NCSRT

Works™ Optimization Procedure

E. Coli lysate by simultaneous processing with *SmartFlow*™ TFF

The *SmartFlow*™ filter *WORKS*™ *E. coli lysate by simultaneous process* Optimization Procedure from NCSRT is intended as a procedure for developing an integrated process for concurrently isolating a target protein from an *E. coli* lysate and concentrating the protein in a second system. In the first step or the isolation step, the product protein is isolated from the lysate by passing the target molecule through the membrane in the first skid. In the second step or concentration step, the product is concentrated in the second skid. The permeate from this concentration step can then be used as the diafiltration buffer for the isolation step (Figure 1). Significant benefits in time savings, process equipment costs, and buffer costs are realized by performing the processes simultaneously rather than sequentially.

The isolation part of the optimization procedure uses either a microfiltration (MF) membrane or a high molecular weight cut off ultrafiltration (UF) membrane that retains the cells and cell debris and allows the desired products to pass freely through the membrane. The passage characteristics of proteins change with different buffers, temperatures, concentrations, and membranes. The concentration step of the optimization procedure uses an ultrafiltration (UF) membrane to retain the desired protein in the retentate while small molecules such as sugars and salts are able to pass through the membrane. The passage characteristics of proteins change with different buffers, temperatures, concentrations, and membranes. By examining the passage characteristics of the different membranes available in the appropriate process conditions, a well defined and executed process development study can identify the most efficient membrane and process conditions to achieve the required performance.

This optimization procedure starts with selecting membrane modules most likely to work with respect to polymer and pore size based upon numerous NCSRT trials. Once the module is selected for each of the isolation and concentration steps, ranges in which to begin optimizing parameters such as membrane capacity, recirculation rate, and pressure are presented. Because of the variability in the products and processes using NCSRT's *SmartFlow* technology, we do not make specific process recommendations on parameters of temperature, pH, buffers, or other variables that may affect the separation process and the target product activity.

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Each parameter of the TFF process: product, membrane type, shear, pore size, temperature, concentration factor, pH, anti-foam, etc. may impact the fermentation broth components passage through the membrane. This is why a systematic experimental plan must be developed and executed to optimize a concentration and diafiltration process.

1) Select the *SmartFlow* filter modules to evaluate for the isolation and concentration steps. The selection requires specifying a combination of membrane type,

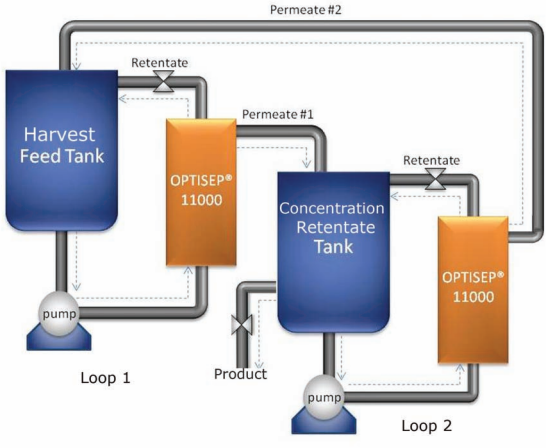

Figure 1 – Simultaneous Processing Schematic

channel height, and membrane area for a given module that will be tested.

a) NCSRT has filtered thousands of solutions and therefore can provide several membrane chemistries and pore size recommendations that will work in the majority of cases. In general the pore size should be 5 to 10 times the size of the molecule to be passed through the membrane in the first step and one half to one third the size of a molecule to be retained in the second step.

b) The combination of the channel height and the fluid velocity through the flow channel created by the recirculation pump produce a shear at the membrane surface. It is this shear that governs the separation performance and efficiency. Care must be taken in selecting and maintaining the shear at the membrane surface.

c) The membrane area also affects the pump size required to achieve the necessary shear rates for a given separation.

2) Select the first membrane to test.

Recommended starting membranes for passage of the desired protein from a cell lysate are a regenerated cellulose (RC) 100 kDa, a polyethersulfone (PES) 500 kD, or a modified polysulfone (MPS) 0.45 µm pore sized membrane. See table 1 to determine which membrane is recommended to test first.

Table 1

Protein Size	Membrane Polymer	Pore Size
< 20 kDa	RC	100 kDa
20 kDa - 80 kDa	PES	500 kDa
> 80 kDa	MPS	0.45 µm

3) Select the channel height for the module.

a) For the passage of proteins from *E. Coli* lysate, a channel height between 0.75 and 1.5 mm is recommended.

b) In most cases, a channel height of 0.75 mm is recommended because it will require the lowest recirculation rate (and thus the smallest pump) and produce the highest flux rate.

c) Cases to use a higher channel height include:

i) If cell aggregation is occurring, the lower height channels may clog. This is not a common problem for cells that have been sufficiently homogenized.

ii) If the channel is clogged by aggregates or process particles, the inlet pressure will increase dramatically and the permeate rate will decrease over a short period of time. This will occur usually in the first five minutes.

iii) In cases where high solids are desired, a channel height of 0.875 mm or above will be necessary.

4) Select the membrane area.

a) The membrane area depends upon the batch size to be processed. For filtration process development trials, usually the smallest size membrane and thus the smallest batch size is desired.

b) For cell harvests, an important parameter is the membrane capacity or LM ratio. The LM ratio is defined as the volume of starting material divided by the membrane area.

c) The range of LM ratios for the passage of proteins from cell lysate we have observed vary from 25 to 150 LM.

i) The typical starting ratio we recommend is 60 LM.

ii) If a fermentation broth is being processed, the presence of antifoam is an important parameter that can impact the starting volume to membrane area ratio. Antifoam agents may significantly

reduce the permeate flux observed with ultrafiltration membranes. In cases where the fermentation broth contains antifoam, use a starting ratio of 30 LM.

iii) The minimum batch size is the system hold up volume times the concentration factor. For a continuous diafiltration, the minimum batch size is simply the system hold up volume.

d) The membrane area needed is the batch size divided by the LM ratio.

5) Determine the shear rate.

a) The typical shear rate for the passage of proteins from an *E. coli* lysate ranges from 7,500 sec^{-1} to 16,000 sec^{-1}.

b) The typical starting shear rate passage of proteins from an *E. Coli* lysate for a process development run is 10,000 sec^{-1}.

c) The benefit of increasing the shear rate is an increased permeate rate.

d) The disadvantages of increasing the shear rate are:

i) Higher pump costs due to higher recirculation flow rates.

ii) Higher pressure drops and TMPs which may decrease the passage of the desired protein.

e) An increase in the shear rate should be balanced by an increase in the flux rate or protein passage for the process to retain the same overall efficiency. The energy costs of running the pump at a higher shear rate must be offset by savings on membranes to make increasing the shear rate cost efficient.

6) Calculate the flow rate needed to operate the selected module at the selected shear rate using the NCSRT Scale-UP LPM GPM spreadsheet. Ensure that a pump is available that can produce this flow rate at the needed pressure. If a suitable size pump in not available, consider either running a smaller trial or calling NCSRT to determine if a suitable size pump is available.

7) Use Table 2 to determine the module(s) part numbers for ordering.

Table 2: *SmartFlow* filter module part numbers

Module Size		Channel Height		Membrane polymer and pore size	
_____	-	___		____-_____	
74	100 ft^2 Optisep 11000	D	0.5 mm	5B-0005	RC 5 kD
72	50 ft^2 Optisep 11000	E	0.75 mm	5B-0010	RC 10 kD
71	10 ft^2 Optisep 11000	G	0.875 mm	5B-0030	RC 30 kD
41	10ft^2 Optisep 7000	H	1 mm	5B-0100	RC 100 kD
40	5 ft^2 Optisep 7000	J	1.5 mm	2B-8010	NF-PES 10
52	2 ft^2 Optisep 3000			2B-0005	PES 5 kD
51	1 ft^2 Optisep 3000			2B-0010	PES 10 kD
				2B-0050	PES 50 kD
				1B-0100	PS 100 kD

8) Select the first membrane to test for the concentration or UF step.
Use Table 3 for general recommendations of what membrane and pore size is needed to retain the desired product. For more specific recommendations, see the specialized optimization procedure for your target molecule.

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Table 3 - Ultrafiltration membranes

Target Molecule	Membrane polymer	Pore size
> 8 kD	RC	5 kD
> 18 kD	RC	10 kD
>50 kD	RC	30 kD
> 180 kD	RC	100 kD
3- 8 kD	NF-PES-10	10% Salt Rejection
>7.5 kD	PES	5 kD
>18 kD	PES	10 kD
>100 kD	PES	50 kD
> 180 kD	PES	100 kD

9) Select the channel height for the module.
   a) For the concentration of a prefiltered protein, a channel height between 0.75 and 0.875 mm is common.
   b) In most cases, a channel height of 0.75 mm is recommended because it will require the lowest recirculation (and thus the smallest pump) and produce the highest flux rate.
   c) Use a higher channel height when high solids are desired (greater than 20% protein), a channel height of 0.875 mm or above will be necessary.

10) Select the membrane area.
   a) The key to sizing the concentration step is to ensure that the permeate rate is equal in the two steps. For the concentration step, this usually requires the area be sized at twice the membrane area that was used in the isolation step.

11) Determine the shear rate.
   a) The typical shear rate for the concentration of proteins ranges from 7,500 sec^{-1} to 20,000 sec^{-1}.
   b) The typical starting shear rate for a process development run is 10,000 sec^{-1}.
   c) The benefit of increasing the shear rate is an increased permeate rate.
   d) The disadvantage of increasing the shear rate is the higher pump costs due to higher recirculation flow rates.
   e) An increase in the shear rate should be balanced by an increase in the flux rate for the process to retain the same overall efficiency. The energy costs of running the pump at a higher shear rate must be offset by savings on membranes to make increasing the shear rate cost efficient.

12) Calculate the flow rate needed operate the selected module at the selected shear rate using the NCSRT Scale-UP LPM GPM spreadsheet. Ensure that a pump is available that can produce this flow rate at the needed pressure. If a suitable size pump in not available, consider either running a smaller trial or calling NCSRT to determine if a suitable size pump is available.

13) Use Table 2 to determine the module part numbers for ordering.

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Filter Operation:

1) After loading the filter modules and making all the connections, the first step is to perform a water and/or buffer rinse of the system directing the permeate to the waste. This step should be performed on both the isolation and concentration systems.
2) After the rinse, direct the permeate lines back to the retentate tanks in both systems so no concentration occurs prior to establishing the desired shear rate and performing the transmembrane pressure (TMP) optimization procedure. See Process Optimization on page 7 of this procedure.

IMPORTANT: Do not permit the permeate line to come in contact with the retentate fluid. This can contaminate the permeate pool in later samples.

3) Slowly increase the flow rate of the isolation system recirculation pump to the calculated rate from step 6 in the previous section or the optimal flow rate determined in the Process Optimization process.
4) Start with the backpressure at zero. The inlet pressure should be at least 5 psig (0.3 bar) and remain below 12 psig (0.8 bar). If the inlet pressure is above 12 psig (0.8 bar), the recirculation flow rate should be reduced such that the inlet pressure remains below 12 psig (0.8 bar). If the inlet pressure is less than 5 psig, then slight backpressure can be added until the inlet pressure increases to 5 psig. Table 4 provides typical TMP values for the different membranes used in *SmartFlow*™ TFF filter modules.
5) Begin concentrating.
   a) Remove the permeate lines from the retentate tank of the isoaltion process and place them in the permeate vessel.
   b) Wait until about 5% of the starting retentate volume has passed through the membrane to the permeate to take the initial samples of the retentate and permeate.
   c) Take permeate and retentate samples when each additional concentration factor is reached.
   d) When each sample is taken, record the permeate flow rate using a graduated cylinder, scale, or flow meter.

Table 4 Typical transmembrane pressure values for *SmartFlow* modules

Membrane Pore Size	Transmembrane Pressure Starting Value PSIG (Bar)	Transmembrane Pressure Ranges PSIG (Bar)	Cell Harvest Inlet PSIG (Bar) Starting Value	Cell Harvest Outlet PSIG (Bar)
Ultrafiltration Membranes				
1 kDa	75 (5)	90 to 150 (6 to 10)		
5 kDa	45 (3)	60 to 90		
10 kDa	30 (2)	45 to 90 (3 to 6)		
30 kDa	15 (1)	30 to 75 (2 to 5)		
100 kDa	15 (1)	20 to 60 (1.37 to 4)	20 (1.37)	12 (0.83)
300 kDa	10 (0.69)	15 to 45 (1 to 3)	20 (1.37)	10 (0.69)
500 kDa	7.5 (0.5)	10 to 45 (1 to 3)	15 (1.0)	8 (0.55)
Microfiltration Membranes				
0.1µ	2 (0.13)	4 to 15 (0.27 to 1.0)	4 (0.275)	0
0.2µ	2 (0.13)	4 to 15 (0.27 to 1.0)	4 (0.275)	0
0.45µ	2 (0.13)	4 to 10 (0.27 to 0.69)	4 (0.275)	0
0.8µ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0
1.0µ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0
2.0µ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0
3.0µ	1 (0.07)	1 to 6 (0.07 to 0.41)	2 (0.13)	0

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6) Once the desired concentration factor is reached, record the volume remaining the in the retentate.
   a) The remaining volume in the retentate can be calculated by subtracting the permeate volume and retentate volume samples from the starting volumes.

$$Retentate\ Volume = Starting\ Volume - Permeate\ Volume - Retentate\ Sample\ Volume$$

7) At this point, the concentration skid recirculation pump should be turned on to provide permeate, which will act as the buffer for the diafiltration of the isolation step, to the recirculation tank of the isolation step.
   a) Slowly increase the pump speed until the flow rate calculated in step 12 above or determined during the Process Optimization process on page 7 is reached.
   b) Place the permeate hose in the retentate vessel from the isolation step. Here, the permeate will provide the diafiltration buffer for the first step.
   c) Increase the TMP until it is in the midpoint of the range given in Table 4 or determined in the Process Optimization process.
   d) Take a permeate flow rate from the concentration step permeate.
   e) If the concentration step permeate flow rate is higher than the isolation step permeate flow rate, then decrease the pressure until they are equal.
   f) If the isolation step permeate flow rate is higher than the concentration step permeate flow rate, then increase the pressure and measure the permeate flow rate.
      i) Keep increasing the pressure as long as the permeate flow rate is increasing or until it is equal to the isolation step permeate flow rate.
      ii) If an increase in the pressure does not increase the permeate flow rate (an indication that the gel layer has compressed on the membrane), increase the recirculation rate until the permeate flow rates from the two steps are equal.

8) Diafiltration – the following describes the procedure for diafiltering the product 3x:
   a) Start to monitor the retentate volume in the isolation step tank.
   b) The level in the tank will remain constant if the two permeate flow rates are equal.
   c) Continue to add permeate back to the tank until 3 times the retentate volume has been added to the system.
   d) Take samples from the permeate hose and both the isolation step and concentration step retentate tanks when each diafiltration factor is reached (i.e. take a sample when the permeate volume is equal to a multiple of the retentate volume such as 1X, 2X, etc.).
   e) For other diafiltration factors, continue the process until the amount of diafiltration buffer added equals number of desired diafiltration factor times the system volume recorded in step 6.
      i) The theoretical recovery from a 3X diafiltration for a molecule with a 100% passage is 95%.
      ii) Increasing the diafiltration factor will increase the yield especially when the target molecule has low passage. However, the cost of increasing the diafiltration volume is that the process time will be greater.
      iii) Decreasing the diafiltration factor will decrease the yield. However, for molecules with high passage and low value, the small decrease in the yield may be worth the faster processing time.

Data analysis:

Sample Analysis:

1) Check the permeate samples from the isolation step for cells.

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2) Use a gel or specific protein assay to check the permeate samples from the concentration step for product passage.
3) Calculate the membrane flux rate or LMH (L/m2/h) by dividing the measured permeate flow rate at each sample by the membrane area.
4) Calculate the instantaneous product passage in the isolation step by dividing the permeate product content by the retentate product content and multiplying by 100.
5) Record the data on the Membrane Test worksheet.

Process Optimization:

The procedure should be repeating under different process conditions to ensure that the optimized conditions are reached.

1) The important variables to optimize are the yield, membrane passage, and membrane flux rate.
2) An important parameter that affects the yield, passage, and flux rate for cell harvest is the membrane capacity or LM ratio for the isolation step.
   a) Increasing the LM ratio decreases membrane performance, which increases processing time and decreases membrane costs. If membrane performance suffers greatly, then saving a little bit on membranes will not offset the costs in higher processing time.
   b) Decreasing the LM ratio increase the membrane performance and increases membrane costs. Increasing membrane performance may decrease the processing time at a small incremental membrane cost, therefore decreasing total cost.
   c) To find the optimal LM ratio:
      i) If the current trial was too fast with very high yield, increase the LM ratio by starting with a larger volume of starting material.
      ii) If the current trial was too slow or had a low yield, decrease the LM ratio by starting with a smaller volume of starting material.
3) The module used is an important optimization parameter. By changing the membrane chemistry or membrane type, optimized flux rates and passage may be found.
4) Using the same membrane, the shear rate can be optimized by increasing and decreasing the shear rate and measuring the effects on the membrane flux rate and passage. If an increase in the shear rate results in a relatively large increase in the flux rate, then the savings in membrane cost will offset the increased energy consumption.
   a) The concentration factor before starting diafiltration should also be optimized.
   b) The goal is to begin the diafiltration with the instantaneous passage of at least 50% to increase diafiltration efficiency.

After analysis of the data, select the best performing modules. The best performing modules will retain the cells and permit the desired media component to pass into the permeate with a high permeate flux for the isolation step. Additionally, the best performing concentration step module will concentrate the desired protein without losses into the permeate.

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Conclusion:

This *SmartFlow*™ filter *E. coli lysate by simultaneous process* WORKS™ Optimization Procedure provides guideline for optimizing the application of NCSRT's *SmartFlow* filters. Additional documents to assist you in developing an optimized separations protocol incluyde the SmartFlow worksheet and the NCSRT Scale Up calculator. To receive the complete application package, please request the *E. coli lysate by simultaneous process* WORKbook.

NCSRT's *SmartFlow* filter technology….It WORKS.

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