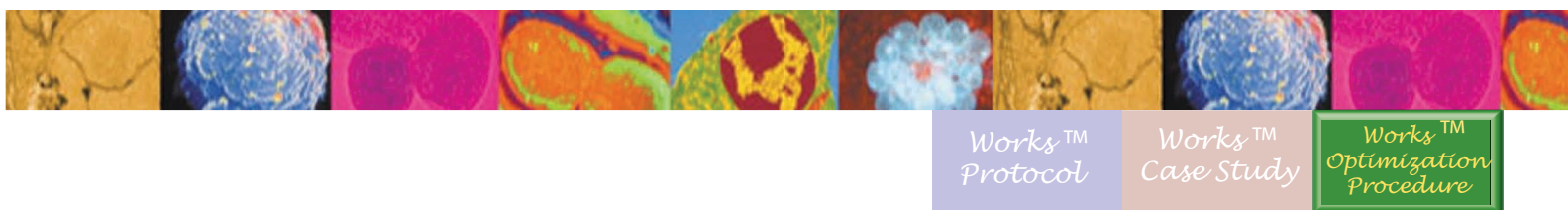# NCSRT

Purification of bacterial phage by continuous diafiltration with *SmartFlow™* TFF

The *SmartFlow™* filter $WORKS$™ *Purification of bacterial phage by continuous diafiltration* optimization procedure from NCSRT is intended to purify phage from heavier more viscous cell cultures where complete autolysis has occurred and/or where the phage is dimensionally larger such as 0.08 micron.

This optimization procedure uses a microfiltration (MF) membrane that retain the cells and cell debris and allow the bacterial phage to pass freely through the membrane. The passage characteristics of bacterial phages changes with different buffers, temperatures, concentrations, and membranes. By examining the passage characteristics of the different MF 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.

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Optimization
Procedure

Purification of bacterial phage by continuous diafiltration with *SmartFlow*™ TFF

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.

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 the velocity that governs the phage passage and efficiency. Care must be taken in selecting and maintaining the velocity at the membrane surface.
   c) The membrane area also affects the pump size required to achieve the necessary velocity for a given separation.
2) Select the first membrane to test.
   a) Recommended starting membranes for isolation of the bacterial phage are the 0.2 µm and 0.45 µm modified polysulfone (MPS) membranes.
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 the isolation of bacterial phage, a channel height between 0.75 and 0.875 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 a 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 high solids are 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 the isolation of bacterial phage will vary from 25 to 120 LM.
      i) The typical starting ratio is 60 LM. See below for additional information on optimizing the LM ratio.
      ii) However, 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 30 LM.
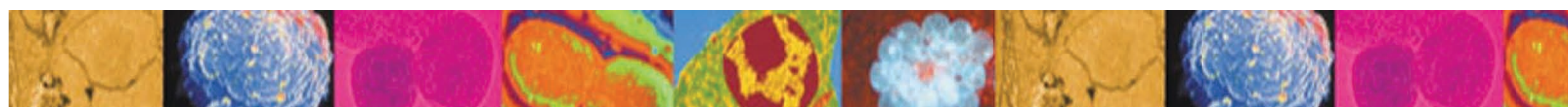
Purification of bacterial phage by continuous diafiltration with *SmartFlow™* TFF

        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 hold up volume.

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

6)   Select the velocity.

    a) The typical velocity for the *Purification of bacterial phage by continuous diafiltration* ranges from 50 cm/sec to 175 cm/sec.

    b) The typical starting velocity for a process development run is 100cm/sec.

    c) The benefit of increasing the velocity is an increased phage passage.

    d) The disadvantages of increasing the velocity beyond the optimum are:

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

        ii) Higher flow rates and TMPs which may decrease the passage of the bacterial phage. Therefore, an increase in the velocity should be balanced by an increase in the phage passage for the process to retain the same overall efficiency. The energy costs of running the pump at a higher flow rate 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 1 to determine the module(s) part numbers for ordering.

Table 1: *SmartFlow™* filter module part numbers

Module Size		Channel Height		Membrane polymer and pore size	
74	100 ft² Optisep 11000	D	0.5 mm	1N-9045	MPS 0.45 μm
72	50 ft² Optisep 11000	E	0.75 mm	1N-9020	MPS 0.2 μm
71	10 ft² Optisep 11000	G	0.875 mm		
41	10ft² Optisep 7000	H	1 mm		
40	5 ft² Optisep 7000	J	1.5 mm		
52	2 ft² Optisep 3000				
51	1 ft² Optisep 3000				

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 shear rate 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 7 above.

4)   Start with the backpressure at zero. The inlet pressure should be at least 5 psig (0.3 bar) and remain below 10 psig (0.7 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

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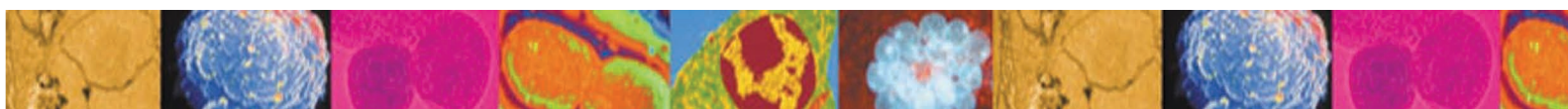
Purification of bacterial phage by continuous diafiltration with *SmartFlow™* TFF

        5 psig, then slight backpressure can be added until the inlet pressure increases to 5 psig. See Table 2 for typical TMP vranges for different membranes.

5)   Diafiltration – the following describes the procedure for diafiltering the product 5x:

    a) Start to monitor the permeate volume with a graduated cylinder or scale.

    b) To start the diafiltration, add 5 to 15% of the starting retentate volume to the retentate tank.

    c) When the permeate volume has increased by the volume added in step ii, 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.

    d) Continue to add buffer at a rate equal to the permeate rate in aliquots equal to between 5 and 15% of starting retentate value. Continue until 5 times the total starting volume has been added to the system.

    e) 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.).

    f) For other diafiltration factors, continue the process until the amount of diafiltration buffer equals the number of the desired diafiltration factor times the starting volume.

      i) The theoretical recovery from a 5X diafiltration for a molecule with a 100% passage is 99%.

      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 anlaysis:

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 phage percent passage by dividing the permeate phage content by the retentate phage content and multiplying by 100.

    a. The theorectical case will see the passage decrease as the experiment continues.

    b. Another common result is the passage increasing to a maximum between the second and third diafiltration volumes.

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)   The important variables to optimize are the yield, membrane passage, and membrane flux rate.

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Procedure
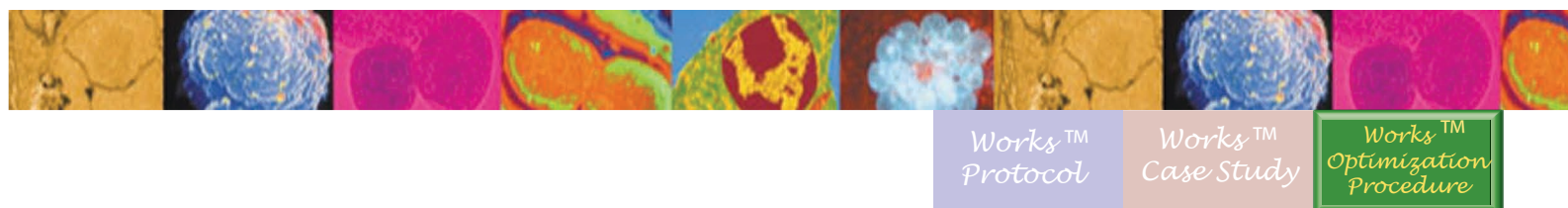
Purification of bacterial phage by continuous diafiltration with *SmartFlow*™ TFF

2)   An important parameter that effects the yield, passage, and flux rate for cell harvest is the membrane capacity or LM ratio.

3)   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.

4)   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.

5)   To find the optimal LM ratio:
   a) If the current trial was too fast with very high yield, 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 yield, decrease the LM ratio by starting with a smaller volume of starting material.

6)   The module used is an important optimization parameter. By changing the membrane chemistry or membrane type, optimized flux rates and passage may be found.

7)   Using the same membrane, the velocity can be optimized by increasing and decreasing the flow rate and measuring the effects on the membrane 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 bacteria phage to pass into the permeate and have a high permeate flux.

Table 2 Typical TMP Pressures for *SmartFlow*™ filter 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 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
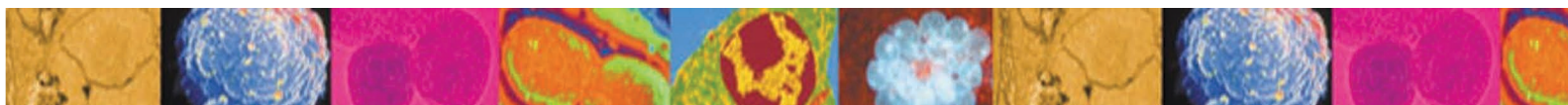
Purification of bacterial phage by continuous diafiltration with *SmartFlow™* TFF

Conclusion:

This *SmartFlow™* filter *WORKS™ Purification of bacterial phage by continuous diafiltration* optimization procedure provides guideline for optimizing the application of NCSRT's *SmartFlow* filters. To receive the complete application package, please request the *Purification of bacterial phage by continuous diafiltration WORK*book.

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

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