high product recovery in shortest time.

Materials

For this clarification of bacillus whole cells an 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 m^2 .

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	0.0193 m^2
No. of Fibers	18
Recommended batch volume per module	250 - 1,500 mL
Diameter Module (cm)	1.3 cm
Feed Retentate connectors	½-inch TC
Permeate connector	¾-inch Hose Barb
Order Code	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. For clarification of bacterial processes, a pore size between 0.2 μm and 0.45 μm normally is used. Besides, the recommended shear rate should be ideally between 6,000 – 9,000 sec^{-1} .

Results

Details of Trial

Feed	98 g/L (OD = 24.944)
Initial Feed Volume & Loading	500 mL = 15.6 Liters/ m^2
Membrane Mass Loading	49 g/320 cm^2 = 1.53 kg/ m^2
Process Objective	3 \times concentration + 3 diavolumes (DV) at 7,000 sec^{-1} shear
Process Time & Flux	Approx. 50 minutes at constant 30 LMH (16.0 mL/min)

At the membrane loading of 15.6 L/ m^2 and a flux of 30 LMH, the overall product recovery was 91%. The transmembrane pressure profile at a constant permeate flux of 30 LMH was steady and was less than 4 psig during the 3 \times concentration.

Conclusion

The lysate clarification at the higher 7,000/sec shear rate compared to the 5,100/sec shear rate decreased the processing time by approximately 20%.

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