SmartFlow Technologies

Mammalian Cell Harvesting Case Study

Mammalian cells have long been a major producer of therapeutic protein in the biotechnology industry. As the batch sizes and titer of mammalian cell products have increased, the downstream processing of mammalian cell cultures has caused a bottleneck in the production of these proteins. One major step that has caused a problem with processing of mammalian cells is the cell harvest. Here, experiments are described that illustrate the superior yield and performance of using *SmartFlow*™ TFF for the harvest of Chinese Hamster Ovary (CHO) cells.

A CHO cell culture producing monoclonal antibodies was chosen for the clarification. The culture was stored in 20 L bags for 8 weeks at 4ºC after harvest until this work was initiated. Before performing the experiments, the cells were placed in a water bath and heated to 17 to 21ºC. The clarification experiments were performed using OPTISEP® 800 and OPTISEP 3000 filter modules. For the OPTISEP 800 experiment, 0.032 m^2 of membranes were used to clarify either 2 or 4 L of CHO cells. Shear rates of 7,600 sec^{-1} or 12,000 sec^{-1} and no back pressure were used for the experiments. Membrane pore sizes of 0.2 and 0.45 µm were used. For the OPTISEP 3000 experiments, 7 different CHO cell harvests were performed. The modules were cleaned using 0.1 M NaOH for 30 minutes. Then the modules were permitted to soak overnight in 0.01M NaOH before clean water flux measurements were taken.

First, the 0.2 µm and 0.45 µm pore sizes were tested in the OPTISEP 800 (Figure 1). Both membranes were able to finish the cell harvest. The 0.45 µm membrane had a 10x higher flux rate and a higher passage rate than the 0.2 µm membrane (Table 1). Therefore, it was selected for additional trials. The next test evaluated the starting volume to membrane area ratio (Figure 2). This ratio was doubled by increasing the starting volume from 2 to 4 liters. Here, it was found that doubling this ratio still permitted the concentration of the cells to 10X with high passage. Therefore, the higher ratio was also used for the final tests that studied the effect of changing the shear rate on the membrane performance. The two shear rates studied were 7,600 sec^{-1} and 12,000 sec^{-1} (Figure 2). The higher shear resulted in slightly higher flux rates with the antibody passage remaining high. Therefore, scale up would utilize the higher shear rate and the higher starting volume to membrane area ratio.

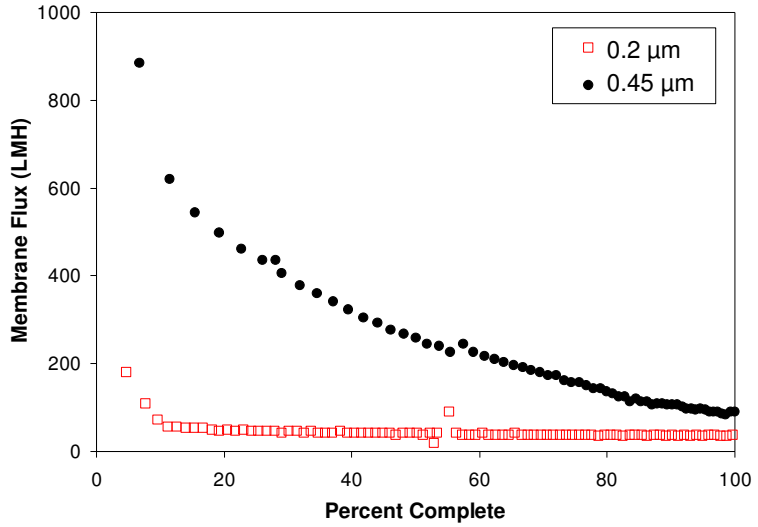

Figure 1 – A comparison of the instantaneous flux rates of the 0.2 µm and 0.45 µm pore size membranes.

Table 1 - Summary of clarification experiments of CHO cell producing antibodies.

Pore Size (µm)	Starting Volume l	Shear (sec^{-1})	Average LMH ($l/(m^2 h)$)	Protein Passage at 2x (%)	Protein Passage Final (%)
0.45	2	7600	405	100	99
0.2	2	7600	43	69	69
0.45	4	7600	213	99	97
0.45	4	12000	278	100	99

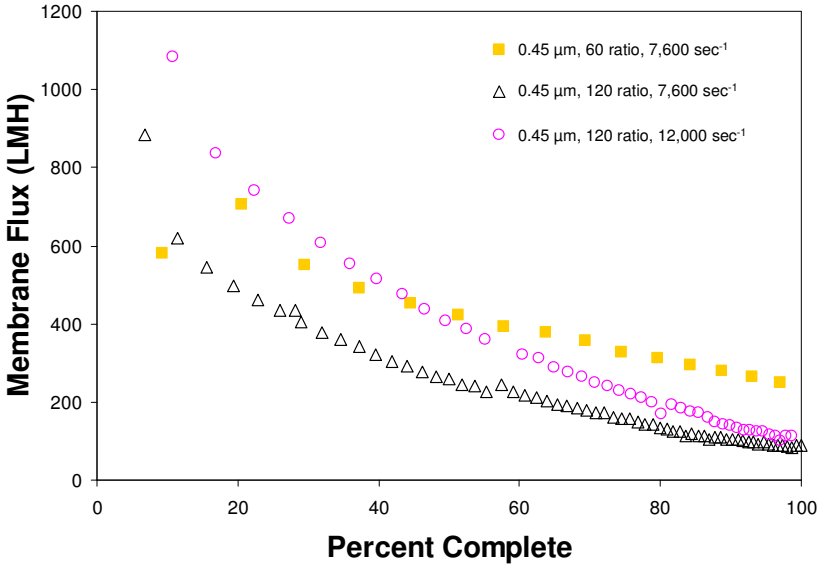

Figure 2 – A comparison of the instantaneous flux rates of the 0.45 µm membrane using two different shear rates and two different starting volume to square meters of membrane ratios.

The final tests involved the filter cleaning. One OPTISEP 3000 module was used for 7 different cell clarification steps. After each step, it was cleaned with 0.1 M NaOH, 3% phosphoric acid, and soaked in 0.01 M NaOH overnight. Then, the clean water flux was compared to the initial clean water flux (Figure 3). The clean water flux after 7 trials was identical to the initial clean water flux, which indicates that this cleaning regimen is sufficient to clean the filter modules.

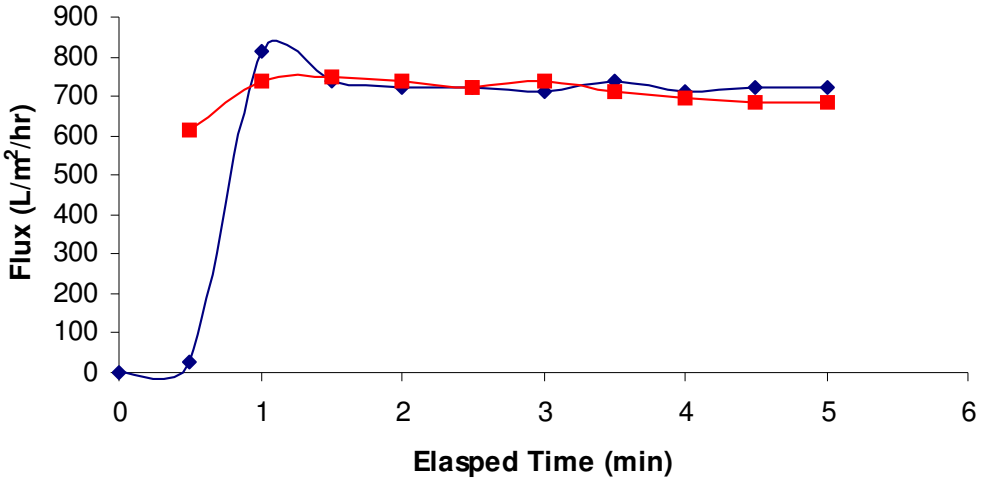

Figure 3 – A comparison of the initial clean water flux rate (■) and the clean water flux rate after 7 CHO cell harvest (◆). The discrepancy in the first data points was due to them not being for the full 30 seconds.

This MF process for the harvesting of monoclonal antibodies from CHO cells illustrates two key elements for biopharmaceutical manufacturing: 1) consistent operation of a clarification process, and 2) improved recovery and yield of the target protein at an upstream point in the process. The patented OPTISEP modules performed with a consistent target protein passage. Also, the modules were cleanable between runs. This reduced fouling results in shorter process times and a more reliable process. Additionally, the high yields reduce the number of bioreactor batches needed over the course of each year, which can greatly reduce processing costs.