

Clarification and Recovery of Recombinant Proteins using a Cascade Tangential Flow Filtration (TFF) System

Introduction

A cascade TFF system is defined as a two stage TFF system. The first stage clarifies the feed stock using a microfiltration (MF) membrane while the second stage concentrates the permeate from the first stage using an ultrafiltration (UF) membrane. The permeate from the second stage is recycled for use as the diafiltration medium for the first stage. The product of interest is in the retentate from the second stage.

Cascading TFF systems are extremely effective for clarification and recovery of recombinant proteins from microbial fermentation (lysates/whole cell suspensions) and cell cultures. Recovery of recombinant proteins from solutions with high concentration of particulate matter (up to 30% solids) is ideal for a cascade TFF system. Normal flow filtration (NFF) methods are impractical for processing fluids with high concentration of suspended solids. Larger surface areas are required to remove the high quantity of solids and these filters may also plug or the solids may even break through. While centrifuges can be employed for primary clarification, additional secondary clarification may be required before the protein is concentrated using ultrafiltration.

The key process parameters considered when designing the cascade system include:

1. Cross flow rate (L/m^2h) for both the MF and the UF stages
2. Permeate flux for the first stage (L/m^2h)
3. Diafiltration: by controlling the permeate flow rate from the second stage UF concentrate step to be equal to the permeate flow rate from the MF clarification step
4. Membrane pore size rating and feed loading for the MF stage (L/m^2)

These parameters are derived through process optimization.



Advantages of a Cascade TFF System

A cascade TFF system has several advantages. The system is robust and scalable, yielding extremely high final protein recovery without significant buffer consumption. Tank requirements for buffer storage or permeate collection are also significantly reduced, thus saving valuable manufacturing space. In addition, the final recovered product from a well-designed cascade TFF system has low turbidity and will require less surface area for downstream sterile filtration, thus making the overall protein recovery process more economical.

- High product recovery
- Minimal buffer consumption
- Scalable and robust
- Low turbidity of final, recovered product

Protein Expression Systems

Microbial fermentation

The process of fermentation occurs when microorganisms obtain energy through the breakdown of glucose. The gene that produces the recombinant protein of interest is introduced into the microbe, and the protein of interest is produced or expressed during fermentation. Bacteria (such as *E. coli*) and yeasts are some of the commonly used microbes. *E. coli* has been an attractive expression system because of its low expression time and established regulatory track record. *E. coli* is easy to grow and has well-understood genetics. However, most of the proteins expressed in *E. coli* are intracellular requiring cell lysis for protein recovery. Yeasts also have well-understood genetics. Some advantages of yeasts include no endotoxin production, and high expression levels of secreted proteins, which are typically properly folded proteins.

Cell Culture

Cell culture involves taking cells from living organisms and growing them under controlled conditions. Several types of cells are used for protein expression, such as insect and mammalian. Typically, the protein of interest is secreted.

Cascade TFF systems are also used successfully for the clarification and protein recovery from other more recent expression systems, such as transgenic animals and plants.

TFF Process Design

Stage One – the MF Clarification Step

The first stage in the cascade TFF system is clarification. Microfiltration membranes such as 0.1 or 0.2 micron PVDF Durapore® are used for this step in the Pellicon® 2 V screen or Prostat™ MF open-channel format. Very open ultrafiltration membranes like Biomax® 1000 kDa are also used for clarification applications.

Stage Two – the UF Concentration Step

For the second stage ultrafiltration step, the rule of thumb is to select a membrane with a nominal molecular weight limit (NMWL) 0.2 to 0.3 times the molecular weight of the target protein. For example, an Ultracel® 5 kDa membrane is typically used for the UF step to concentrate and recover 15–30 kDa proteins.

Operating the MF and UF steps requires two pumps:

1. Feed pump
2. Permeate pump for flux control

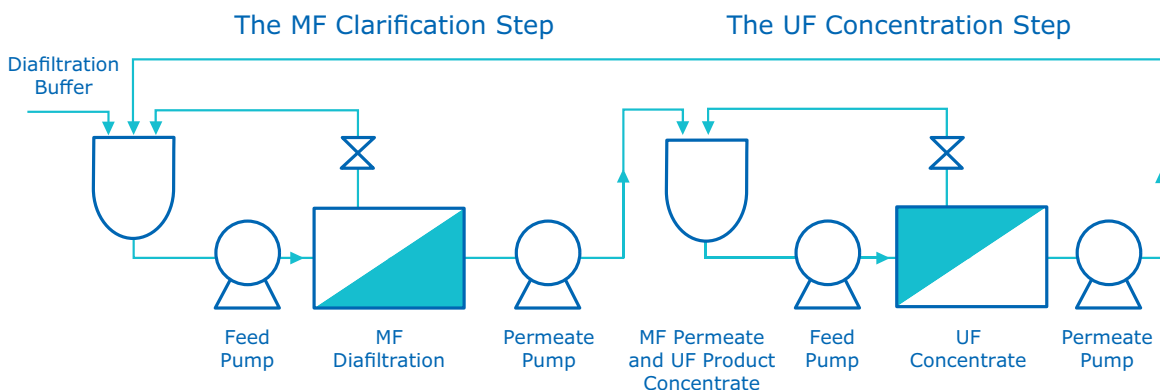
It is essential to optimize the cross flow rate and the transmembrane pressure. A successful cascade system has the same permeate flow rate from both the MF and UF steps.

A Typical Cascade TFF System

A Cascade TFF system is illustrated in Figure 1.

1. The MF feed pump recirculates the feed across the first membrane. Due to the high solids in the feed, diafiltration is typically required at the beginning of the process. Permeate flux through the MF membrane is controlled at previously optimized conditions using a permeate pump.
2. About 1–2X diafiltration is performed to generate enough permeate to start the subsequent concentration step. The permeate from the MF clarification step is then fed using a second feed pump across the UF membrane.
3. The permeate from the UF concentration step is used as the diafiltration medium for the first stage clarification step. Permeate flow rate from the UF step is controlled to maintain a constant feed volume in both the feed vessels, i.e., the permeate from the MF step has the same flow rate as the permeate from the UF step.
4. Experiments are conducted to determine the number of diafiltration volumes necessary to achieve the desired protein recovery.

Figure 1. A Typical Cascade TFF System



Typical processing parameters presented in **Table 1**.

Table 1. Typical Processing Parameter

	Microfiltration/ Clarification	Ultrafiltration/ Concentration
Cross flow for Pellicon® 2 device (L/m ² h)	360-720	240-480
Cross flow for Prostat™ MF device (L/min/channel)	1-4	N/A
Loading (L/m ²)	20-100 (depending on whether the feed is a microbial lysate or whole cells)	50-150 (membrane area is determined by the permeate flux required to maintain the same flow rate as the MF step)
Final TMP (psi)	<5	Determined by the permeate flow rate
ΔP (psi)	<5	10-15
Permeate flux (L/m ² h)	10-15	Flow rate to be the same as the microfiltration step
Permeate pump	Yes	Yes

Predicting Protein Recovery

The following formula can be used (assuming complete protein retention in the UF step) to predict protein recovery in the MF permeate after a given number of diafiltrations in the clarification step.

$$\text{Product in the MF permeate (\%)} = 100 * \{1 - (\exp [(-S) * (\ln VCF + N)])\}$$

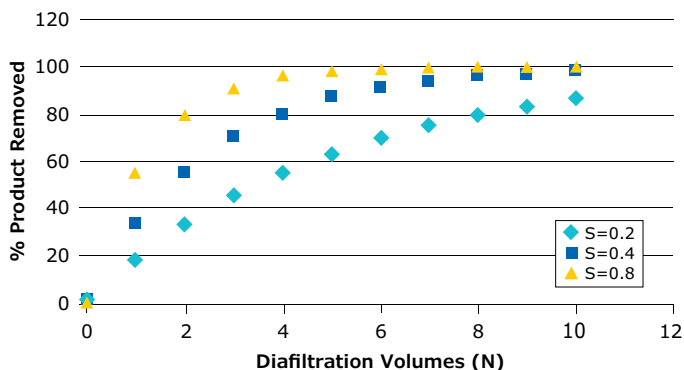
N = the number of diavolumes using the permeate from the UF concentration step as the diafiltration medium

S = is the protein sieving (the fraction of the protein that passes through the membrane into the filtrate stream) in the MF clarification step

VCF = the volumetric concentration factor for the MF clarification step

For example, the following graph shows the product in the MF permeate/UF retentate as a function of the number of diavolumes for different S values. VCF = 1 for this example, i.e., the diafiltration is performed right from the beginning for the clarification step.

Figure 2. Product Recovery in UF Retentate as a Function of N for the MF Step



Conclusion

A cascade TFF system is a two-stage process including a clarification step using a microfiltration membrane followed by an ultrafiltration step to concentrate the clarified permeate. The permeate from the UF step is recycled and used as diafiltration medium to enhance protein recovery from the clarification step. This format minimizes diafiltration buffer usage in spite of carrying out a large number of diafiltrations.

Typically, 1X diafiltration is required to be able to start the ultrafiltration (or concentration) process. Once the concentration is started, the permeate flow rates from the clarification and concentration steps are maintained equal.

A cascade TFF system can be successfully used for maximum protein recovery from solutions containing high concentrations of suspended solids (e.g., microbial fermentation lysates, whole cell suspensions, etc.), where NFF may not be a feasible alternative.

The main advantages of a cascade TFF system include:

- Improved clarity (low turbidity) for the final recovered product
- Reduced sterile filter area sizing
- Better column lives for the downstream chromatographic steps
- Higher protein recoveries
- Proven reduction in diafiltration buffer usage
- Scalability and robustness
- Reduced manufacturing space requirements

References

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8. "Protein Concentration and Diafiltration by Tangential Flow Filtration". Technical Brief TB032, Millipore Corporation.

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