NCSRT

Works™ Optimization Procedure

Isolation of small molecules with *SmartFlow*™ TFF

The *SmartFlow*™ filter *WORKS*™ *Isolation of small molecules* optimization procedure from NCSRT is intended for separating a target small molecule such as a sugar or an amino acid from a fermentation or extraction process stream. The procedure is designed to have the small molecule pass freely through the membrane while cells, proteins, and large molecular weight process stream components are retained by the membrane.

This optimization procedure uses ultrafiltration membranes that retain the cells and allow the small molecules to pass freely through the membrane. The process can often be run at high flux rates by using a high MW UF membrane that retains the cells but still permits the free passage of the small broth components.

The passage characteristics of broth components change with different buffers, temperatures, concentrations, and membranes. By examining the passage characteristics of the different UF 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 a membrane module most likely to work with respect to polymer and pore size based upon thousands of NCSRT trials. Once this module is selected, 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.

To learn how others have applied the *SmartFlow* filter technology to similar separations, please review the *WORKS Isolation of small molecules* case study.

The *SmartFlow* filter *WORKS Isolation of small molecules* protocol provides a detailed instructions on how to execute the process under conditions that should provide >90% yield without a comprehensive optimization program. For many customers, this provides the opportunity to rapidly implement the *SmartFlow* technology and realize significant yield improvements over current operations and exceed their internal targets for the process step. Subsequent optimizations studies that use this *SmartFlow* filter *WORKS Isolation of small molecules* optimization procedure will result in further yield improvements and process efficiencies.

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Each parameter of the TFF process: product, membrane type, velocity, 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.

Module and System Selection:

1) Select the *SmartFlow*™ filter module to evaluate. The selection requires specifying a combination of membrane type, 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 sizes that will likely 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 and one half to one third the size of a molecule to be retained.
   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.
   a) Recommended starting membranes for concentrating whole cells are the regenerated cellulose (RC) series, Polysulfone (PS) or Polyethersulfone (PES). Use Table 1 to select a starting membrane.

Table 1. Recommended membranes for *Isolation of small molecules* procedure.

Target Molecule	Membrane polymer	Minimum Pore size
Protein < 5 kD	RC	30 kD
Protein > 5kD	PS	100kD
Carbohydrate ≤ 1 kD	RC	10 kD
Carbohydrate ≤ 1 kD	PES	10 kD
Carbohydrate (greater than 1 kD and less than 5 kD)	RC	30 kD
Organic Acids (i.e. Amino)	RC	10 kD
Organic Acid	PES	10 kD

3) After selecting the type of membrane, the variables needed to determine the module to buy are the membrane area and channel height.
4) Select the channel height for the module.
   a) For cell harvest a channel height between 0.75 and 1.0 mm is recommended.
   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) Cases to use a higher channel height include:

i) If cell aggregation is occurring, the lower height channels may clog. 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.
ii) In cases where concentration to high solids is desired, a channel height of 0.875 mm or above will be necessary.

5) 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 membrane capacity or LM ratio is defined as the volume of starting material divided by the membrane area.
   c) A typical LM ratio for a cell harvest will vary from 30 to 200 LM.
      i) The typical starting ratio is 100 LM. See below for additional information on optimizing the LM ratio.
      ii) If a fermentation broth is being concentrated, 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 the cases of fermentation broths containing antifoam, use a starting ratio of 60 LM.
   d) The minimum batch size is the system hold up volume times the concentration factor. The target concentration factor will affect the process yield, process time, diafiltration time, and buffer consumption.
      i) Minimum starting volume = Membrane test system hold-up volume X concentration factor.
      ii) Example: 500 mL hold up volume x 8X concentration = 4000 mL minimum starting volume.
   e) The membrane area needed is the batch size divided by the LM ratio.
6) Select the linear velocity.
   a) The typical velocity for the isolation of small molecules ranges from 75 cm/sec to 200cm/sec.
   b) The typical starting shear rate for a process development run is 100 cm/sec.
   c) The benefit of increasing the velocity may be an increase in target molecule passage.
   d) The disadvantages of increasing the velocity beyond the optimum are:
      i) Higher pump costs due to higher recirculation flow rates.
      ii) Higher pressure drops and TMPs which may decrease the passage of desired molecules. Therefore, an increase in the flow rate should be balanced by an increase in the small molecule passage for the process to retain the same overall efficiency. The energy costs of running the pump at a higher velocity must be offset by savings on membranes to make increasing the velocity efficient.
7) Calculate the flow rate needed to operate the selected module at the selected velocity using the *WORKS*™ 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.
8) Use Table 2 to determine the module(s) part numbers for ordering.

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.
2) After the rinse, direct the permeate line back to the retentate tank so no concentration occurs prior to

establishing the desired velocity and performing the transmembrane pressure (TMP) optimization 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 recirculation pump to the calculated rate from step 6 above.

Table 2: SmartFlow filter module part numbers

Module Size	Channel Height		Membrane polymer and pore size	
_____ -	____		____ -	_____
74 100 ft² Optisep 11000	D	0.5 mm	5B-0010	RC 10 kD
72 50 ft² Optisep 11000	E	0.75 mm	5B-0030	RC 30 kD
71 10 ft² Optisep 11000	G	0.875 mm	2B-0010	PES 10 kD
41 10ft² Optisep 7000	H	1 mm	1B-0100	PS 100 kD
40 5 ft² Optisep 7000	J	1.5 mm		
52 2 ft² Optisep 3000				
51 1 ft² Optisep 3000				

4) Optimize the TMP.

a) TMP is optimized by setting the back pressure in the retentate loop downstream of the filter module using a back pressure valve. While increasing the back pressure, maintain a constant retentate recirculation rate. It is normal to increase the pump speed in order to maintain the desired recirculation rate as the backpressure is increased.

b) Increase the TMP to the lowest operational value for the current membrane. This value can be found in Table 3.

c) Measure the permeate flow rate and the passage of the target molecule by taking a permeate sample. This permeate flow rate is the base rate. Record these values.

d) Increase the TMP by 3 PSIG (0.2 bar) and measure the permeate flow rate and passage of the desired small molecule. Record these values. Compare the permeate flow rate to the base rate or the previous permeate flow rate reading. If the rate has increased from the previous measurement go to step e, otherwise go to step g.

e) Wait three minutes and measure the permeate flow rate again. If the permeate rate has remained above the rate of the reading go to step f.

f) Repeat steps d and e until the permeate rate no longer increases with increasing pressure or does not hold that increase for three minutes or until the passage of the small molecule decreases.

g) Lower the TMP to the pressure that was measured before the permeate flow rate stopped increasing or to the pressure that still permitted high passage of the small molecule. This is the optimal TMP.

5) Remove the permeate line from the retentate tank and place back in the permeate vessel. Do not allow the permeate lines to contact the permeate fluid pool in the reservoir.

a) Take a retentate sample from the retentate tank and a permeate sample directly from the permeate hose simultaneously. Record the data on the Membrane Test Worksheet.

i) With each sample, record the permeate flow rate using a graduated cylinder, scale, or flow meter.

ii) It is critical to collect the data to be able to properly analyze the experimental results and develop an optimized procedure.

b) Concentrate the solution to be to the desired concentration factor.

i) Take a retentate sample from the retentate tank and a permeate sample directly from the permeate hose simultaneously at each concentration factor processed.
Note: for concentrations to a high concentration factor, not every concentration factor needs to be recorded.

ii) With each sample, record the permeate flow rate using a graduated cylinder, scale, or flow meter.

Table 3 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)	55 (3.0)	40 to 50
30 kDa	15 (1)	30 to 75 (2 to 5)	45 (3.0)	30 to 45
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 30 (0.7 to 2)	7.5 (0.5)	0
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

6) After the final concentration samples are taken, record the volume of liquid remaining in the system at this time. There are two alternative methods for determining the end of concentration system volume.
a) The system volume can be determined by subtracting the volume of the permeate and the volume of all of the samples taken from the starting volume.
b) *System Volume = Starting Volume – Permeate Volume – Retentate Sample Volume.*
c) If the system hold up volume is known and the volume in the retentate reservoir is known, adding these two values will produce the current system volume.

7) Diafiltration:
a) The following describes the procedure for diafiltering the product 3X.
b) Start to monitor the permeate volume with a graduated cylinder or scale.
c) To start the diafiltration, add 5 to 15% of the retentate volume calculated in step 6 to the retentate tank. Optional - Remove the permeate line from the first permeate collection tank to a second permeate collection tank. By doing this, the effect of the concentration can be isolated from the effect of the diafiltration.
d) When the permeate volume has increased by the volume added in step c, take a retentate sample from the retentate tank and a permeate sample directly from the permeate hose simultaneously. With each sample, record the permeate flow rate using a graduated cylinder, scale, or flow meter.
e) Continue to add buffer at a rate equal to the permeate rate in aliquots equal to between 5 and 15%

of the retentate volume calculated in step 6. Continue until 3 times the total volume of system recorded in step 6 has been added to the system.

f) Take samples from the permeate hose and retentate tank 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.)

g) For other diafiltration factors, continue the process until the amount of diafiltration buffer equals the 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 and a larger supply of buffer will be needed.

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 and the saving on buffer.

Data Analysis:

Sample Analysis:

1) Check the permeate samples for cells.
2) Calculate the membrane flux rate or LMH (L/m²/h) by dividing the measured permeate flow rate at each sample by the membrane area.

$$LMH = Permeate\ \ Flow\ \ Rate\ (mL/min) * \frac{1L}{1000\ mL} * \frac{60\ min}{1hr} \div Membrane\ \ Area\ (m^2)$$

3) Calculate the instantaneous small molecule percent passage by dividing the permeate small molecule content by the retentate small molecule content and multiplying by 100.
4) Record the data on the Membrane Test Worksheet.

Process Optimization:

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

1) An important parameter for cell harvest is the membrane capacity or LM ratio.
2) 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 membrane will not offset the costs in higher processing time.
3) Decreasing the LM ratio increases 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.
4) To find the optimal LM ratio:
   a) If the current trial was too fast and had high small molecule passage, increase the LM ratio by starting with a larger volume of starting material.
   b) If the current trial was too slow or had a low small molecule passage, decrease the LM ratio by starting with a smaller volume of starting material.

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5) The module used is an important optimization parameter. By changing the membrane chemistry or membrane type, optimized flux rates and passage may be found.
6) Using the same membrane, the velocity can be optimized by increasing and decreasing the flow rate and measuring the effects on the membrane flux rate and passage. If an increase in the velocity results in a relatively large increase in the passage, then the savings in membrane cost will offset the increased energy consumption.

After analysis of the data, select the best performing membrane. The best performing membrane will retain the cells, permit the desired small molecule to pass into the permeate, and have a high permeate flux.

Conclusion:

This *SmartFlow*™ filter *WORKS*™ *Isolation of small molecules* optimization procedure provides guideline for optimizing the application of NCSRT's *SmartFlow* filters. To see how others have applied the technology to their separations operations, please refer to the *WORKS Isolation of small molecules* case study. NCSRT has developed a simple step-by-step protocol that has been proven to deliver >90% product yield in the applications to which it has been applied. Along with the *WORKS Isolation of small molecules*, NCSRT has developed a Scale Up Component listing providing the part numbers and ordering information for the *SmartFlow* filter modules to execute the protocol at all process stages and volumes. To receive the complete application package, please request the *Isolation of small molecules WORK*book.

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

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