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Technical Brief

Evaluation of TFF Operating Control Strategies and Scalability for Viral Vector Process Development

Viral gene therapies are relatively new and complex therapeutics. A standard manufacturing process template for these novel products does not currently exist. This presents challenges during process development of viral vectors as the industry continues to navigate and learn in this area. One such challenge is finding the right technology solutions for manufacturing. Technologies that are reliable and provide process predictability accelerate the path from development to commercialization. Further, viral vector manufacturing requires a way to reduce the risk of introducing adventitious agents and cross-contamination between production cycles. Single-use technologies are well suited to mitigate such risks, and capabilities to process closed further enhance process and product safety.

In this technical brief, we discuss use of our new single-use technology for tangential flow filtration (TFF) of viral vectors during the evaluation of two TFF operating control strategies. Scalability and comparability evaluations to assess predictability at scale-up are also discussed. The insights from these studies provide a starting point for process development considerations to help you with achieving your goals faster.

Study Background

Permeate-control TFF systems are typically used for microfiltration applications, where highly permeable membranes can lead to excessively high fluxes that degrade or destabilize performance[1]. For tighter ultrafiltration membrane applications, such as 30 kDa for antibody retention, TMP-control systems are used, where only feed-side pressures are adjusted. Membrane cutoffs typically used in viral vector manufacturing, e.g., adeno-associated virus (AAV) and lentivirus (LV), are 100 and 300 kDa. The openness of these membranes falls between the tighter ultrafiltration (UF) and more open microfiltration applications. In this study, we applied both TMP and permeate control strategies to evaluate and compare performance of the TFF1 step (post-clarification/pre-capture) in viral vector manufacturing **(Figure 1)**.

For the TMP-control operation, a feed pump was used and there was free flow of permeate **(Figure 1a)**. For the permeate-control operation, a two-pump TFF system was used, which included a feed pump and a permeate pump that was used to restrict and control flow of the permeate **(Figure 1b)**. Note that permeate-control operation can also be achieved using a flow-controlled permeate valve instead of a permeate pump.

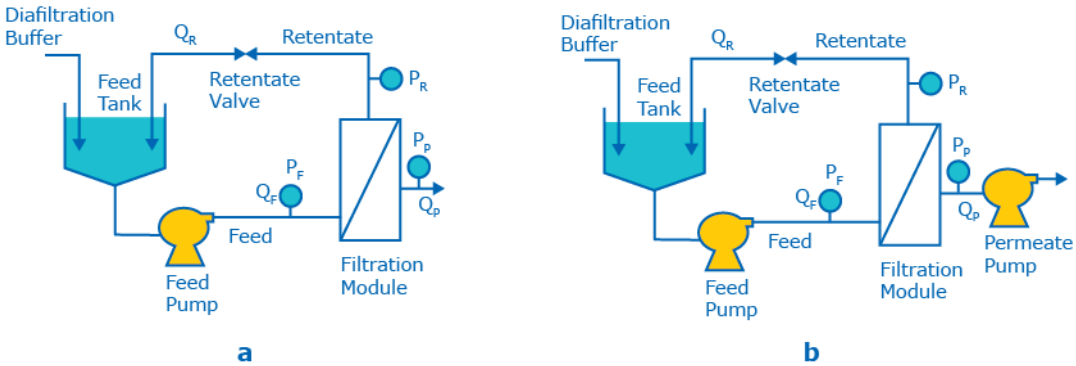

Figure 1. Schematic of TFF systems used in this study: a) TMP control and b) permeate control with permeate pump.

Materials

Feed

Development and process simulation studies for the TFF1 step were performed using a model feed. Final studies to confirm trends were performed with AAV2. The model feed was produced in the same way as the AAV stream, with a detergent-lysed, depth filter-clarified HEK293 cell culture. However, instead of transducing the HEK293 cells, the clarified model feed was spiked with a bacteriophage of similar size to AAV, at $1e^7$ phage/mL. Performance comparability of the virus model feed versus in-house AAV2 was demonstrated based on flux, yield, and impurity reduction for a 5× concentration step **(Figure 2)**.

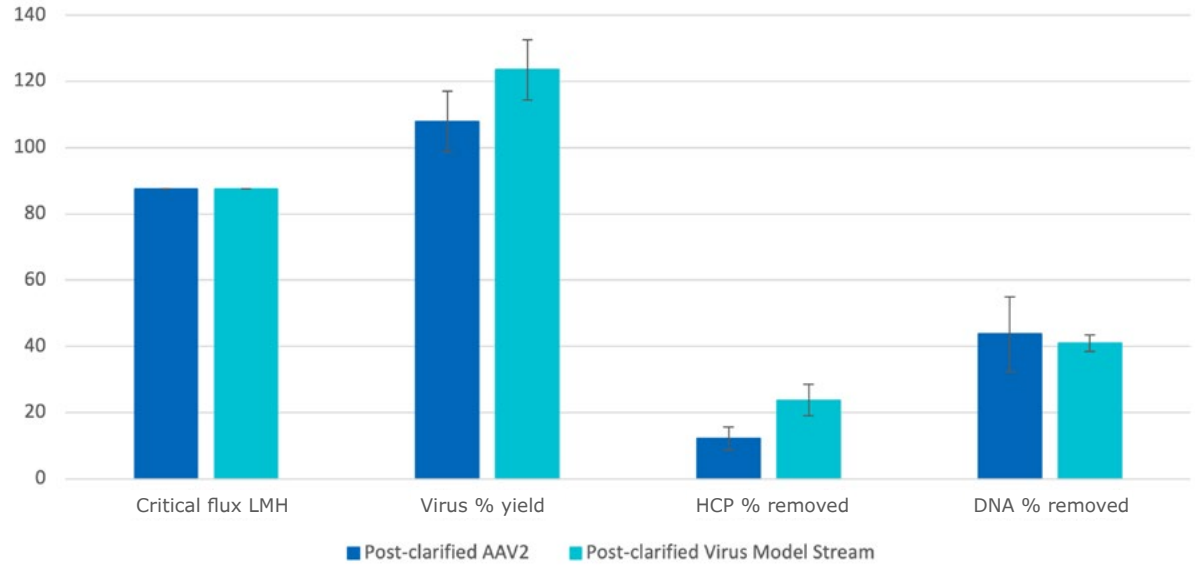

Figure 2. Comparability of bacteriophage model feed versus AAV2 feed using Pellicon® XL 50 cassette with 100 kDa Ultracel® membrane during a post-clarification 5× concentration.

TFF Filters

Pellicon® Capsules and scale-down Pellicon® XL 50 cassettes with 100 and 300 kDa Ultracel® membrane were evaluated in this study. Reusable Pellicon® 2 cassettes were also included for performance comparability evaluation.

Study Design and Methods

Initial development experiments were performed to characterize permeate flux of the TFF filters for each control strategy (TMP and permeate control). Then, UF/DF process simulations were run to evaluate average flux, processing time, TMP, virus yield, and impurity reduction **(Table 1).** The process simulation goal was to concentrate four-fold (4×) in batch mode, diafilter with five diavolumes (5 DV) of HEPES buffer at constant volume, and then concentrate two-and-a-half-fold (2.5×) in batch mode for an overall volumetric concentration factor (VCF) of 10×.

Table 1. Study design flow chart.

Clarified Virus Model Feed	Flux Characterization	• Permeate Flux Characterization • Target Initial Flux for Processing
	Process Simulations UF1 – 4× DF – 5 DV UF2 – 2.5×	• Pressure and Flux Trends by Control Strategy • Time, Yield, Impurity Reduction by Control Strategy • Impact of Feed Loading by Control Strategy
Clarified AAV2 Feed		• TMP Versus Permeate Control • Pellicon® Capsule Versus Pellicon® XL 50 Cassette Scaling

Development experiments were run on virus model feed in total recycle to characterize flux versus TMP for TMP-control systems and TMP stability versus flux for permeate-control systems. Cassettes were run in co-flow mode (permeate port closed on feed end and open on retentate end). Operating conditions were determined and target initial fluxes were compared for all TFF filters to assess scalability at the start of processing. Target operating conditions for Pellicon® XL 50 cassettes were then used for Pellicon® Capsules and Pellicon® 2 cassettes to assess performance and scaling during process simulations, except where noted.

The loading target was 35 liters of virus model feed per m^2 of membrane (L/m^2) for all tests and the crossflow (avg. of feed and retentate flow) rate was set to 5 Liters/min/m^2 (LMM). After completing studies at 35 L/m^2, the effect of high loading (120 L/m^2) was evaluated for process robustness of either control strategy. Final studies were then performed with in-house AAV2 feed to confirm performance, scaling, and virus yield.

Feed and permeate samples were collected during each process simulation. Virus was assayed by infectivity for bacteriophage (model feed) and ELISA for AAV, host-cell proteins (HCP) by ELISA, DNA by PicoGreen™, and Benzonase® endonuclease by ELISA. All error bars shown are combined standard error from initial and final samples, n=2.

TMP Control Method

Flux stability for the TMP-control system was demonstrated during a 15-minute total recycle of virus model feed, where the flux declined less than 20%. Crossflow rate was set to give 5 LMM at the beginning of the flux stability test, and the retentate valve was throttled to give ~2 psi backpressure. After flux stability was reached, a TMP excursion was run, where permeate flux was measured at 1-psi TMP increments by throttling the retentate valve. Following the TMP excursion, the target initial operating TMP was then determined as the last point before the plateau (3 consecutive points of increasing TMP which fluxes differ less than 10%).

Permeate Control Method

Operating conditions for the permeate-controlled system were determined via a critical flux excursion. Permeate flux on clarified feed was progressively increased in 5-10 liters/m^2/h (LMH) increments for each permeate flux level by increasing the permeate pump speed, and a crossflow rate of 5 LMM was maintained by adjusting the feed pump. Retentate pressure started at ~5 psi and was increased as needed to maintain a positive permeate pressure. Each flux was held for 15 minutes, pending TMP stability. TMP was considered unstable when it increased ≥1.5 psi within 10 minutes. The flux point at which the TMP becomes unstable is called the critical flux. The average of the last two fluxes is used as the critical flux when the critical flux starts between both points.

The operating flux for the process simulation was set to 50% of the critical flux (50% CF) to provide stability of TMP through the first concentration step (UF1). For the subsequent diafiltration (DF) and concentration steps (UF2), the permeate flux was reduced to 25% of the critical flux (25% CF). The crossflow rate was set to 5 LMM and the retentate pressure to 5 psi for each process step. To further challenge the permeate-control process, higher fluxes were also evaluated in some cases as noted later.

Development Results

Permeate Flux Characterization

Exemplary data for the scaling filter, Pellicon® XL 50 cassette, is shown for a TMP excursion **(Figure 3)** and a critical flux excursion **(Figure 4)**. These characterization tests were run on virus model feed to assess flux and help set operating conditions for the process simulations.

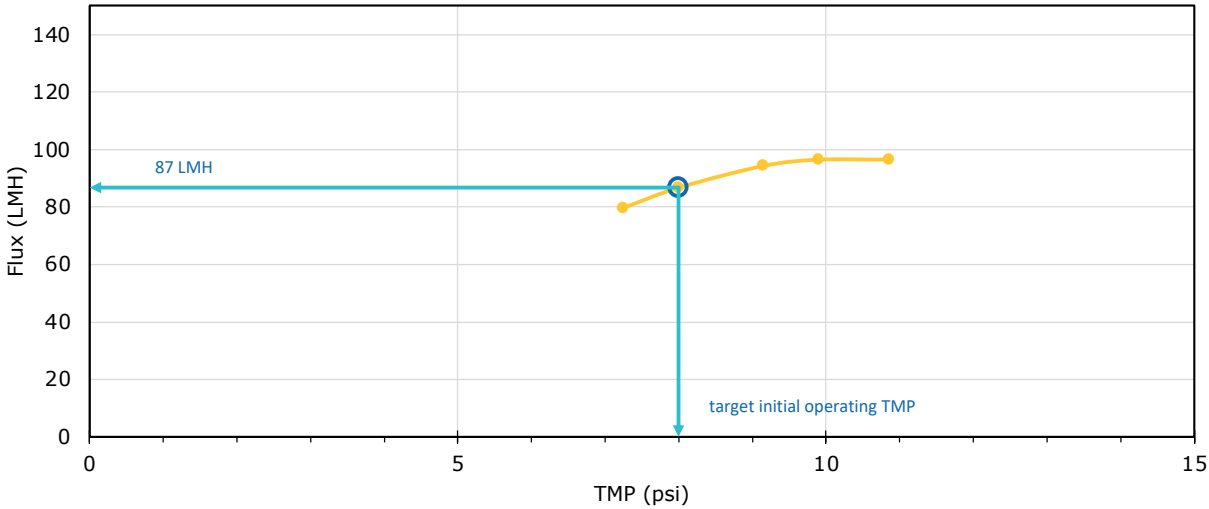

Figure 3. TMP excursion for the TMP-control system using virus model feed. Shown for two 100 kDa Pellicon® XL 50 cassettes run in parallel for 100 cm² membrane area.

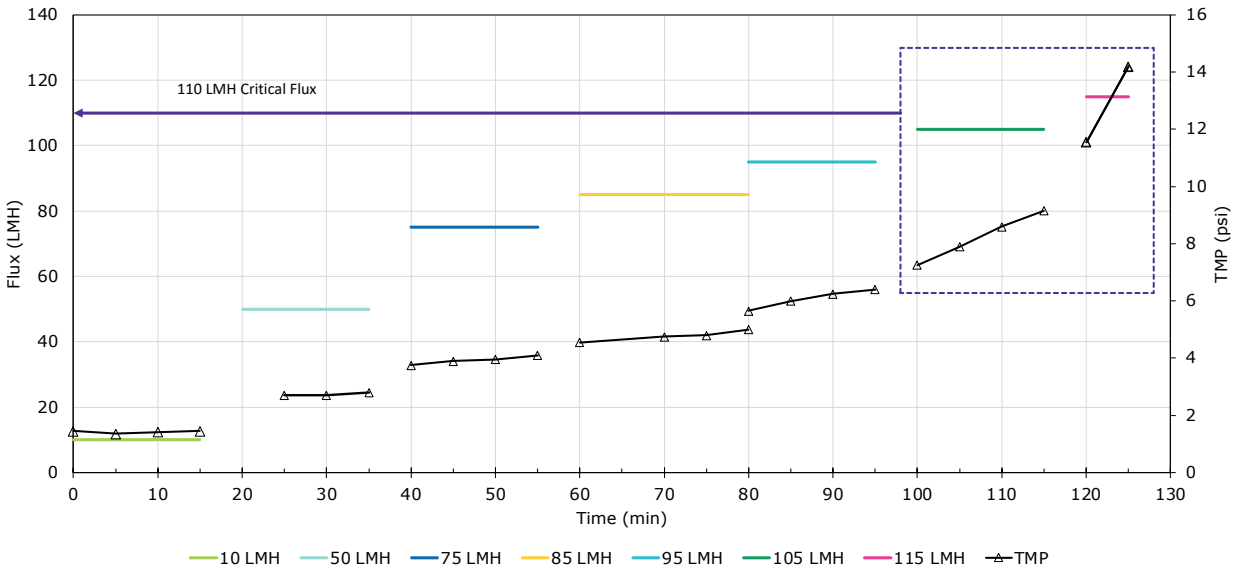

Figure 4. Critical flux excursion for the permeate-control system using virus model feed. Shown for two 300 kDa Pellicon® XL 50 cassettes run in parallel for 100 cm² membrane area.

Target Initial Permeate Flux for Processing

Target initial operating fluxes based on characterization data with virus model feed for all filters are shown in **Figure 5**. Fluxes for Pellicon® Capsules and the Pellicon® 2 cassette fell within 20% of the Pellicon® XL 50 cassette flux. Flux was similar or slightly higher for 300 kDa compared to 100 kDa membranes. As expected, TMP-control operation gave higher flux than the permeate-control operation. The previous critical flux excursion sample data **(Figure 4)** shows permeate-control systems could run stably at the TMP-control operating flux, but only at the start of the concentration step. Because flux drops naturally during the concentration step, trying to maintain the initial flux constant would lead to a large rise in TMP. Hence, the permeate-control flux was set lower than the target initial flux for TMP-control to enable stable operation throughout the UF/DF process.

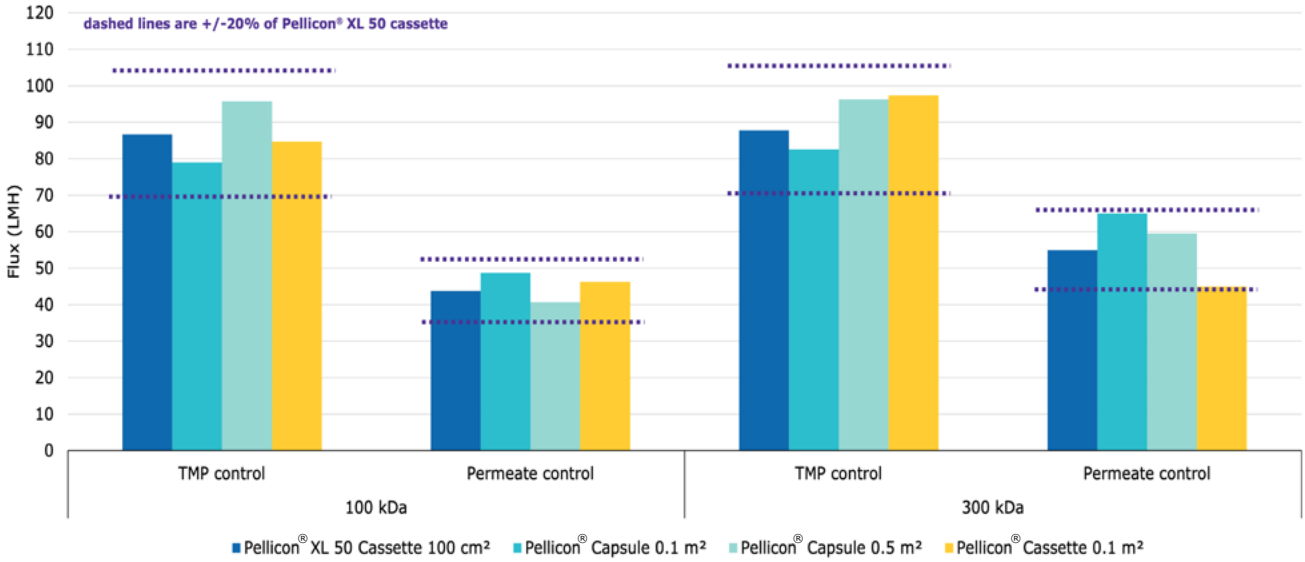

Figure 5. Target initial flux for process simulation based on TMP and critical flux excursions using virus model feed at 35 L/m² loading. Two Pellicon® XL 50 cm² cassettes were used in parallel for 100 cm² membrane area.

Virus Model Feed Process Simulations

Pressure and Flux Trends by Control Strategy

Exemplary chart-recorder plots of flux and TMP during the TFF process simulation are shown for the TMP-control case **Figure 6**, where flux declines naturally, and the permeate-control case **(Figure 7)**, where the flux is fixed and TMP can rise.

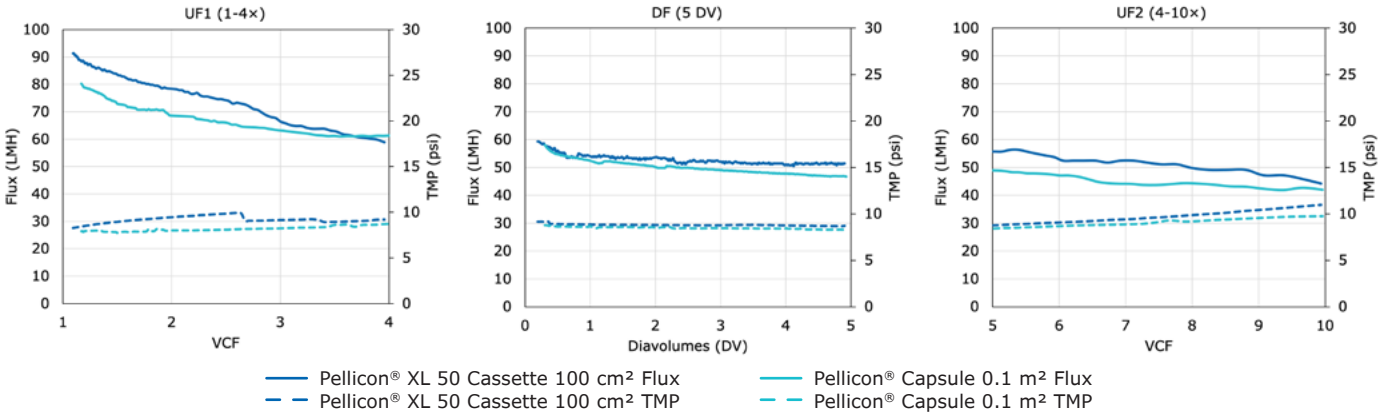

Figure 6. Flux and pressures during the 100 kDa TMP-control process simulation using virus model feed at 35 L/m² loading.

Note that a higher flux was attempted at the start of the second concentration step in **Figure 7** for the Pellicon® XL 50 cassette, but since the TMP rose too quickly, it was reduced back to the initial target (25% CF). It should be noted that the impact of the lower UF2 flux on run time was low since process time requirements for the secondary concentration step were minimal in comparison to the first concentration and diafiltration steps. TMP trends during the permeate-control process were similar for the Pellicon® Capsule and Pellicon® XL 50 cassette for all steps run at similar fluxes.

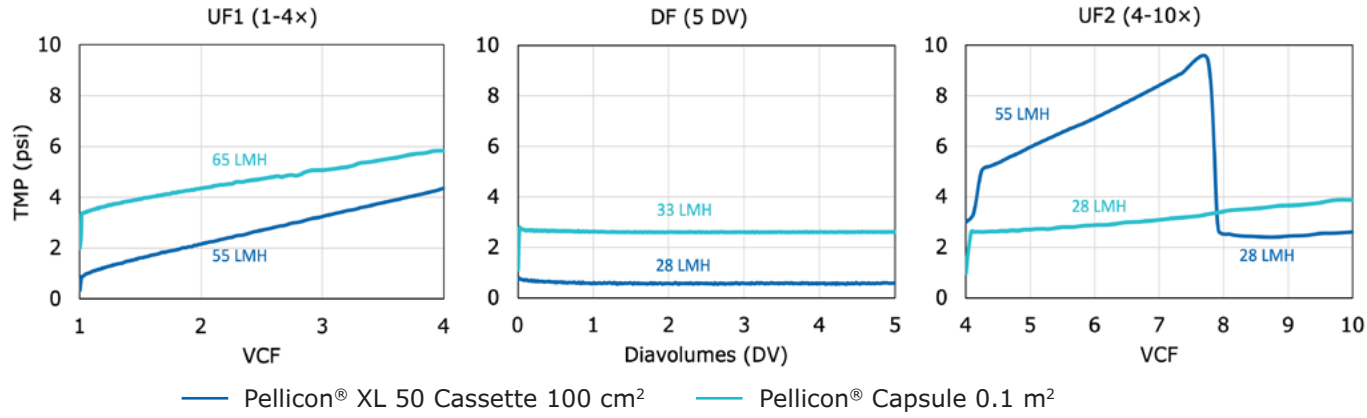

Figure 7. Flux and pressures during the 300 kDa permeate-control process simulation using virus model feed at 35 L/m² loading. Flux was set to 50% of critical flux for UF1 and 25% for DF and UF2 for each filter (50%/25% CF).

Process Run Time by Control Strategy

Because the operating point for TMP control was set near the plateau, the flux stays close to its maximum at every point during the process, while naturally decreasing with concentration. This gives TMP-control operation an advantage of shorter TFF processing time due to higher average flux compared to permeate-control operation. In this study, TMP control had ~30-35% less processing time than permeate control operating at 50%/25% of critical flux **(Table 2)**. If a permeate-control TFF run is set up with higher flux (i.e., >50%/25% of critical flux), this time advantage can of course be smaller. Faster processing time observed with 0.5 m² capsule may be related to higher feed pulsations generated by the larger pump head used.

Table 2. Run time for process simulations at 35 L/m² loading.

TFF Fliter	TMP Control, 100 kDa (min)	TMP Control, 300 kDa (min)	Permeate Control, 300 kDa (min)
Pellicon® XL 50 (100 cm²)*	74	74	112
Pellicon® Capsule 0.1 m²	79	77	112
Pellicon® Capsule 0.5 m²	67	54	ND
Pellicon® 2 Cassette 0.1 m²	78	76	ND

*Two Pellicon® XL 50 cassettes in parallel. ND = no data.

Yield and Impurities Reduction by Control Strategy

For both permeate-control and TMP-control process simulations, yield and impurities reduction were measured. For the TMP-control process, Pellicon® Capsules 0.1 and 0.5 m² were tested along with Pellicon® 2 Cassette 0.1 m² and Pellicon® XL 50 cassettes (100 cm² total area). Yield and impurity removal levels for all filters with both 100 and 300 kDa Ultracel® membranes are shown in **Figure 8**.

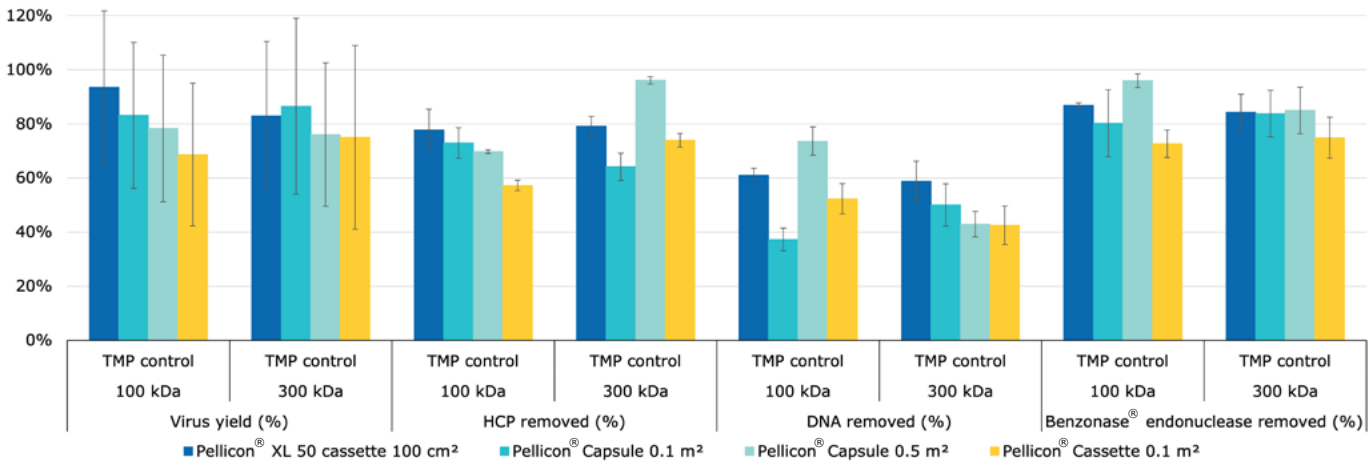

Figure 8. Yield and impurity reduction for TMP-control system using virus model feed at 35 L/m² loading, 100 and 300 kDa, after 4×/5 DV/2.5×.

For the permeate-control case, data is shown for Pellicon® Capsule 0.1 m² and Pellicon® XL 50 cassettes (100 cm² total area) with 300 kDa Ultracel® membrane **(Figure 9)**. Compared to the TMP-control run **(Figure 8)**, the impurity reduction level was similar or somewhat greater for the 300 kDa membrane when using permeate control.

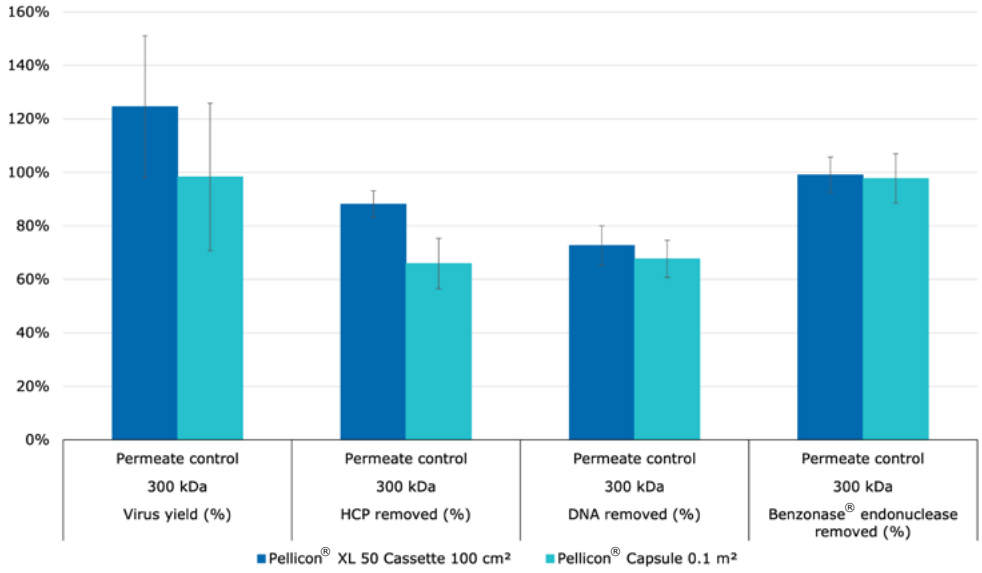

Figure 9. Yield and impurity reduction for permeate-control system using virus model feed at 35 L/m² loading, 300 kDa, after 4×/5 DV/2.5×.

Impact of Feed Loading by Control Strategy

Loading levels of 35 and 120 L/m² of virus model feed were assessed in process simulations with TMP and permeate control using Pellicon® XL 50 cassettes with 300 kDa Ultracel® membrane. For both loading cases, permeate-control simulations were run as described in previous studies with conservative flux rates of 50% of critical flux for UF1 and 25% of critical flux for DF and UF2 (10×, 5 DV), labeled as 50%/25% CF in **Figure 10 and 11.** An additional permeate-control process simulation was conducted with 35 L/m² loading, in which permeate rates were higher: 60% of critical flux for UF1 and 40% of critical flux DF and UF2 (60%/40% CF).

For all cases TMP control had higher average flux, and consequently shorter process times than permeate control **Figure 10**. The average and maximum TMP were higher for the TMP-control process, but still the maximum TMP stayed within reasonable process limits, even for 120 L/m² loading. This demonstrates a stable process.

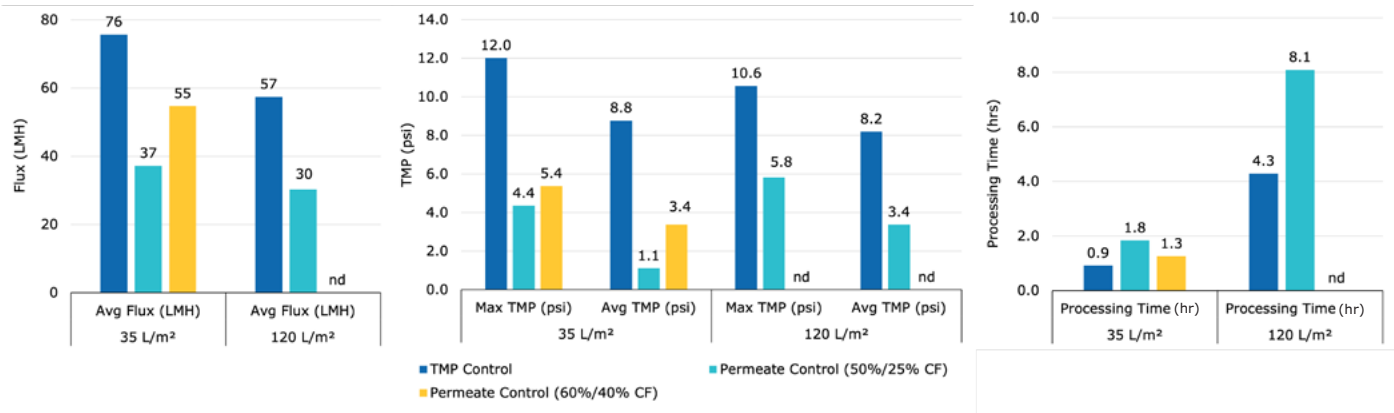

Figure 10. Impact of control strategy and feed loading on average process flux, TMP and process time using virus model feed and 300 kDa Pellicon® XL 50 cassette, 4×/5 DV/2.5×.

Virus yields, Benzonase® endonuclease clearance, and HCP and DNA reduction are shown for permeate and TMP-control operations for both low and high loading scenarios **(Figure 11)**. Yield and impurity reduction levels seemed to be maintained even when feed loading increased from 35 to 120 L/m^2.

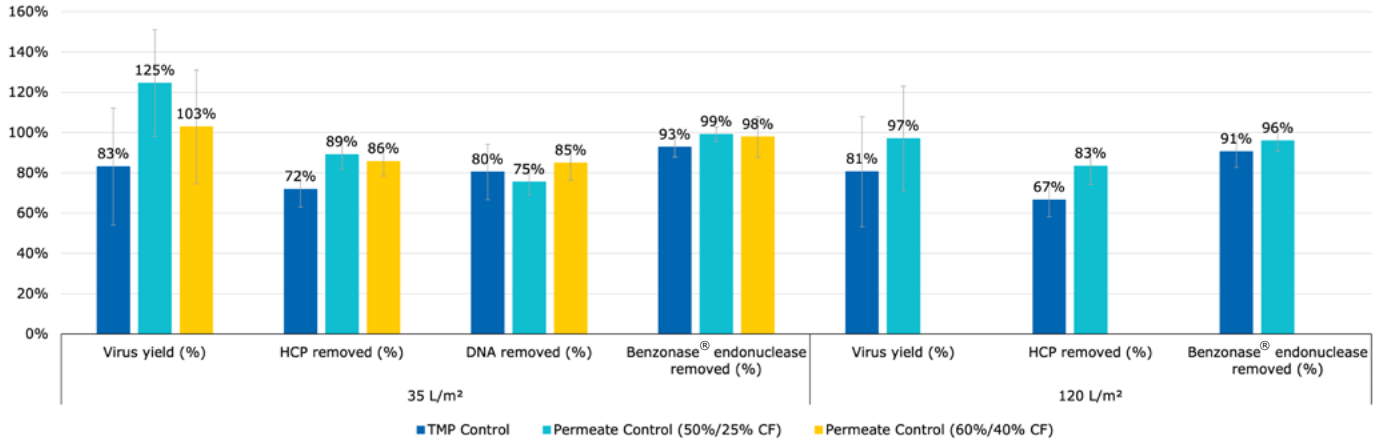

Figure 11. Impact of operating control strategy and feed loading on yield and impurity reduction using virus model feed and 300 kDa Pellicon® XL 50 cassette, 4×/5 DV/2.5×. No DNA data for 120 L/m^2 due to sample contamination.

AAV Process Simulations

TMP versus Permeate Control Study

Process simulations (4×, 5 DV, 2.5×) with in-house clarified AAV2 feed were run mainly to confirm yield, considering that assay variability is much lower for AAV (ELISA) than for the model stream with bacteriophage (infectivity). TMP-control and permeate-control processes were performed with 80 L/m^2 loading of AAV2 using Pellicon® XL 50 cassette with 300 kDa Ultracel® membrane. Crossflow rate was 5 LMM.

Process flux, pressures, and processing time when operating with TMP control and permeate control are compared in **Figure 12**. As in the previous study with model feed, TMP-control mode had higher average flux and TMP than the permeate-control mode; however, the flux advantage is lower because the TMP-control flux drops at the higher loading, and at the same time the permeate flux in this case study was controlled to give a higher flux (67%/40% CF).

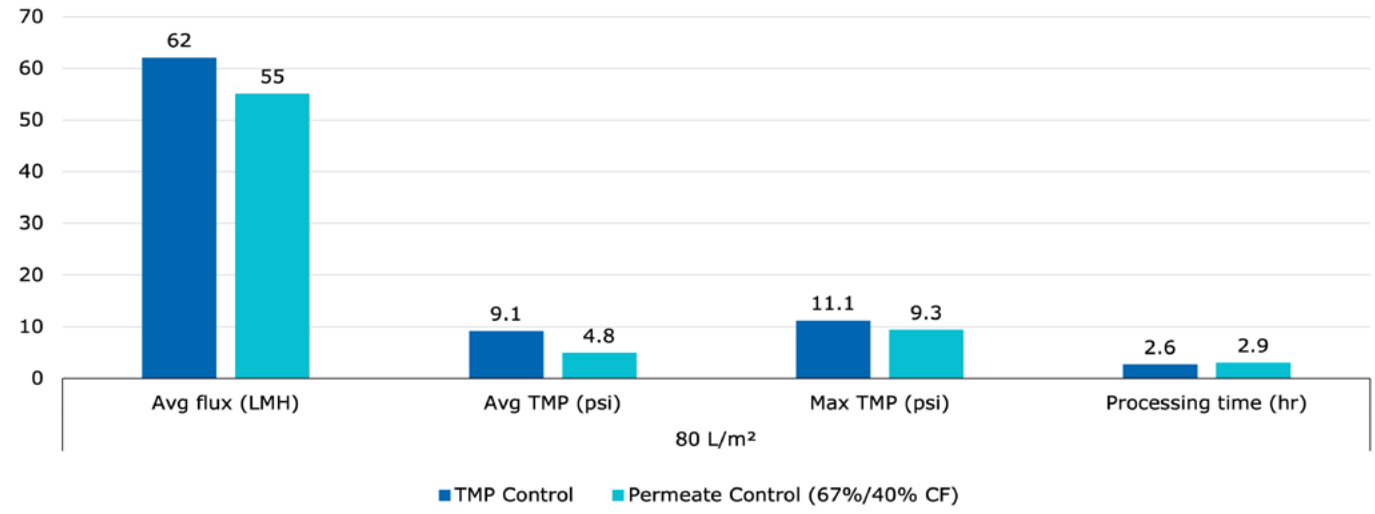

Figure 12. TMP and permeate control process comparison using AAV2 at 80 L/m^2 loading with 300 kDa Pellicon® XL 50 cassette, 4×/5 DV/2.5×.

AAV yield was comparable between both control strategies. However, impurity removal of the clarified AAV2 feed was higher for the permeate-control process simulation **(Figure 13)**. Results from the virus model feed work **(Figure 11)** can therefore predict such trends for the AAV stream performance.

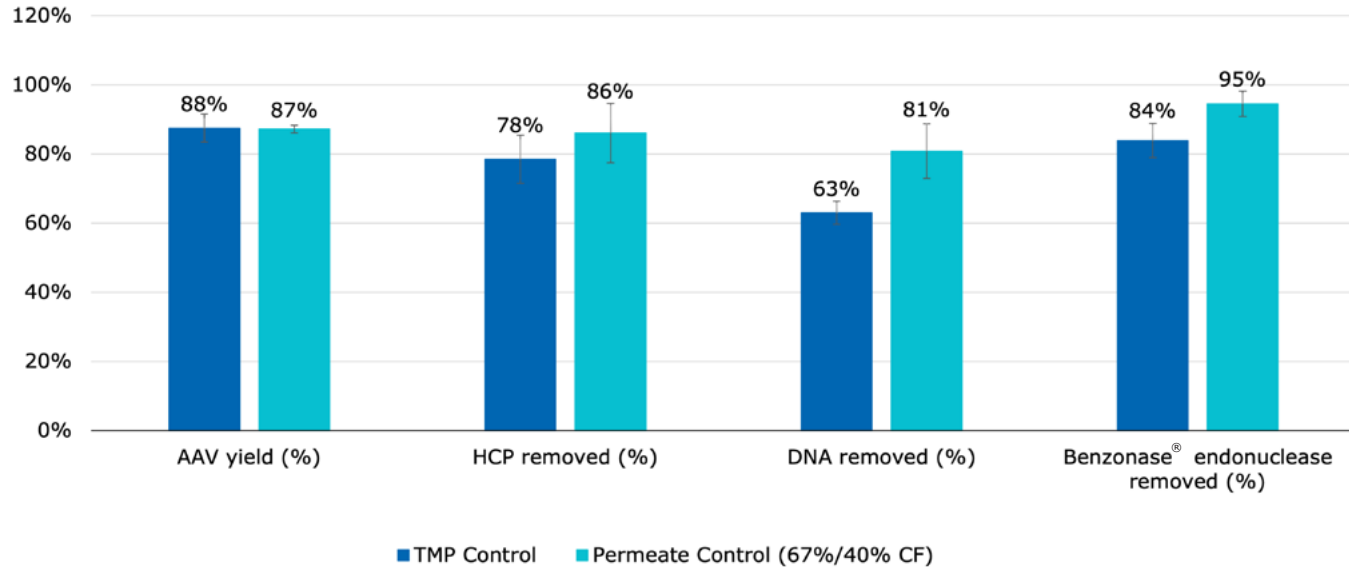

Figure 13. Impact of operating control mode on yield and impurity reduction for AAV2 at 80 L/m² loading with 300 kDa Pellicon® XL 50 cassette, 4×/5 DV/2.5×.

Pellicon® Capsule and Pellicon® XL 50 Cassette Scaling Study

Next, performance of Pellicon® Capsule versus Pellicon® XL 50 cassette was evaluated using a clarified AAV2 feed stream with 60 L/m² feed loading. The feed stream for Pellicon® XL 50 cassette was generated in a shake flask, while the feed for Pellicon® Capsule was generated in a bioreactor. This resulted in a 3.6-fold higher AAV2 titer for the capsule. Permeate control was selected to process the viral vector at 5 LMM and flux was controlled to 50%/25% CF. Flux, pressures, and processing time for both filters are compared in **Figure 14**. Flux was similar between both filters, TMP was higher for the capsules due to higher titer compared to the feed used for the cassette. AAV2 yield and impurity removal levels for the 0.1 m² capsule are similar to the Pellicon® XL 50 cassette results **(Figure 15)**.

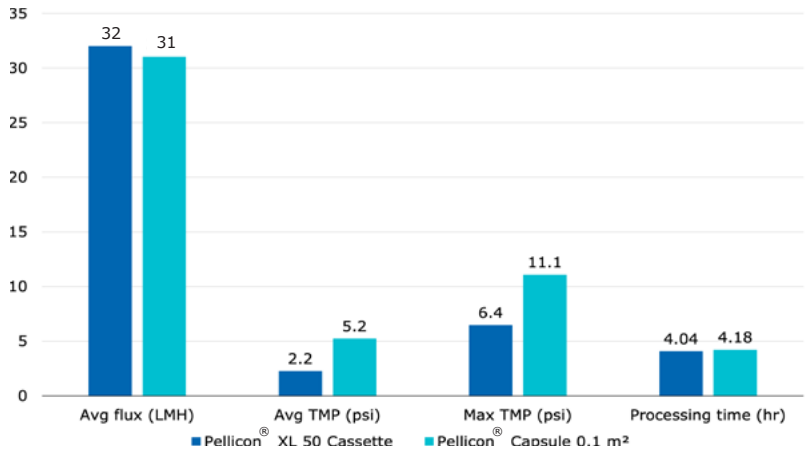

Figure 14. AAV flux and TMP scaling study: AAV2 60 L/m², 300 kDa membrane, permeate control, 4×/5 DV/2.5×.

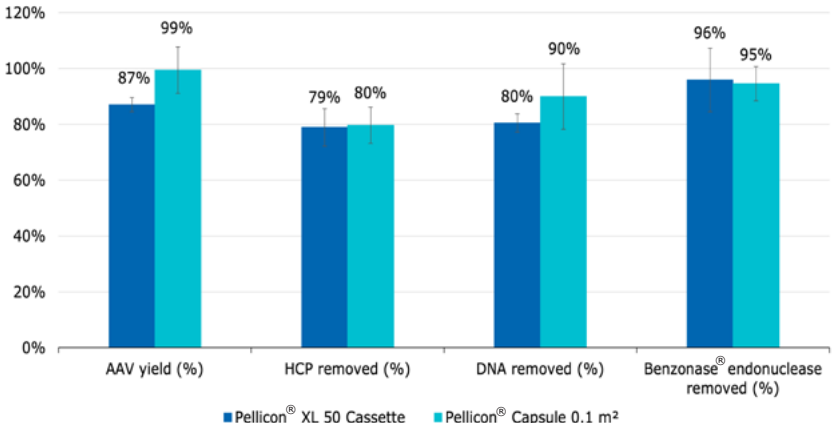

Figure 15. AAV yield and impurity reduction scaling study: AAV2 at 60 L/m², 300 kDa membrane, permeate control, 4×/5 DV/2.5×.

Discussion

Control Strategies Comparison

TMP-control systems are the simplest to run: they only require setting feed flow and the retentate valve to give a target TMP. When the operating point is set near the plateau, the flux will be close to the maximum at every point during the UF/DF process, naturally decreasing with concentration. This approach can give the shortest process time due to higher average flux. However, if the process becomes unstable or performance is too low while using TMP control, then restriction of permeate flow may be needed.

Permeate-control systems can restrict permeate flow to operate well below the minimum flux of TMP-control systems and reduce polarization at the membrane, which can improve performance such as impurity reduction. However, permeate-control systems require additional control elements over TMP-control systems (permeate pump or control valve) and could provoke an exponential rise in TMP at the critical flux, potentially preventing run completion due to feed pressure or TMP limitations.

In this study, based on 300 kDa membrane comparisons with Pellicon® Capsule and Pellicon® XL 50 cassette, TMP-control operation demonstrated a flux (or time) advantage as high as two-fold, which can cut required membrane area in half; while permeate-control operation demonstrated a higher impurity removal advantage in general. Results for virus yield were mixed: yield was similar for AAV2 when using both control strategies, but bacteriophage yield was generally higher for the permeate-control model feed studies.

Future studies can be run to optimize performance of each control strategy, which may influence preference for one control mode over the other. You should determine the best mode of operation using a feed stream and process conditions representative to your specific applications.

Scaling within Pellicon® Capsule and Cassette Families

Scaling of capsules and cassettes was demonstrated by comparing flux, TMP profile, yield, and impurity reduction using a virus model feed and AAV2. Our family of Pellicon® TFF filters enables linear scaling over a wide range of feed batch sizes. **Table 3** shows an example of how TFF batches up to 2,000 liters can be handled with single-use Pellicon® Capsules. If scaling to larger, multi-use batches is needed, switching to Pellicon® 2 cassettes with the same membrane and screen combination can be done seamlessly.

Table 3. Scale-up sizing with Pellicon® TFF filters. Example based on Figure 10 data (4×/5 DV/2.5×), except where estimates were extrapolated (*).

			Batch size (L) at loading (L/m²) of		
Filter	**Total Area (m²)**	**TFF System²**	**35**	**120**	**150**
Pellicon® XL 50 Cassette	0.0050	Cogent® Lab	0.18	0.60	0.75
Pellicon® Capsule	0.1	Cogent® Lab	3.5	12	15
	0.5	Mobius® TF2S	17.5	60	75
	1	Mobius® TF2S	35	120	150
	1.5	Mobius® TF2S	52.5	180	225
	3	Mobius® TF2S	105	360	450
	9	Mobius® TFF80	315	1080	1350
	13.5	Mobius® TFF80	472.5	1620	2025
Pellicon® 2 Cassette	80	Cogent® Process Scale	2800	9600	12000
		Permeate control process time (hr)	1.8	6.2*	7.7*
		TMP control process time (hr)	0.9	4.3	6.1*

Summary

Two different operating control strategies for UF/DF in the processing of viral vectors were evaluated: TMP control and permeate control. Both control strategies were successfully applied in process simulations with model feed and AAV2 to achieve 10× concentration and 5 DV diafiltration. TMP control was found to be simpler and faster, while permeate control maintained lower pressures and showed potential for higher impurity reduction. These trends were shown to be maintained during high feed loading evaluations (shown up to 120 L/m^2).

While using either control strategy, Pellicon® XL 50 cassette was shown to be an excellent scale-down tool for Pellicon® Capsules—flux, virus yield, and impurity removal levels for the capsule were comparable to the Pellicon® XL 50 cassette values. Scalability within the Pellicon® Capsule family was further demonstrated with sizes 0.1 and 0.5 m^2. For processes where conversion from Pellicon® 2 cassettes may be considered, performance comparability between Pellicon® 2 cassette and Pellicon® Capsule was also presented.

References

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2. Tangential Flow Filtration Holders & Systems, www.sigmaaldrich.com/TFF-systems

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