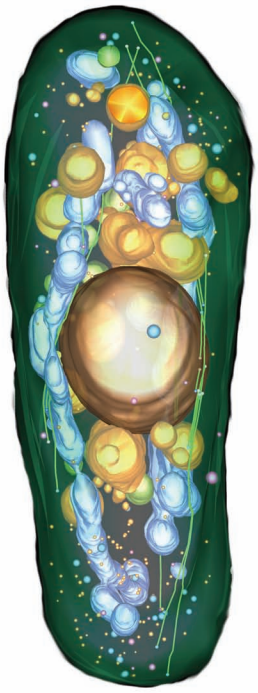
NCSRT

Works™ Case Study

Concentration and diafiltration of Whole Cells with *SmartFlow*™ TFF



NCSRT, Inc.
1000 Goodworth Dr.
Apex, NC 27539
USA

Phone: +1 919 387 8460
Fax: +1 919 387 8540
email: info@ncsrt.com
Web: http://www.ncsrt.com

Concentration and diafiltration of whole cells

The *Works™ Concentration and diafiltration of whole cells* Optimization Procedure provides a systematic method for optimizing the harvest of intact cells from bacterial and fungal fermentation broths. Isolation of the cells in high concentration is a critical starting point for the recovery and purification of recombinant products sequestered intracellularly or in inclusion bodies. Diafiltration prepares the cells for the subsequent lysis or storage. The procedure is also important for the development of cell paste for plasmid DNA transfer.

This *Concentration and diafiltration of whole cells* Case Study describes how a customer evaluated the *SmartFlow*™ TFF filter technology, comparing it to the cost and efficiency of a large scale centrifuge to concentrate the *E. coli* from their fermentor. The customers evaluated two conditions, a best case scenario where the process output would be equivalent to the centrifuge option and an enhanced process where the output would result in significant savings of time, material, and provide less volume for the cell lysis step that follows.

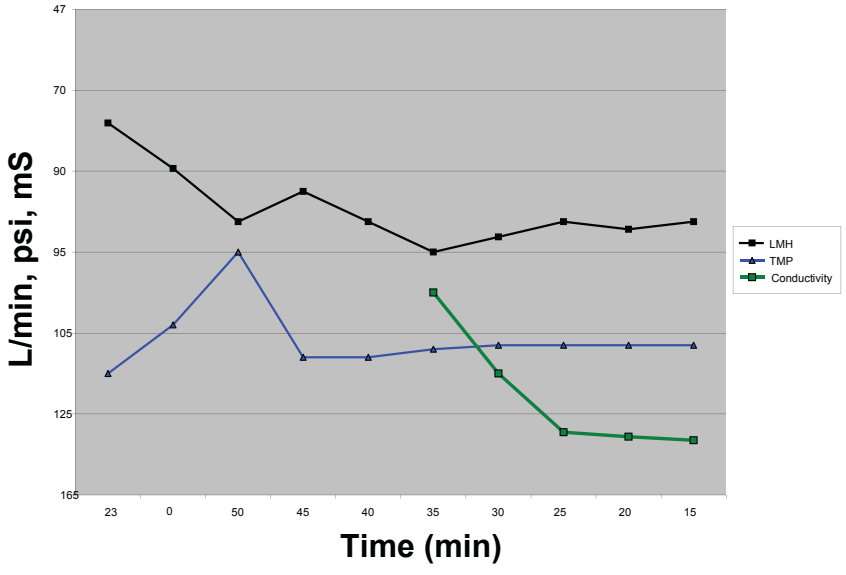
The case study describes the cell harvest from the fermentation broth using a regenerated cellulose (RC) 100 kD ultrafiltration membrane to pass the broth components into the permeate and retain the cells. The customer set an initial target for the cells to be concentrated 4X and followed by a diafiltration to below 7 millisiemens (mS) with the lysis buffer for the downstream step. A second test was performed to examine the process performance impact of increasing the challenge volume and targeting an 8X concentration followed by diafiltration to a point below 7 mS.

Case Study: *Concentration and diafiltration of whole cells*

Process development scientists at a major biopharmaceutical company evaluated a tangential flow filtration (TFF) process to harvest *E. coli* and transfer the concentrated cells into a buffer for subsequent lysis. The process was evaluated as an alternative to their planned centrifugation and depth filtration process. Historically, industrial scale cell harvest with traditional TFF cassette or hollow fiber formats have been subject to low flux rates and unacceptable product yields. The harvest processes are prone to plugging if the fluid flow at the membrane surface is not managed properly. However, a properly optimized *SmartFlow* TFF process improves product yield and significantly reduces both initial capital cost and operational costs when compared to the centrifuge/depth filter combination. Centrifugation combined with depth filtration is reported to provide acceptable yields but requires significantly higher initial capital investment to implement than SF-TFF. The goal of this study was to determine the ability to achieve high cell mass and transfer the cell paste into a lysis buffer using SF-TFF more efficiently than the current process. A 5X diafiltration is used to achieve the transfer into the lysis buffer. The buffer transfer was monitored by the conductivity of the TFF permeate. A conductivity level of < 7 mS indicated acceptable buffer transition.

In the first test, a 100 kDa regenerated cellulose membrane was used to filter 3 liters of cells. This volume challenged the membrane at a level of 50 LM (L of media per square meter of membrane). The pump was adjusted so a shear of 5000 sec^{-1} was obtained. The cells were concentrated 4X and then diafiltered 5X using a continuous diafiltration process. Conductivity was measured at the start of diafiltration and at 2X, 3X, 4X, and 5X.

Figure 1



Concentration and diafiltration of whole cells

A second test was performed increasing the membrane challenge (higher LM ratio) and increasing the concentration of the cell mass to an 8X concentration. A 5 liter sample was used in the process for this test, resulting in a 75 LM challenge level for the RC100 kDa membrane. The higher concentration of the cell mass significantly reduces the lysis buffer required for the diafiltration process.

Results:

The first test demonstrated a stable cell harvest system with an average permeate flux of 35 LMH for the entire process. The 4 X concentration was complete at the 60 minute point and the 5X diafiltration was complete in 150 minutes for a total process time of 210 minutes (Figure 1). Once the diafiltration began, the membrane flux remained fairly constant between 30 and 33 LMH indicating that the *SmartFlow*™ TFF module was not fouling. The second test demonstrated that the *SmartFlow* TFF process was able to increase the cell concentration to 7X. System hold up volume constraints limited further concentration with this challenge. 5X diafiltration achieved the desired conductivity of < 7 mS (final data point missing). The 7 X concentration was complete in 95 minutes and the 5 X diafiltration took an additional 165 minutes for a total process time of 4 hours and 20 minutes (Figure 2). This corresponds to an average flux rate of 28 LMH for the entire experiment. Again the *SmartFlow* TFF module maintained a constant flux, this time around 22 LMH during the diafiltration. This constant flux again indicates that the module was not fouling during the experiment. The flux rate was slightly lower than in the previous case because of the higher cell to area (LM) ratio in the second experiment.

Conclusion:

The *SmartFlow* TFF system provided improved ability to concentrate and diafilter the cells when compared to the current system of centrifugation and depth filtration. Additional benefits of reduced buffer volumes and their associated costs are attainable when implementing the SF-TFF concentration and diafiltration process. Additional efficiencies can be achieved by performing a systematic evaluation of the process conditions using the NCSRT *Concentration and diafiltration of whole cells* Optimization Procedure.

SmartFlow TFF provides a preferred alternative platform to centrifugation/depth filtration for E. coli harvest prior to lysis to recover inclusion bodies or intracellular products. NCSRT *SmartFlow* technology provides unparalleled value in downstream process for cell harvest applications in *E. coli* fermentation systems.

Figure 2