Bacterial Cell Harvesting Case Study

Biopharmaceutical processing has required optimization of the use of microorganisms to produce therapeutic proteins. A common organism used in recombinant technology is *Escherichia coli (E. coli)*. Whether the protein is secreted or remains within the cell, a fundamental first step in clarification is the separation of cells and/or cell debris from the target protein. To date, the use of microfiltration (MF) membranes has provided the industry a simple, yet time-consuming, method for separation. Filters frequently become clogged, increasing processing time and decreasing the recovery of the target protein.

SmartFlow Technologies has developed a method for the simultaneous processing of recombinant proteins using their novel OPTISEP® filter modules that can improve both processing time and yield. Figure 1 illustrates a simultaneous process set-up where the first system is used as a MF unit to separate whole cells / debris from protein while the second is an ultrafiltration (UF) step to concentrate the target protein.

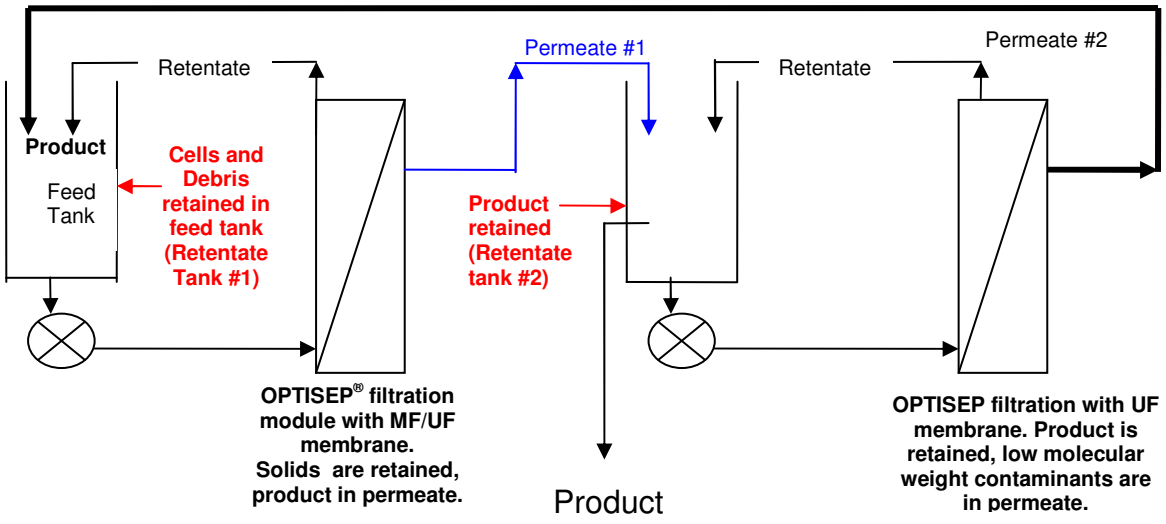

Figure 1: Set-up for a simultaneous process

By combining these steps into a simultaneous process, facilities decrease the movement of material between unit operations and decrease the amount of buffer required during clarification and subsequent concentration. Here, we will focus on the MF step.

The experimental starting material was 8.7L of whole cell *E. coli* broth with an optical density (OD) of 73. The MF unit was a SmartFlow Technologies OPTISEP 3000 module, 0.4m^2, with 0.2µm pore size and 0.75mm channel height. The *E. coli* broth underwent a 5 volume diafiltration during MF. Flow and pressure measurements and retentate and permeate samples were collected after each diafiltration volume. Retentate and permeate samples were assayed for protein via ELISA to determine target protein levels in each process stream.

Trending of the MF permeate flux rate throughout the experiment demonstrated that after 9.75 hours of operation, the flux remained near constant with an average of 13LMH (Figure 2).

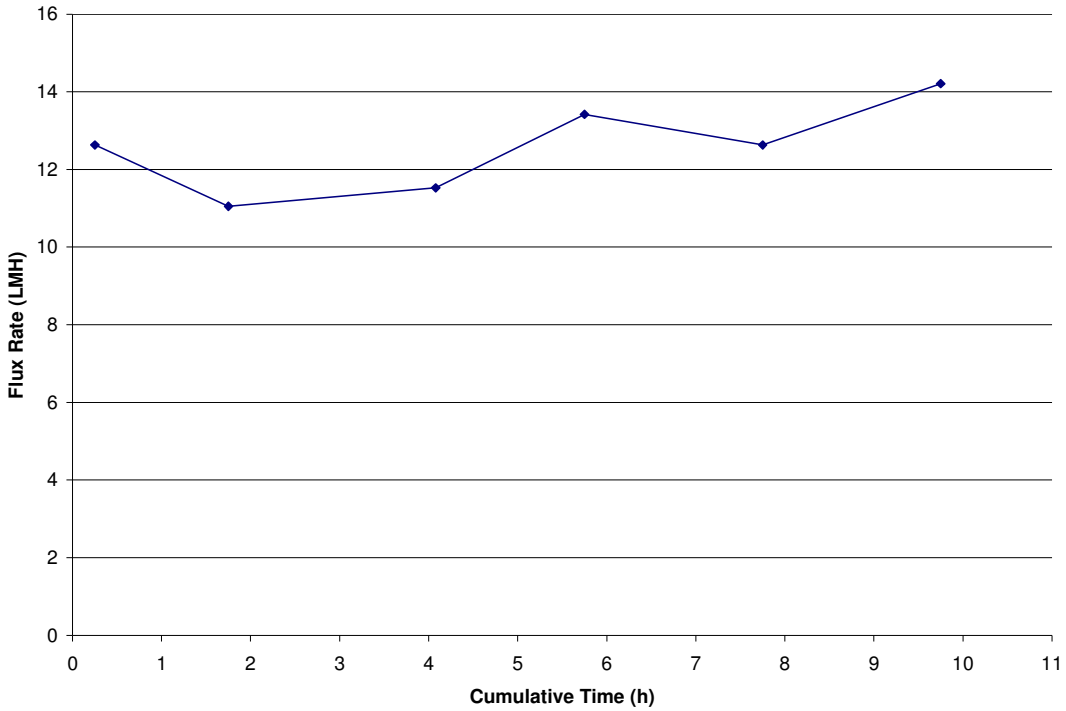

Figure 2: Flux Rate verus Run Time

In addition, the transmembrane pressure (TMP) for the OPTISEP 3000 module remained at an average of 4.25 psig (0.29 Bar). Based on these parameters, the OPTISEP module showed no decrease in operational capability during the 5X diafiltration process.

The ELISA assay results from the retentate and permeate pool confirm the efficiency of the MF process. Figure 3 trends percent recovery versus diafiltration volumes. By the end of the experiment, the process had recovered 66.5% of the original amount of target protein. Additional diafiltration volumes would have further increased this recovery.

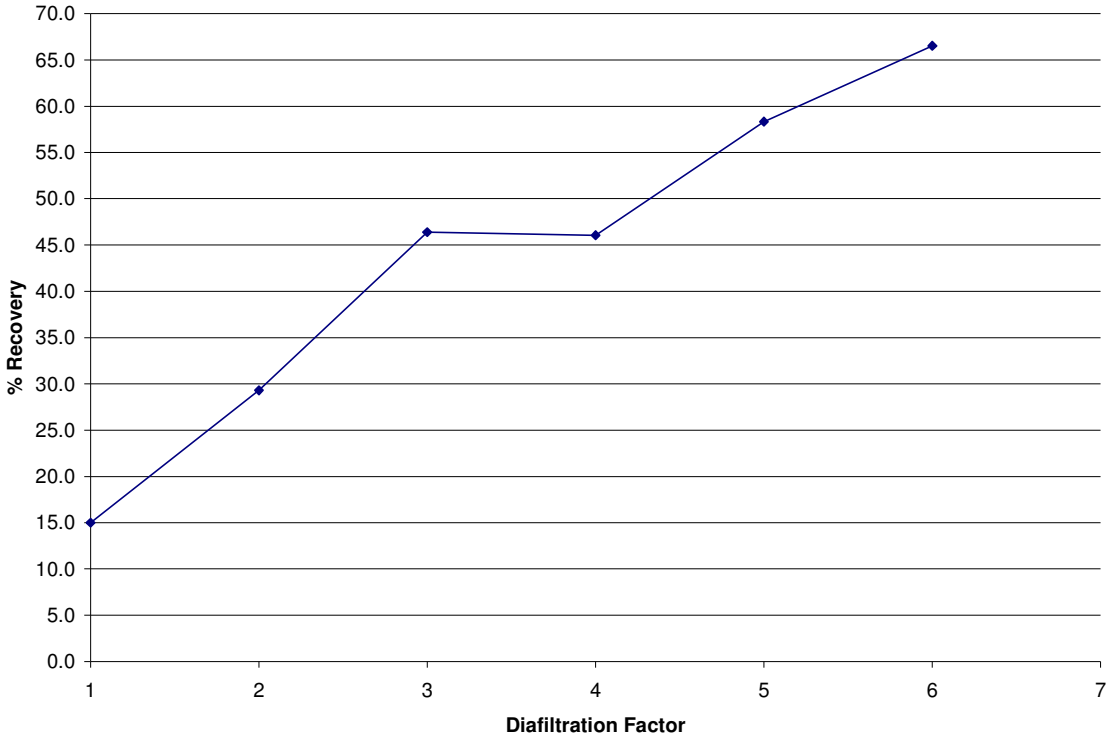

Figure 3: Percent Protein Recovery versus Diafiltration Volume

The MF process operation illustrates two key elements for biopharmaceutical manufacturing: 1) consistent operation of a clarification process, and 2) improved recovery of the target protein at an upstream point in the process. The patented OPTISEP modules performed with a consistent flux rate and membrane passage. This reduced fouling results in shorter process times and a more reliable process. Additionally, the high yields reduce the number of fermentation batches needed over the course of each year, which can greatly reduce processing costs.