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White Paper

Single-pass tangential flow filtration, a versatile application to streamline biomanufacturing

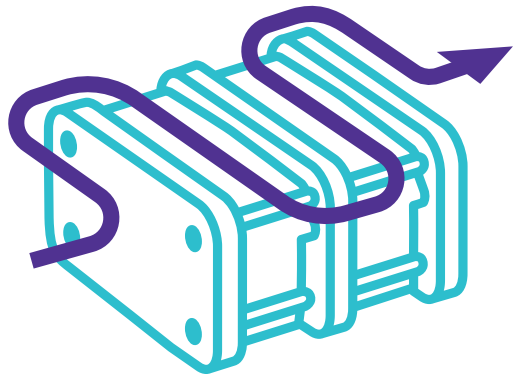
Current trends in the biopharmaceutical industry are driving the implementation of high capacity processes capable of reducing costs and footprint without sacrificing manufacturing efficiency and product quality. A step in the biomanufacturing process that enables such convenience is ultrafiltration with single-pass tangential flow filtration (single-pass TFF).

Single-pass TFF is an enhanced approach to the existing TFF technology that has been used for the purification of monoclonal antibodies, therapeutic proteins, and vaccines at different steps within downstream processing. In this mode of TFF, the feed solution is pumped once through a set of filters arranged in series to produce permeate and concentrated retentate. The once-through flow path of single-pass TFF offers the advantages of reduced footprint, shear exposure, and working and hold-up volumes, which in turn lead to higher plant productivity.

Processing challenges addressed with single-pass TFF include:

- pool volumes increased beyond tank capacities
- limited plant floor space
- unachievable target concentrations due to high dilutions
- product loss due to hold-up volume constraints

Integration of single-pass TFF into a biomanufacturing process is easily accomplished with existing Pellicon® cassettes. Merck's extensive variety of proven Pellicon® cassettes offers user-configurable single-pass TFF capabilities and applicability to a wide range of molecules and conversion targets.



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What is single-pass TFF

Traditional TFF operates in batch mode by recirculating the feed/retentate through filters assembled in parallel until the desired conversion is achieved (**Figure 1A**; termed hereafter batch TFF[1]). Single-pass TFF is a different approach to this filtration process. This method utilizes a longer feed channel path to reach the desired conversion after one pump pass (**Figure 1B**). Absence of a recirculation loop allows the retentate to flow downstream and thus, reduce hold-up volumes, eliminate pool tank requirements, and enable continuous processing.

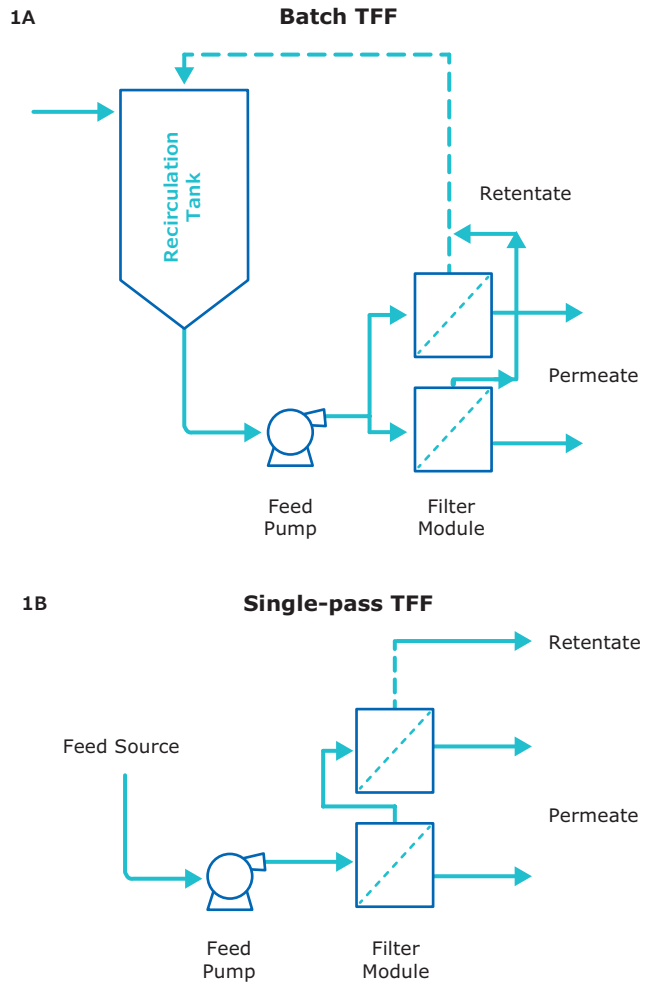


Figure 1. Compared to the traditional parallel configuration of batch TFF (A), single-pass TFF (B) produces concentrated retentate after one pass through filters in series, eliminating the space-limiting recirculation loop.

In single-pass TFF, product conversion is improved by increased feed residence time on the membrane. Cassettes configured in series have a longer feed channel flow path than that of cassettes in parallel with the same total membrane area. This provides the feed with a longer residence time for more efficient permeation, enabling high concentration factors (>10×) to be achieved after a single pump pass.

The feed residence time is also controlled with a steady, low feed flow rate (approximately 1/10th that of batch TFF). For example, a feed volume of 0.3 L/m^2 running at a feed flow rate of 0.5 L/min/m^2 would yield a residence time of approximately 35 seconds. At lower feed flow rates (<0.5 L/min/m^2) the residence time would be greater and correspondingly result in higher retentate concentration after one pass (**Figure 2**).

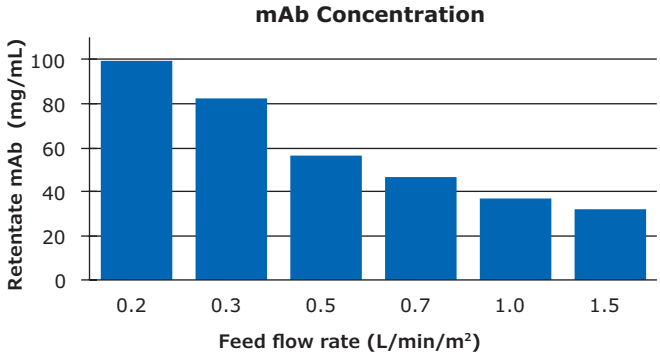


Figure 2. At low feed flow rate, the residence time of the feed increases, resulting in higher retentate concentration.

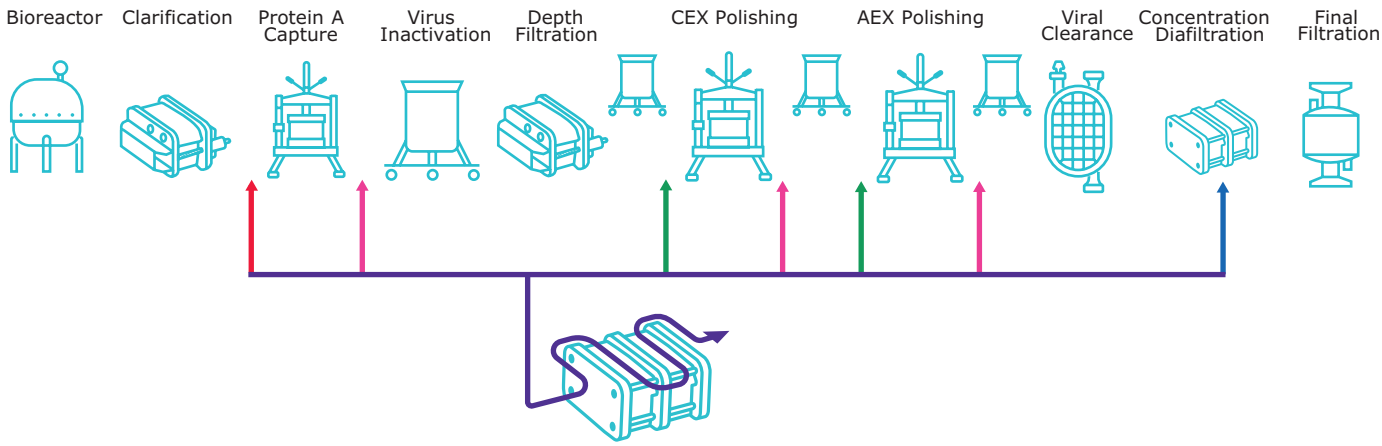
Industry adoption

Single-pass TFF has historically found utility in whey protein manufacturing, water purification, and gas separation processes.[2-6] Although single-pass TFF has been known for some decades in these industries, this alternative approach to batch TFF has just recently generated interest within the biopharmaceutical industry to enable downstream platforms capable of handling increasing market demands.[7] However, while single-pass TFF can bring significant benefits to the bioprocessing community, its widespread adoption has been slower than expected due to mischaracterizations as a commercially restricted technology.[8]

Integration of single-pass TFF in the manufacturing of biologicals does not require custom, limited modules nor is it restricted to a single vendor. Single-pass TFF can be easily configured using Pellicon® cassettes as recommended by Merck.[9,10] The broad range of existing and proven Pellicon® 2 and Pellicon® 3 cassettes, available in a variety of membrane materials, pore sizes, and feed screens, offers flexibility to the end user, allowing tailored assembly of precise filter modules that address both molecule and process needs. The following sections will describe single-pass TFF with Pellicon® cassettes, highlighting wide and versatile applicability, ease-of-use, and benefits in biomanufacturing.

Versatility in bioprocessing

In the manufacturing of biologics, single-pass TFF enables inline volume reduction to reduce tank requirements, reduce size of subsequent steps, concentrate with inline buffer addition for desalting, and facilitate high final concentrations with high product recovery. The small footprint of single-pass TFF is ideal to easily accommodate an inline concentration step anywhere within a biomanufacturing process where volume reduction is needed, including before or after a column chromatography step or at final concentration and formulation (**Figure 3**).



- **Intensified capture:** Load faster; concentrate low titer-high volume product pools to improve downstream productivity
- **In-process volume reduction:** Concentrate expanded product volumes to overcome pool tank constraints
- **Intensified polishing:** Improve column productivity and process economics with intensified polishing
- **Final ultrafiltration:** Achieve higher final concentrations and higher yields

Figure 3. Single-pass TFF can be integrated anywhere within a biomanufacturing process to address volume challenges.

Before chromatography

Inline concentration before column chromatography enables faster loading and increased column capacity for improved plant run rate and productivity (**Figure 4A**). Pre-protein A single-pass TFF is ideal to process large volumes of molecules that have low titers in order to reduce column loading time and debottleneck the manufacturing process when post-harvest tank size is limiting.[11]

Moreover, single-pass TFF before ion exchange chromatography enhances the efficiency of the polishing step.[12] Intensified polishing offers reduced dilution requirements to adjust pH and conductivity, which ultimately increases column productivity and reduces the use of consumables, contributing to improved process economics.

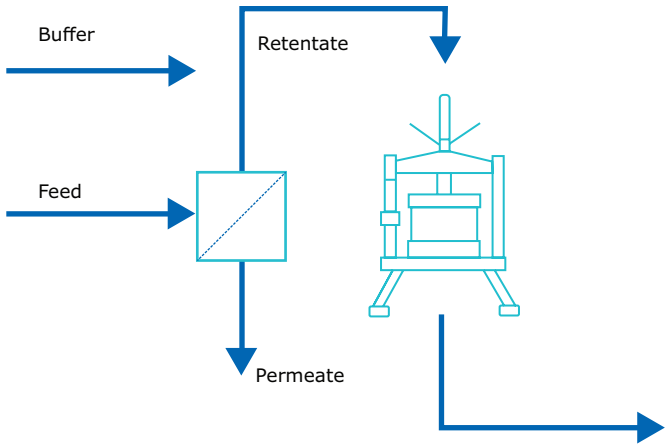


Figure 4A. Before Column

After chromatography

Concentration of in-process product pools is suitable to eliminate volume constraints that emerge from substantial volume expansion after a column chromatography step or sequence of steps (**Figure 4B**). High dilutions required to desalt product pools can result in volumes increased beyond tank capacities, requiring product to be discarded in order to properly condition. In such cases, single-pass TFF is ideal to reduce volumes to tank capacity to debottleneck a process and maximize product mass recovery.[7]

Although upgrading equipment may seem a direct solution, single-pass TFF is an immediate solution that, in comparison, requires little capital investment and space. More so, single-pass TFF improves facility fit by eliminating intermediate pool tank requirements and reducing size of subsequent steps.

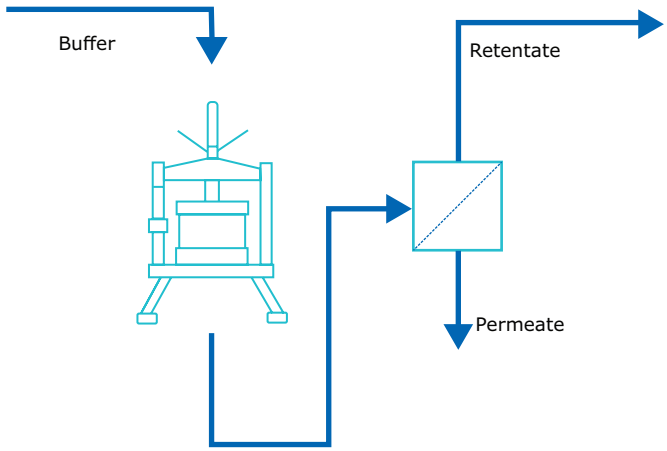


Figure 4B. After Column

Final concentration

For high-value final products, single-pass TFF is used to maximize product recovery due to low working volumes (**Figure 4C**). The smaller footprint of single-pass TFF offers lower hold-up volumes per square meter of membrane compared to batch TFF. This reduces recovery flush volumes and minimum working volume constraints.

Furthermore, higher concentration formulations are achieved during final ultrafiltration with single-pass TFF as it minimizes recovery dilutions, a capability that is ideal for highly viscous injection formulations.[13]

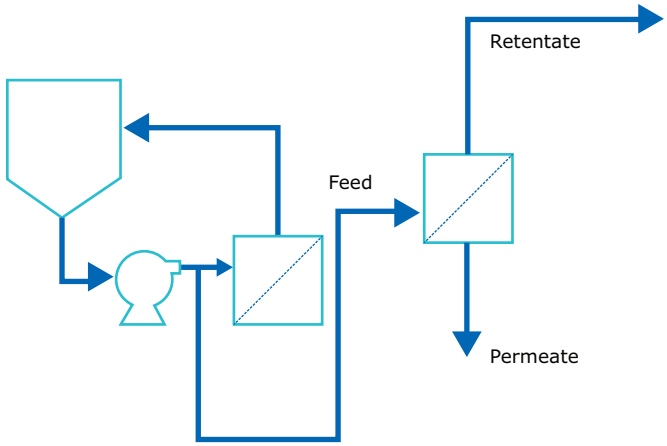


Figure 4C. Final Concentration

Easy module assembly

Existing, proven Pellicon® components can be quickly and easily configured by the end user to generate a serial flow path. Two main configurations are recommended with Pellicon® cassettes: cassettes in series within one holder and cassettes stacked in parallel within holders connected in series.

To generate a serial feed flow path within one holder as shown in **Figure 5**, specially designed diverter plates[14] are inserted between individual Pellicon® cassettes of equal membrane area. These separator plates seal the retentate ports to allow the retentate of one cassette to serve as the feed for the next. This setup can be arranged with Pellicon® 2 or Pellicon® 3 cassettes and available Pellicon® components listed in **Table 1**.

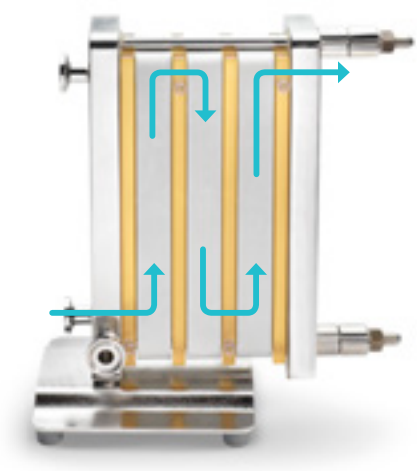


Figure 5. Single-pass TFF setup with three cassettes configured in a 1:1:1 series by use of diverter plates.

Table 1. Pellicon® components used to configure cassettes for single-pass TFF within one holder.

Accessories and Hardware
For Pellicon® 2 0.1 m², Pellicon® 3 88 cm², Pellicon® 3 0.11 m²
Diverter plates and silicone gaskets, mini kit
Stainless steel holder (mini) with extended tie rods
For Pellicon® 2 0.5 m², Pellicon® 3 0.57 m², Pellicon® 3 1.14 m²
Diverter plate
Retentate collection plate
Stainless steel holder (standard)

Alternatively, Pellicon® holders can be serialized by successively connecting a common feed/retentate pipe between holders (**Figure 6**).[15, 16] This assembly does not require diverter plates and allows for multiple cassettes, any Pellicon® 2 or Pellicon® 3 cassettes, to be stacked in parallel within each holder for high capacity processes. The cassettes are equally distributed for equal membrane area per section.

Because single-pass TFF with Pellicon® cassettes is user-configurable, the end user has operational flexibility[17] to assemble a precise module that addresses both molecule and process needs without any customization requirements, in contrast to approaches[18] that call for fixed modules with limited selection of membrane materials and configurations.

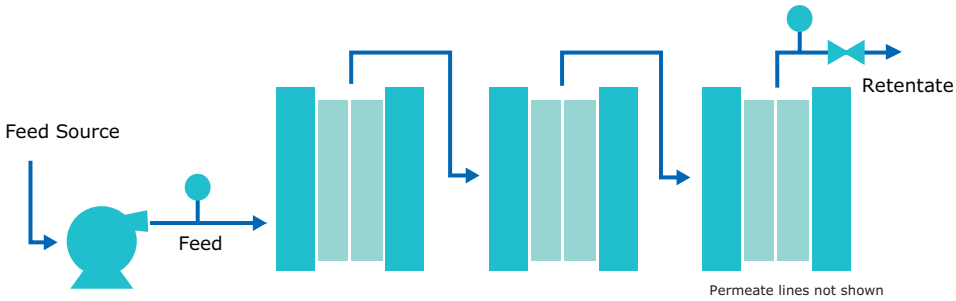


Figure 6. Single-pass TFF setup with three holders in series housing two cassettes each, corresponding to a 2:2:2 configuration.

Cassette selection

Pellicon® 2 and Pellicon® 3 cassettes can be configured by the end user to run a single-pass TFF process. The Pellicon® family of cassettes offers devices with regenerated cellulose Ultracel® or polyethersulfone-based Biomax® membrane in several surface areas and nominal molecular weight cut-offs, and with a range of feed channel screen options to suit different application challenges. **Table 2** summarizes the wide range of options within the families of Pellicon® cassettes available for single-pass TFF use.

Table 2. Summary of available options within the Pellicon® family of cassettes.

	Range of available options			
Cassette family	**Membrane type**	**Membrane area (m²)**	**Membrane cut-off**	**Feed screens***
Pellicon® 2	Biomax® Ultracel®	0.1-2.5	1-1000 kDa	A, C, V
Pellicon® 3	Biomax® Ultracel®	0.088-1.14	3-50 kDa	A, C, D

*Each feed screen is available for select membrane areas and cut-offs. For specific cassette options, consult the corresponding Datasheet.

This comprehensive selection of cassettes enables bench-to-process scale filtration of solutions containing monoclonal antibodies, recombinant and non-recombinant proteins, albumin, hormones, vaccines, growth factors, and viruses. Importantly, because Pellicon® cassettes have been extensively used for batch TFF and thus have proven performance for high product yield, purity, and quality, the transition into single-pass TFF with established cassettes offers greater reliability than approaches requiring new customized modules.

Flexible User Configuration

User-configurable single-pass TFF modules with Pellicon® cassettes enable unique capabilities, including

- fast evaluation of optimum feed flow rate and module configuration over a wide range of conversion targets
- precise linear scale-up/down by leveraging existing, scalable Pellicon® cassettes available in several sizes
- easy implementation of a flexible and efficient cleaning method, specially developed for single-pass TFF

Process development trial

Controlling the feed residence time is critical to reach the desired conversion. Increased residence time is achieved with a low feed flow rate and increased path length using a serial configuration. To determine the required feed flow rate and number of sections in series, the performance of multiple sections at varying feed flow rates is evaluated in a bench-scale trial.[9] The Pellicon® single-pass TFF mini kit (**Table 1**) is ideally suited for start-up trials in order to simultaneously evaluate the conversion profile of one, two, and three sections in series.

The single-pass TFF process development trial offers flexibility with designing an assembly tailored for any given process and target conversion. The trial simply requires measurement of the permeate flux of each section at varying feed flow rates. The necessary data can be gathered with little feed volume (~0.5 L) at the anticipated initial concentration in just a few hours of run time. Once the feed flow rate excursion graph is generated, the user may determine the required feed flow rate and number of sections according to the desired conversion (**Figure 7**).

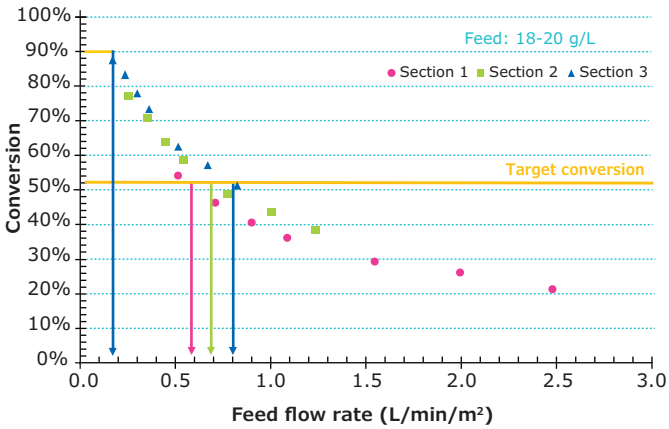


Figure 7. Using the feed flux excursion graph to determine the required feed flow rate for a one-section assembly (●), two-section assembly (■), or three-section assembly (▲) according to the desired conversion (—).

It is worth noting the user may choose any number of sections (1 to 3, or more); however, a reduction in conversion return is observed with each added section. The first section contributes the most to the conversion (>50%), and typically, the second and third sections amount the remaining conversion (~40%) to reach the target value. Because each in-series section after three sections contributes minimally to conversion, three sections are typically used to achieve an optimum tradeoff between conversion and membrane use.

Scaling

Scale-up of single-pass TFF is straightforward with Pellicon® cassettes. The total membrane area is proportionally increased according to the changing feed volume, while maintaining the same feed flow rate and number of in-series sections (residence time and path length) determined at bench scale. Thus, in practice, scaling of single-pass TFF with Pellicon® cassettes is approached in a similar manner as that of batch TFF, offering end users a sense of familiarity while implementing this new process.

Cleaning

Adequate cleaning of cassettes in series has required either flushing with large volumes of chemical solution, which increases costs, or recirculation of cleaning agent to conserve solution, which counterbalances system simplicity.[19] To maintain the simplicity of single-pass TFF concentration with the cleaning efficiency of batch TFF, Merck developed a static cleaning method, which uses a combination of static soaks and dynamic flushing to efficiently clean cassettes without a recirculation loop.[20,21]

Figure 8 shows how the recirculation-free, static cleaning method, using sodium hydroxide as the cleaning agent, consistently restored membrane permeability for all sections of Pellicon® 3 cassettes with Ultracel® membrane over 20 cycles of antibody concentration.

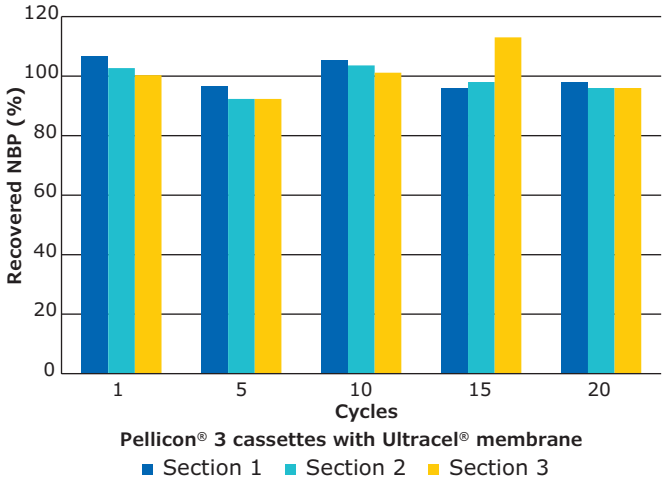


Figure 8. Membrane recovery over 20 runs of antibody concentration and static cleaning.

Case study: intensified polishing

The strategic adoption of single-pass TFF in a biomanufacturing process can be showcased during an intensified polishing step. Intensified polishing with Pellicon® cassettes and Eshmuno® Q anion exchange (AEX) resin is a linked process where feed material is continuously concentrated before it is loaded onto the column. The intrinsic benefit of pre-column concentration is volume reduction, which extends to

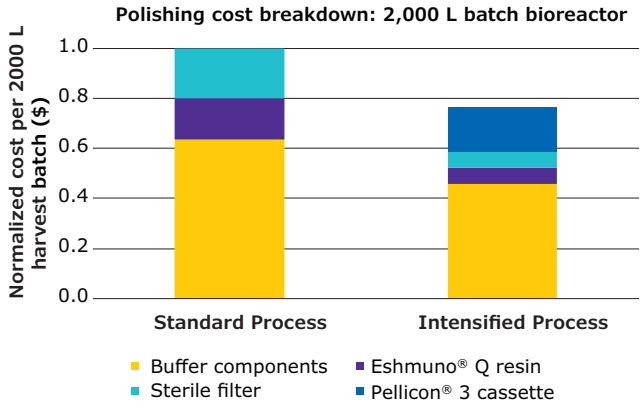
- faster loading time
- easy manipulation of pH or conductivity
- greater column capacity
- reduced post-column pool tank volume
- savings in materials and operational costs

To assemble an intensified polishing step, the column is simply connected to the single-pass TFF retentate port. Inline dilution is accommodated before or after the concentration step. In a case study, single-pass TFF with Pellicon® cassettes significantly reduced dilution volume requirements to adjust conductivity before running the column, enabling higher column mass loading and productivity compared to standard polishing (**Table 3**).[22]

Table 3. Efficiency of AEX polishing is improved with pre-column concentration using single-pass TFF.

Case study: 2,000 L fed-batch bioreactor with 3 g/L titer, single harvest		
AEX polishing step	**Standard process**	**Intensified process**
Pre-column concentration	None	Single-pass TFF
Dilution buffer volume	1508 L	435 L
Eshmuno® Q resin column loading	150 g mAb/L resin	400 g mAb/L resin
Eshmuno® Q resin productivity	24 g/L/h	59 g/L/h

With improved mass loading, resin and process volume requirements are reduced in an intensified polishing step. From a facility design perspective, reduced buffer and product volumes can considerably decrease tank size and footprint requirements for optimum facility fit. Single-pass TFF also improves process economics despite the additional cost of membrane, which is more than offset by savings associated with buffer, resin, and sterile filtration (**Figure 9**). Thus, the overall benefits of single-pass TFF concentration extend beyond volume reduction for a more productive and cost-effective manufacturing process.



100 re-use cycles max per Eshmuno® Q packed resin column
50 re-use cycles per Pellicon® 3 cassette

Figure 9. Intensified polishing with single-pass TFF offers improved process economics.

Summary

Use of single-pass TFF with Pellicon® cassettes is an effective and streamlined strategy to tackle volume challenges within downstream processing. As a continuous operation, single-pass TFF concentration can be linked with other unit operations to eliminate tank bottlenecks, increase the efficiency of polishing steps, or optimize facility fit. Pellicon® cassettes offer unique user-configurable single-pass TFF capabilities, including easy module configuration, precise linear scaling, and cleaning flexibility.

Single-pass TFF with Pellicon® cassettes is applicable to a wide range of molecules and conversion targets, and users can quickly and easily determine module design and scalability requirements according to their specific process needs. With simplified hardware and low footprint, single-pass TFF is easily accommodated anywhere within a biomanufacturing process. The operational flexibility provided with Pellicon® cassettes offers facilities the accessibility to integrate single-pass TFF without major capital investments in order to quickly mitigate in-process volume challenges and meet market demands.

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