E.coli Lysate Cell Debris Removal

An *E. coli* lysatge was processed to recover an intracellular protein

Introduction

Genetically engineered *E. coli* was fermented and then mechanically lysed. The desired product was an intracellular protein, an interferon with cancer treatment potential.

Process Conditions

The initial batch size was 245 liters of lysate. A single 0.2 µm Krosflo® module (catalog number K22M-130-01W) containing 1.0 m^2 of membrane surface was used in a batch separation configuration. Recirculation rate was 57 liters per minute throughout the run. The permeate port was kept closed until circulation was well established, then slowly opened. The downstream circulation was never restricted. the initial batch temperature was 4°C and rose to 12°C by the end of the run. The starting inlet pressure was 12 psig, which dropped to 11 psig because of product warming and then rose to 13 psig due to increasing solids concentration near the end of the run.

Results

As is typical for bacterial lysates, the flow decay curve exhibited a steep initial decline with a rapid transition to equilibrium flux. Collection of 237 liters of purified filtrate was accomplished in 65 minutes. The result was a 30-fold volume reduction and an equilibrium flux of 200 L/m^2hr. Passage of the desired protein was 99%.

Discussion

Choice of the proper filtration module and process conditions are essential to cost-effective separation of the component of interest from cell debris. Spectrum KrosFlo® filtration modules are an effective means of separating soluble proteins from cell debris subsequent to lysis. Single use modules offer several advantages over multiple use filters. Starting each batch with a disposable non-pyrogenic filter eliminates concerns about inadequate cleaning and rinsing of re-used filters. The filter is no longer a possible source of batch-to-batch contamination. Finally, consistent high performance is assured during each run.

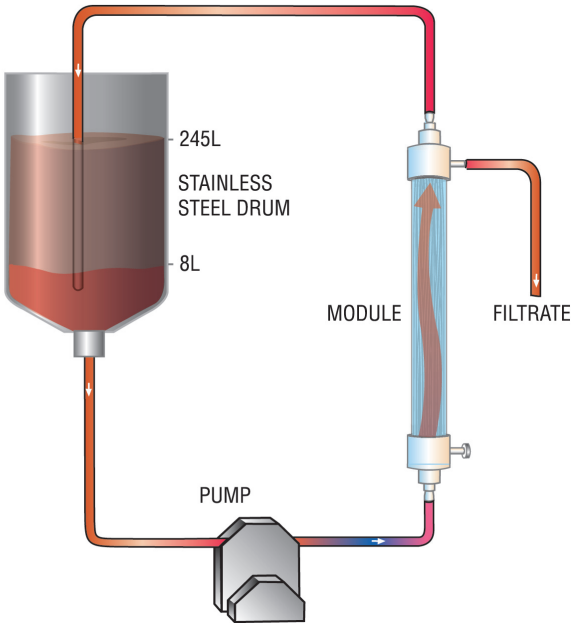

Figure 1 System set-up for *E. Coli* Lysate Cell Debris Removal

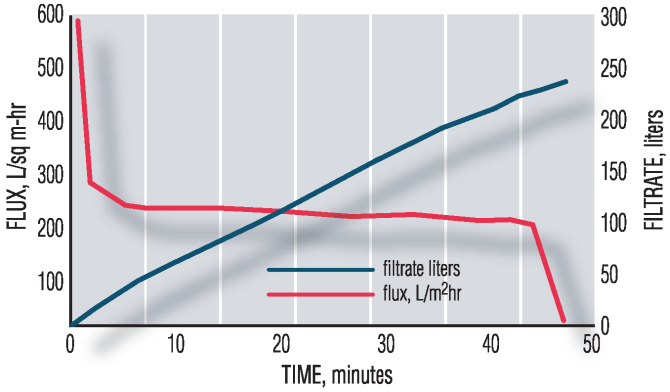

Figure 2 Flux and Filtrate Flow vs. Time for *E. Coli* Lysate Cell Debris Removal