Single-Pass Tangential Flow Filtration

Introduction

Tangential flow filtration (TFF) is widely used in the biopharmaceutical industry for downstream processing applications. Typical TFF steps concentrate product through volume reduction, and buffer exchange through diafiltration, to achieve high yields. TFF typically utilizes ultrafiltration membranes ranging from 1 – 1000 kD nominal molecular weight limit (NMWL) to retain different size molecules. Traditional TFF requires multiple passes through a system, using a pump to drive feed through a filter and sending the retentate back to a tank for another pass through the system.

Single-pass TFF runs at constant operating conditions throughout the process, simplifies the required hardware, allows higher concentration factors and higher product recovery without significant dilution by reducing hold-up volume, and reduces the risk of product damage associated with recirculating TFF operations. Single-pass TFF is also a continuous process and can be run together with another step. This is convenient to reduce volumes to eliminate tank bottlenecks and reduce column sizes, especially in existing facilities where space may be limited.

Principles of single-pass TFF

Traditional TFF operates in batch mode, where the feed/retentate is recirculated through the filter assembly (Figure 1A). Typically, TFF cassettes operate in parallel, with multiple passes through membranes required to achieve the desired concentration.

Our single-pass TFF is a different application of an existing technology (Figure 1B). Single-pass TFF uses existing Pellicon® 2 or Pellicon® 3 cassettes with standard holders. The TFF step is sufficiently concentrated after a single-pass through the filter assembly such that retentate recycle is not required.

The basic single-pass TFF underlying principle is that increased residence time in the feed channel results in increased conversion. Increased residence time can be accomplished by reducing flow rate or increasing path length in a serial configuration. Configuring TFF cassettes in series can improve conversion. Cassettes in series have a higher mass transfer when compared to parallel configurations at equivalent residence times (Figures 1C, 1D).

Figure 1. Compared to traditional TFF configuration (A), a single-pass TFF configuration (B) does not recycle the retentate. Conversion increases with residence time with a serial feed channel configuration (C), offering a slight advantage over a parallel configuration (D) due to higher flow velocities and mass transfer. Permeate is not shown in 1D.
Applications of single-pass TFF

Single-pass TFF has several applications, including:

- **Product concentration/volume reduction:** single-pass TFF can be used in between other unit operations to reduce intermediate pool volumes. In turn, this can de-bottleneck a process limited by tank volumes and/or reduce column sizes and/or number of cycles required for downstream chromatography (Figure 2A).

- **In-line dilutions/de-salting:** single-pass TFF can be used for in-line desalting before ion exchange chromatography, membrane adsorbers or virus prefilters without expanding the pool volume by dilution (Figure 2B).

- **Final formulation/concentration (post-batch UF/DF):** single-pass TFF reduces working volume limitations compared to traditional TFF, allowing the process to achieve higher final concentrations and minimizing post-use recovery dilution (Figure 2C).

**Figure 2.** Applications for single-pass TFF setup include product concentration and volume reduction (A), in-line dilutions and de-salting (B), and final concentration (C).

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Evaluation of single-pass TFF at small scale

Bench scale experiments using single-pass TFF are typically performed with three identical Pellicon® cassettes in conventional mini holder(s) (Figure 3A). Specially designed single-pass TFF diverter plates are used to allow the three cassettes to be configured in series within a single holder (Figure 3B and Figure 3C). This experimental setup allows the operator to evaluate the performance of one-, two- and three-section single-pass TFF processes at the same time. Alternatively, three Pellicon® mini holders can be configured in series.

Trials typically require 1 – 2 L of feed material and 4 – 6 hours of run time. The experiment is run in either total recycle mode or as a true single pass, depending on the volume of feed available. If the experiment is run in total recycle mode, a well-mixed tank is required to ensure proper mixing of the concentrated retentate with recycled permeate.

Single-pass TFF trials require measurement of feed and retentate pressures and retentate and permeate flow rates, at varying feed flow rates. Typically, the trial starts at feed flux rates of 1 L/min/m², and is stepped down to near 0.1 L/min/m², until the desired target conversion is reached or a gel point is reached and the product cannot be further concentrated. Each feed flow point is stabilized for 5 – 10 minutes prior to taking measurements. Longer times are required at lower flow rates to allow for displacement of channel volumes.

**Figure 3A.** Bench-scale setup for single-pass TFF system.

**Figure 3B.** Bench-scale setup for single-pass TFF system using diverter plates.

**Figure 3C.** A diverter plate for bench-scale single-pass TFF setup.
It is convenient to plot the experimentally obtained conversion $Y = Q_{p,\text{total}}/Q_F$ vs the feed flux $J_F$. For any number of sections in series, one uses the total permeate in calculating $Y$ and the total area in calculating $J_F$.

$$Y_n = \frac{\sum_{i=1}^{n} Q_i}{Q_F}$$

Where $Y_n$ is the conversion for an $n$-section SPTFF process, $Q_i$ is the permeate flow measured for sections 1 through $i$, and $Q_F$ is the total feed flow into the system measured as inlet or sum of retentate and permeate. The volume reduction factor (VRF) is defined as:

$$\text{VRF} = \frac{1}{1-Y}$$

By conservation of mass, the steady-state retentate concentration is expressed as:

$$C_{\text{ret}} = C_{\text{Feed}} \cdot \text{VRF}^R$$

Evaluation of single-pass TFF with model feed of 20 g/L and 75 g/L demonstrates how the conversion increases with decreasing feed flow rate, and increases with the number of sections in series (Figure 4A). Measured retentate concentrations confirm predicted concentrations using the mass balance (Figure 4B).

**Cleaning in single-pass TFF**

Cleaning of single-pass TFF systems can be performed in single-pass mode without a recirculation loop, using typical cleaning agents (Figure 5). Cleaning does not require different pump or piping.
Scaling up single-pass TFF

Scale-up uses existing holders and cassettes, including Pellicon® cassettes that are commercially available, and requires no additional customized equipment.

To scale up single-pass TFF, first define the feed flow (equals batch volume divided by the desired process time) and target conversion or concentration. Figure 6 shows an example in which conversion greater than 52% was required, with a feed flow of 25 L/min. Using the data in Figure 6, a 52% conversion (yellow line) gives feed flux values of 0.6 L/min/m² for one-section, 0.7 L/min/m² for two-section, and 0.8 L/min/m² for three-section single-pass TFF.

Next, define the required membrane area by dividing the desired feed flow rate by the feed flux. In the Figure 6 example, the calculation for a two-section process is: 25 L/min ÷ 0.7 L/min/m² = 36 m² total area. This corresponds to a two-section process with 18 m² area per section.

Case study at manufacturing scale

An example of a single-pass TFF application for an existing manufacturing facility, where improved cell culture titers placed increased demand on downstream operations and tank capacities, was reported by Teske et. al. (Biotechnol. Prog. 26(4):1068-72). In this process, the product pool following virus filtration exceeded the available tank capacity by 14%. The use of 4 m² of cassettes in a single section met the 20 L/min flow and volume reduction requirements.

Summary

Use of Pellicon® cassettes in a single-pass tangential flow filtration mode is an effective way of concentrating biomolecules. Small-scale evaluations are simple to execute and analyze. The power of implementing single-pass TFF lies in its ability to reduce in-process volumes in a simple and easy-to-use step. Single-pass TFF enables higher concentration formulations and allows facilities to meet the demands of higher titer processes without major investments in new equipment.

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