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Technical Brief

Pellicon® 3 Cassettes Cleaning Methodology for Reuse in Single-Pass Tangential Flow Filtration Mode

Introduction

Single-pass tangential flow filtration (SPTFF) is a mode of ultrafiltration where feed material flows through TFF cassettes arranged in series at much longer residence times than in batch TFF. The feed material reaches the target concentration after just one pass through the membranes, eliminating the recirculation loop and reducing system hold-up volumes. SPTFF can be used to achieve higher final concentrations, optimize product recovery, control volume of intermediate steps, and enable intensified or continuous processing with chromatography or filtration operations.

To achieve longer residence times, the feed path length is increased and the feed flux to the modules is reduced. Pellicon® cassettes, in a traditional TFF holder, can be run in SPTFF mode simply by diverting the flow path in a serial configuration through sections of equal membrane area. This can be achieved by either serializing the feed flow through multiple holders or by installing diverter plates between each cassette in a holder to divert the feed path through the membranes.[1] This setup, combined with processing at a low flowrate under pressure, results in higher retentate concentration after one pass. SPTFF operation utilizes smaller pump and piping and reduces facility footprint when compared to batch processing, making it well suited for implementation throughout various steps of the purification process.

TFF cassettes used in batch mode are commonly cleaned and reused to reduce costs. For SPTFF operations, the same practice is expected. However, cleaning procedures developed for batch operations employ higher recirculation flows compared to SPTFF. An effective SPTFF cleaning and reuse strategy must take into consideration the lower flowrates, in-series membrane installation, increased residence time of concentrated material in the cassettes as well as the simplicity of the SPTFF system (i.e. no recirculation, reduced footprint/piping).

Previously, we described a static cleaning methodology for efficient cleanability of cassettes over multiple cycles of SPTFF processing.[2] Cassettes were installed in multiple holders and switching valves were used to alternate between in-series flow for protein processing and in-parallel flow for cleaning via a combination of flushing and static hold steps without recirculation. In this study, we explored cleaning strategies for Pellicon® 3 cassettes with Ultracel® and Biomax® membranes installed in a TFF holder with diverter plates (**Figure 1**), where an in-series flow is maintained for both protein processing and cassette cleaning.

Figure 1: SPTFF benchtop system with Pellicon® 3 cassettes installed in holder with diverter plates.

Study Design

Before implementing cleaning procedures at the manufacturing scale, process simulations and cleaning strategies should be evaluated using bench-scale systems. In this study, three cleaning methods were evaluated: one method for a Pellicon® 3 cassette with 30 kD Ultracel® membrane SPTFF system and two methods for a Pellicon® 3 cassette with 30 kD Biomax® membrane SPTFF system. The cassettes for each SPTFF system were configured in series with three sections of equal membrane area, example of setup shown in **Figure 1**. These three systems and respective cleaning methods are described in **Table 1**.

Table 1. SPTFF systems and cleaning conditions evaluated in this study

Setup	SPTFF System	Cleaning Conditions
U	3× Pellicon® 3 cassettes with 30 kD Ultracel® membrane 0.11 m^2 and C screen	0.5 N NaOH
BB	3× Pellicon® 3 cassettes with 30 kD Biomax® membrane 0.11 m^2 and A screen	0.5 N NaOH, 200 ppm NaClO (bleach)
BN	3× Pellicon® 3 cassettes with 30 kD Biomax® membrane 0.11 m^2 and A screen	1.0 N NaOH

A model protein feed stream of human polyclonal IgG was prepared at an initial concentration of 15 g/L in 10 mM phosphate buffered saline at pH 7.2. The feed was sterile filtered prior to each of the 20 process/cleaning cycles to remove any potential particles. Typical process preparation prior to concentration included sanitization of the system, flushing with purified water, and equilibration with buffer. The concentration step was conducted for 3 hours with a target concentration factor of 10 (i.e. 90% conversion). After processing for 3 hours, protein was recovered from the system by flushing buffer through the devices followed by cleaning, flushing with purified water, and final storage in 0.1 N NaOH. Normalized water permeability (NWP) was measured before and after the concentration step as a measure of cleaning efficiency for every cycle of use. Additionally, carryover analysis was performed by measuring endotoxin levels, total protein content, and total organic carbon (TOC) at predetermined time points throughout the study. **Table 2** summarizes the order of operations for a process run and cleaning cycle.

The targeted NWP recovery for the Ultracel® membranes was ≥ 80% of the initial NWP and ≥ 70% for the Biomax® membranes. Initial NWP is the normalized water permeability of a new device post-sanitization and flushing with water. If the NWP recovery was within the specifications, the system was cleaned in static hold mode, otherwise cleaning in total recycle was executed.

A cleaning cycle consisted of flushing the cleaning solution through the system as described in **Table 2**, then either turning off the pump, closing retentate and permeate lines and allowing the system to soak in cleaning solution for 1 hour (static hold), or continue cleaning in recirculation for 1 hour with the last 5 L/m^2 of cleaning solution.

Carryover samples for endotoxin, TOC, and total protein testing were collected at the beginning of the study (before and after the first process run) and every 10 runs. Additional samples were collected if NWP recovery was lower than the targeted value.

Table 2. Steps during SPTFF operation

Step	Solution	Feed Flow (L/min/m^2)	Volume (L/m^2)‡	Flow path†
Water Flush	Purified Water	1.0	20	3.3 L/m^2 SPFC, then 16.7 L/m^2 SPFO
NWP	Purified Water	1.0	10	TRFO
Air Integrity Testing	30 psig Air	-	-	Retentate closed, permeate open
Buffer Equilibration	10 mM PBS Buffer pH 7.2	1.0	10	SPFO
Protein Concentration	15 g/L IgG	Set to achieve 90% conversion	Amount to enable 3-hour processing	SPFO
Protein Recovery	10 mM PBS Buffer pH 7.2	Process flowrate	2-3 hold-up volumes	SPFC
Buffer Flush	10 mM PBS Buffer pH 7.2	1.0	20	10 L/m^2 SPFC, then 10 L/m^2 SPFO (retentate fully open)
Cleaning Cycle	U: 0.5 N NaOH BB: 0.5 N NaOH with 200 ppm bleach BN: 1.0 N NaOH	1.0	20	10 L/m^2 SPFC, 5 L/m^2 SPFO (retentate fully open), 5 L/m^2 SPFO Static hold or total recycle
Water Flush	Purified Water	1.0	20	3.3 L/m^2 SPFC, then 16.7 L/m^2 SPFO
NWP	Purified Water	1.0	10	TRFO
Storage	0.1 N NaOH	1.0	10	SPFO

† SPFO: Single-pass filtrate open at a retentate pressure that results in an overall permeate conversion of 60-80% unless otherwise stated, SPFC: Single-pass filtrate closed, TRFO: Total recirculation with a retentate pressure that results in an overall permeate conversion of 60-80% unless otherwise stated.

‡ Some volume amounts are a function of hold-up volume or based on a targeted time at a given flow rate.

Results

PROCESS PERFORMANCE

After determining optimal conditions to concentrate the model protein solution,[3] 20 consecutive process runs and subsequent cleaning cycles were executed. The process results are summarized in **Table 3** and **Figure 2**.

Table 3 and **Figure 2** demonstrate that the overall conversion of the process remained relatively consistent across all three systems despite variations in retentate pressure, which emphasizes the robust nature of the SPTFF applications.

Table 3. Overall performance during SPTFF processing

Setup	Feed Flow Rate [L/min/m²]	Initial Concentration [g/L]	Final Concentration [g/L]	Concentration Factor	Recovery [%]
U	0.1 ± 0.01	15.3 ± 0.5	156.8 ± 5.5	10.2 ± 0.5	101.5 ± 1.4
BB	0.1 ± 0.02	15.5 ± 0.5	151.4 ± 5.6	9.6 ± 0.5	103.6 ± 3.8
BN	0.1 ± 0.01	15.5 ± 0.3	139.8 ± 11.3	9.0 ± 0.8	103.3 ± 1.9

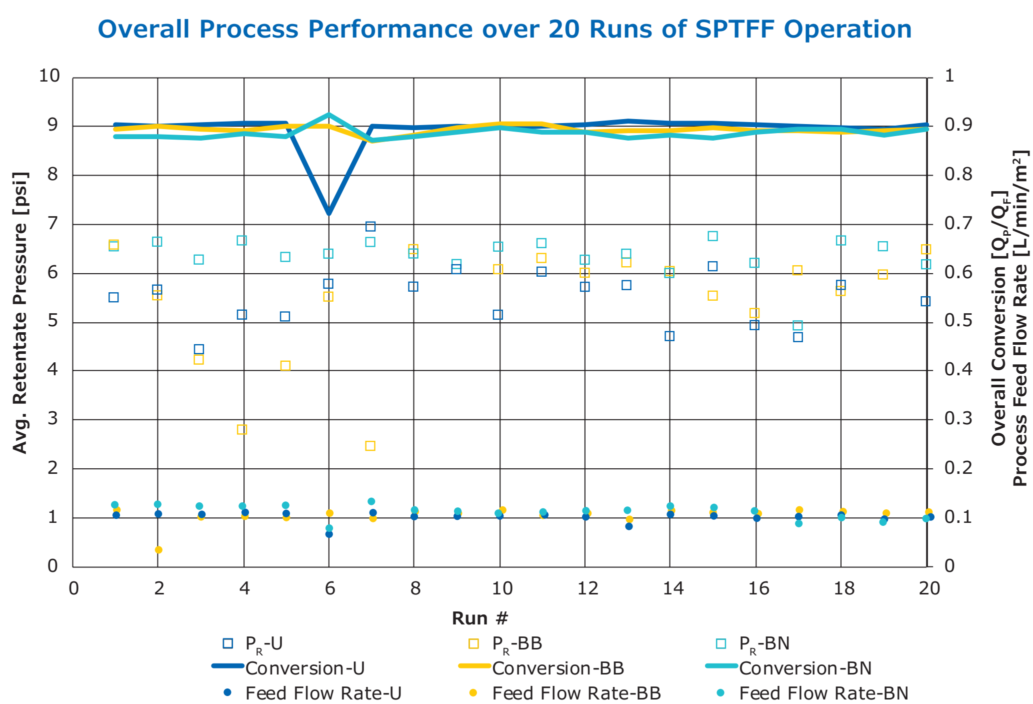

Figure 2. Overall conversion, average retentate backpressure, and average feed flow rate versus process run number for each of the three systems.

MEMBRANE CLEANABILITY AND REUSE

At process scale, SPTFF systems are designed with a single permeate line, therefore it is not feasible to measure the permeability of each section separately. Since measuring the water permeability of each section provides valuable information about the system, both combined water permeability and the water permeability of each section of the benchtop system were individually measured. **Figure 3** shows the NWP recovery as a function of run number for all three systems.

The results in **Figure 3** demonstrate that a static hold cleaning strategy is effective when coupled with the proper cleaning solution, extending the processing simplicity of SPTFF to cassette cleaning by avoiding recycle. In cases where bleach cannot be introduced into a Biomax® membrane cleaning process and the system must be cleaned with NaOH alone, higher concentrations of NaOH and cleaning in total recycle is recommended. Even though 70% target for NWP

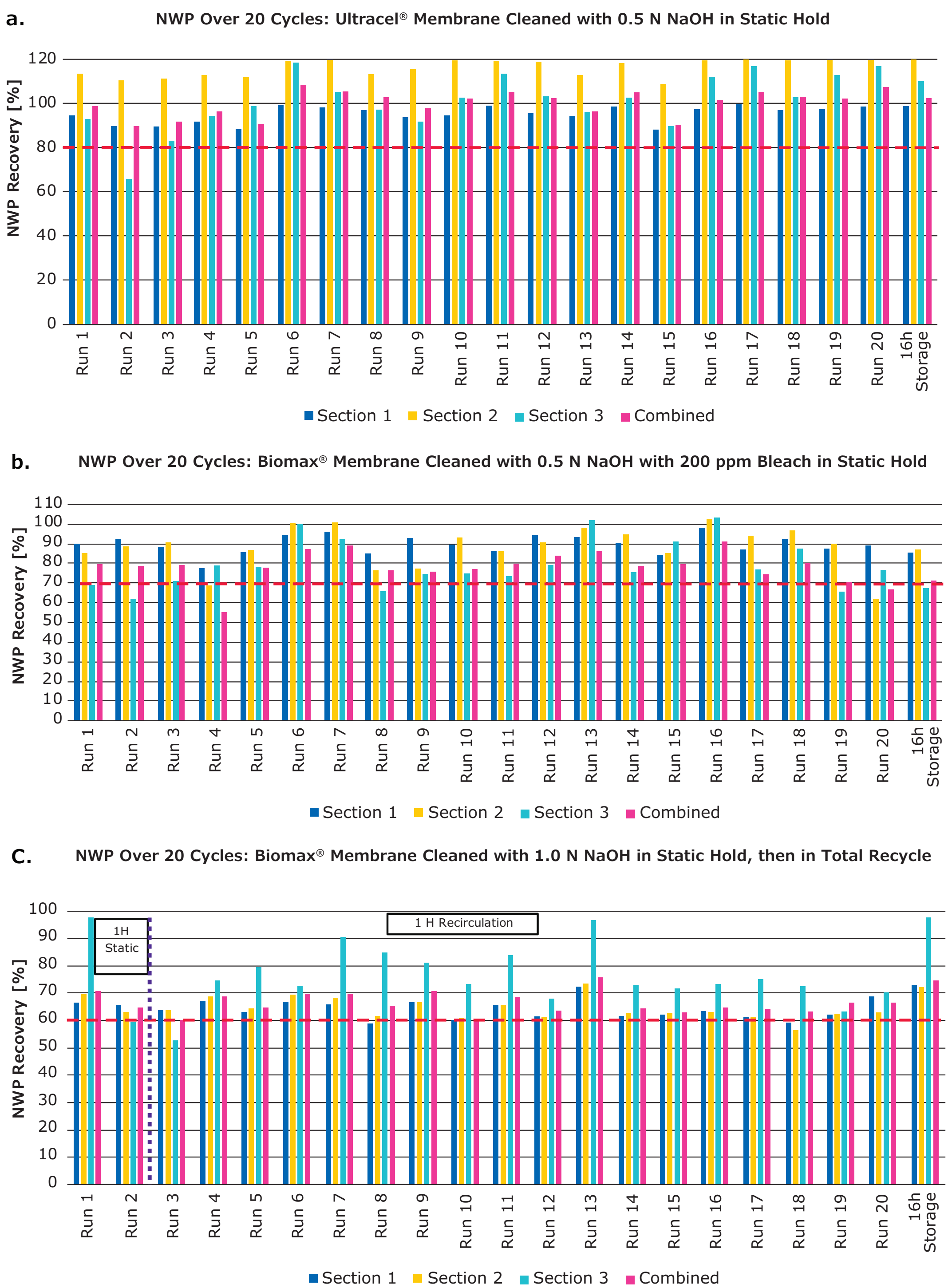

Figure 3. NWP versus process run number for: a) Ultracel® membrane cleaned with NaOH, b) Biomax® membrane cleaned with NaOH and bleach, c) Biomax® membrane cleaned with NaOH.

recovery was not achieved for Biomax® membranes cleaned with NaOH alone (**Figure 3c**), NWP recovery stabilized at around 60% throughout the study and the performance during process runs remained consistent.

Cleanliness of each system was also evaluated by performing carryover analysis, in which samples were collected and analyzed for endotoxin, TOC, and total protein. The samples were collected directly from the retentate and permeate lines after flushing with 20 L/m² of purified water. The results of the endotoxin assay are summarized in **Figure 4**.

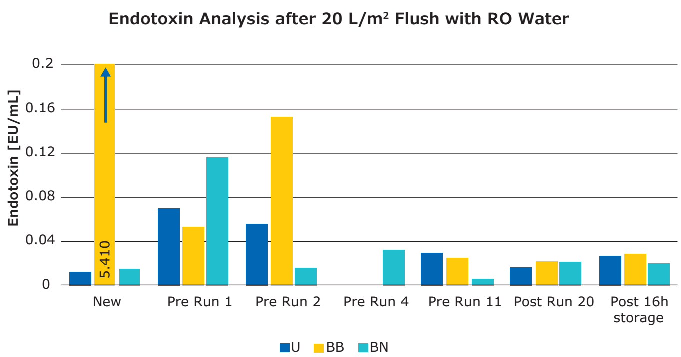

Figure 4. Endotoxin levels in the retentate after flushing with 20 L/m² purified water. Permeate data not shown.

The levels of endotoxin in samples collected from the retentate lines were below 0.08 EU/mL in most cases. In some samples, higher levels of endotoxin were detected, but additional flushing with purified water resulted in levels decreasing below 0.05 EU/mL. The endotoxin levels in samples collected from the permeate lines were below limit of quantification (LOQ) of 0.005 EU/mL (data not shown).

In all but one case, levels of TOC collected from the retentate and permeate lines were below 3.2 ppm after a 20 L/m² flush with purified water. The one exception was a sample collected from the retentate line of the Ultracel® system, post process run 20. For this run, the TOC value was 6 ppm, but decreased to 0.1 ppm after overnight storage and additional flush with purified water. The results are summarized in **Figure 5**.

In addition, total protein concentration for all tested samples was below the LOQ of 2.0 µg/mL. The results of the assays suggest little to no carryover from run to run, demonstrating the effectiveness of the tested flushing and cleaning methods.

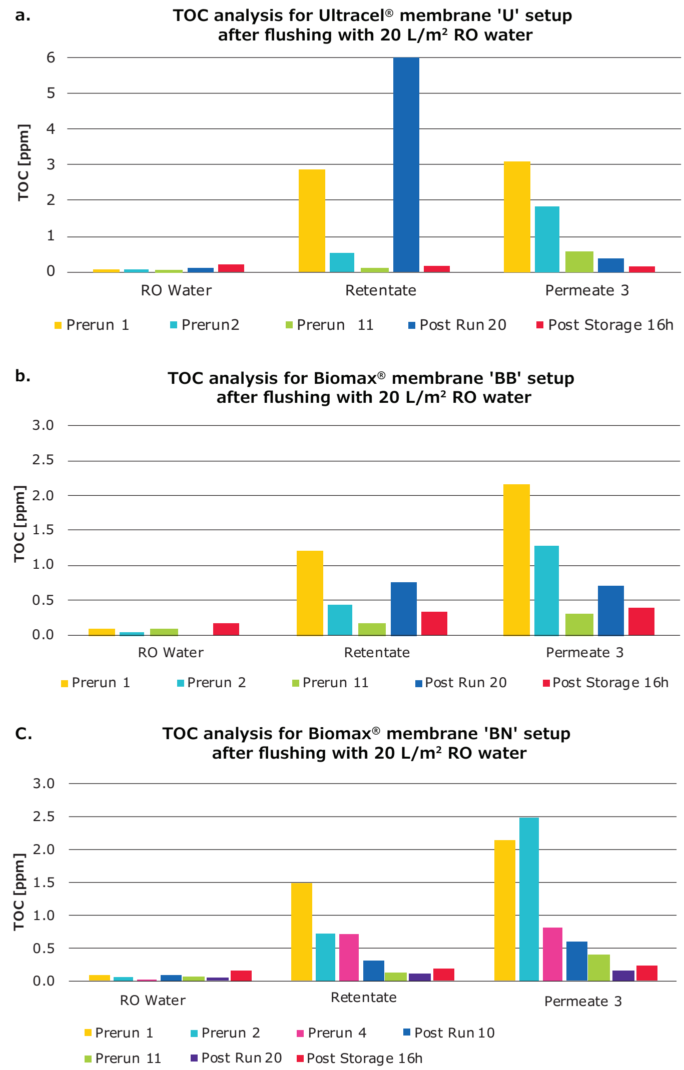

Figure 5. TOC results for purified water source, retentate, and the permeate line of section 3 for Ultracel® membrane (a) and Biomax® membranes (b and c).

Conclusions

This study demonstrates efficient cleaning strategies to enable multiple reuse of TFF cassettes for high concentration applications with SPTFF. Using the described flushing and cleaning methods, both NWP recovery and SPTFF operation was stable and consistent over 20 processing runs for a 10× concentration of a 15 g/L IgG feed solution. Total protein, total organic carbon, and endotoxin levels remained low throughout the study, which indicates the described cleaning methods were effective in minimizing carryover. Considering feed and process requirements vary, it is recommended that cleaning strategies be developed independently and that the methods described in this study be used as a guide.

References

1. White Paper, Single-Pass Tangential Flow Filtration, A Versatile Application to Streamline Biomanufacturing, Lit. No. WP2787EN.
2. T. Bisschop et al., "Efficacy of a Simple Static Cleaning Procedure for SPTFF," BioPharm International 32 (1) 2019.
3. Technical Brief, Optimization of Operating Pressure for Single-Pass Tangential Flow Filtration, Lit. No. AN5572EN00.

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