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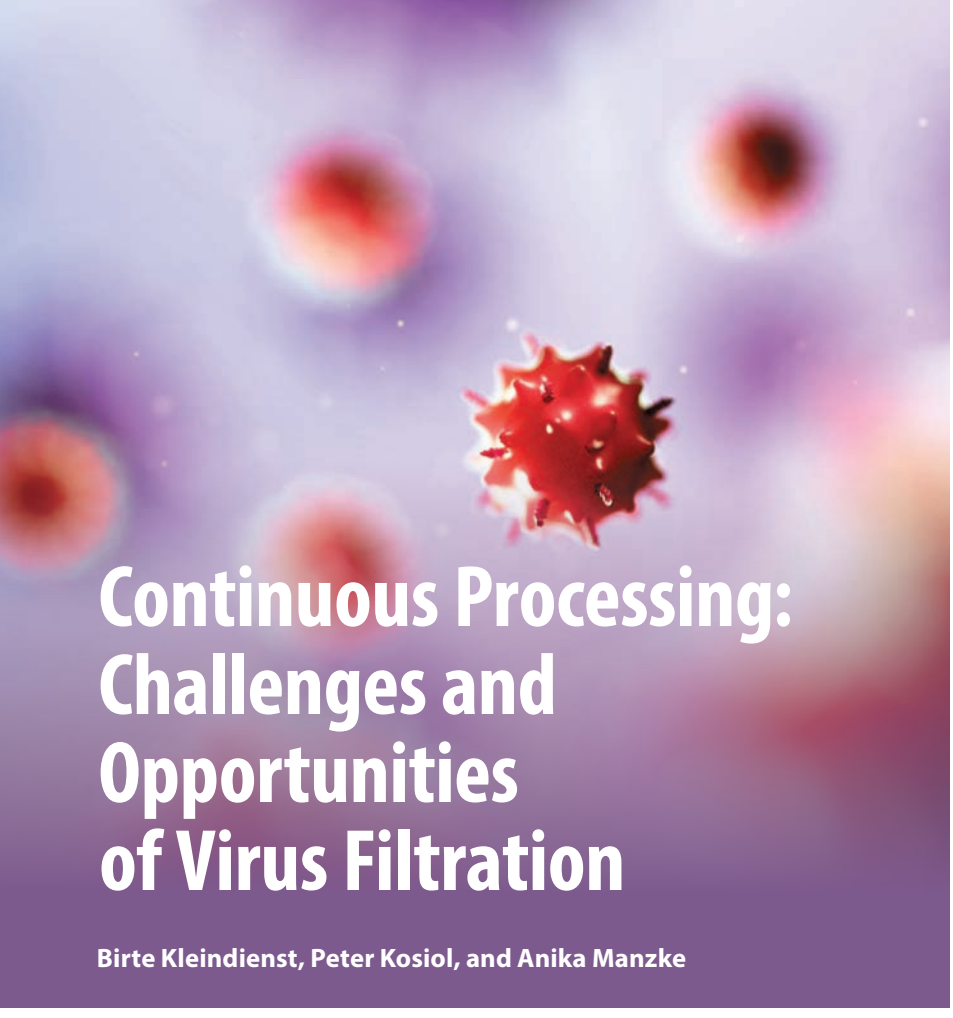

Continuous Processing: Challenges and Opportunities of Virus Filtration

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Requirements for virus filtration must be considered in developing continuous downstream processes.

Bioprocessing technologies have evolved rapidly and significantly during the three decades the biopharmaceutical sector has been in existence. Despite the success of operational improvement programs and measurable increases in productivity, biomanufacturing continues to face challenges (1). Increased cost, quality and production pressures, oncoming competition from biosimilars, and the growing importance of emerging markets and personalized medicines are creating the need for further evolution in bioprocessing technologies (2, 3).

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Steady-state conditions with continuous process approaches have been introduced to decrease cycle times, reduce capital and operating costs, and enable faster scale-up with more consistent quality and greater manufacturing flexibility (3, 4). At this point, end-to-end, fully integrated continuous processing has not been implemented outside the laboratory. Solutions are still being investigated for realizing enclosed, bioburden-free, fully automated, fully continuous processes from bioreactor to formulated drug product with global process control that run for long durations (1, 2). In the meantime, hybrid or semi-continuous approaches are being implemented by early adopters of continuous bioprocessing.

Batch vs. continuous virus filtration

Virus filtration is a crucial downstream processing operation that must be carefully considered when implementing continuous bioprocesses to ensure patient safety. There are several differences between batch and continuous virus filtration process parameters. The unit operations in batch mode typically last for four to six hours, while continuous processes can be performed for days. Operating pressures are also much lower during continuous virus filtration, and an adsorptive pre-filter is essential for the removal of potential aggregates that might lead to fouling of the virus filter. Batch systems are open with manual or semi-automated control, while continuous processes are closed, more complex, and highly automated. The feedstream for a batch process is homogeneous, but in continuous virus filtration, variability in protein concentration, pH, and conductivity from the elution peaks of the previous chromatography step will challenge the virus filter (1).

Design space of continuous virus filtration

In batch processing, it is known that protein concentration, pH, conductivity, buffer type, viscosity, additives, operating pressure, and pressure release times can affect virus filter performance. The question is: which of these process variabilities are relevant for continuous virus filtration? To begin answering this question, a design-of-experiment (DoE) study was conducted to define the design space for continuous virus filtration.

DoE. A full factorial DoE (2^3) was performed including a total of 10 experiments that varied the length of the run, the operating pressure, and either a monoclonal antibody (mAb) or buffer feed. Depending on the total length of each run, pressure was applied for 24 or 48 hours twice with a 30-minute pressure release after each filtration period as shown in **Figure 1**. For the 48-hour runs, an additional pressure release of 60-minutes was conducted. Fractions were collected in the beginning of each filtration and before and after each filtration pe-

Figure 1: Pressure profile over the virus filter during a design-of-experiment study.

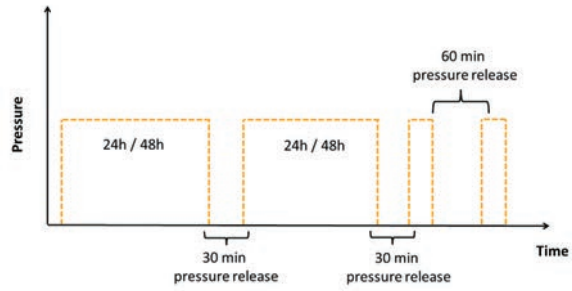
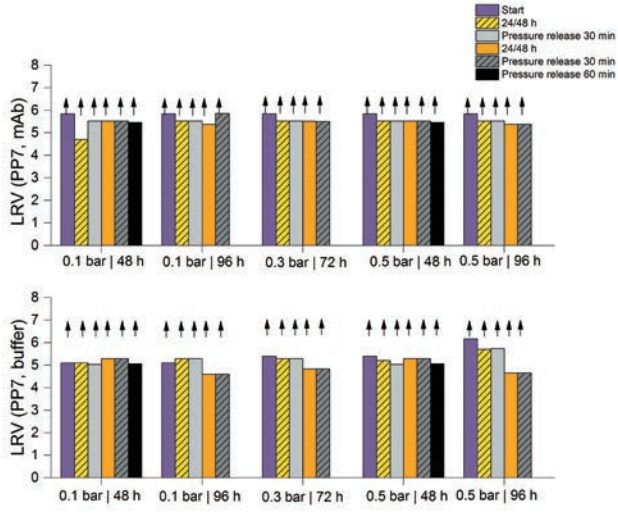


Figure 2: Design-of-experiment results of virus retentive bacteriophage PP7 capacities of a filter (Virosart HF, Sartorius Stedim Biotech), in either monoclonal antibody (mAb) or buffer feed, over the course of a continuous virus filtration. LRV is log_{10} reduction value.



riod and pressure release to evaluate any impacts of the pressure profile.

Filtration parameters. Because continuous virus filtration is operated at much lower flow rates, longer filtration times often involve longer pressure releases than are observed with batch filtration; these operating parameters were included in the DoE study. Although continuous filtration is typically run at constant flow rather than constant pressure, for ease of experimentation, a constant pressure range of 0.1 bar (1.5 psi) up to 0.5 bar (7.2 psi) was covered to represent a maximum of 25% of the flow used in batch operations at 2.0 bar (30 psi). Filtration times of 48 to 96 hours were used to keep the operating time within the normal five-day work week.

Virus model. *Pseudomonas aeruginosa* bacteriophage PP7 (ATCC 15692-B2), a single stranded, 20–25 nm, non-enveloped, ssRNA bacteriophage from the Leviviridae family, was used. PP7 bacteriophage is an established model system that is often used to evaluate the removal capabilities of virus filters (5). The filters were challenged with a minimum titer of 10^6 pfu/mL.

Product feed. A mAb feed (non-optimized after ion exchange chromatography at 0.3 g/L in 20 mM, pH 7.2 TRIS hydrochloric acid and 150 mM sodium chloride) and a buffer solution (20 mM KPI buffer, pH 7.2) were used to test virus retention in the presence and absence of protein to exclude the possibility of interactions between the mAb, the PP7, and the virus retentive membrane (commercial, down-scaled 1.7 cm^2 Virosart HF filter with a down-scaled 5.0 cm^2 Virosart Max adsorptive 0.1 µm inline pre-filter, both from Sartorius Stedim Biotech). It was determined upfront that the pre-filter did not remove bacteriophage PP7 in a significant amount.

Results. Results for the DoE study using the buffer and mAb feed are shown in **Figure 2**. Notably, in both cases, retention without any virus breakthrough was achieved over the entire filtration period for each experiment. Therefore, a robust log_{10} reduction value (LRV) of greater than four was achieved independent of operating pressure, pressure release time, and overall filtration time. The titer of PP7 bacteriophages declined over the course of 96 hours from 10^6 down to 10^5 pfu/mL, whereas the mAb feed seemed to stabilize the titer.

Separately, the stability of typical model viruses used for validation studies of virus filters was investigated under the long processing time present in continuous manufacturing. Simple infectivity tests were conducted for Minute virus of mice (MVM) and Murine leukemia virus (MuLV) in the buffer and mAb feed used for the DoE study. The results over 96 hours are shown in **Figure 3**.

MVM and MuLV infectivity decreased during the 96-h operation time. The decline in MVM infectivity of 0.5 LRV is within the variation of the assay. MuLV, a large enveloped virus known to be a less stable virus, showed a higher decrease of titer with 1 LRV.

Virus clearance validation

New ways of manufacturing, such as continuous processing, bring up new challenges for process validation. A representative feedstream for the virus validation studies needs to be defined. In addition, while the DoE results presented here indicate that filtration parameters do not have a significant impact on virus retention, such performance must be confirmed by end users under their specific process conditions. One possible approach is to conduct a DoE type of validation by identifying the critical parameters (e.g., concentration, flow, pH, conductivity) and then validating only the representative worst-case conditions.

The manner in which the virus should be spiked has also to be addressed. Typi-

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Figure 3: Infectivity of Murine leukemia virus (MuLV) (top) and Minute virus of mice (MVM) (bottom) over 96 hours in monoclonal antibody (mAb) and buffer feeds. LRV is log_{10} reduction value.

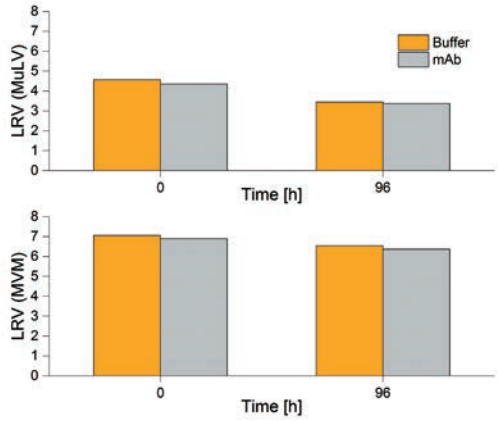
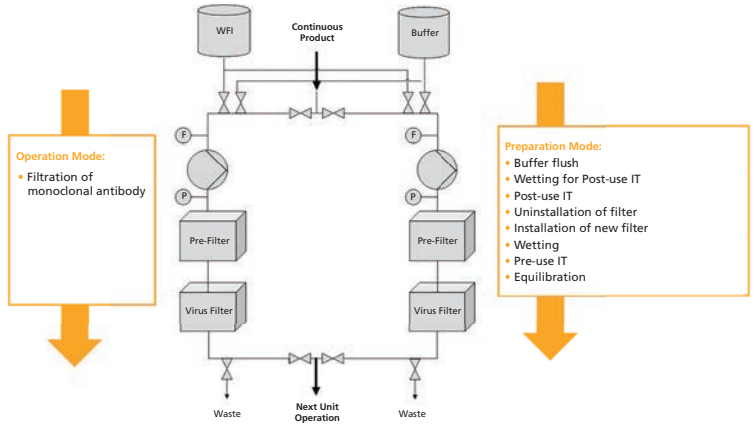


Figure 4: Possible process implementation of continuous virus filtration showing operation mode (left) and preparation mode with integrity testing (IT) (right).



cally, in batch processing, the "spike and run" method is used, in which the spike is added to the pooled feedstream prior to the virus filtration. This approach is difficult to realize with a continuous flow of product. Inline spiking for continuous dosing into the feed seems to be the most likely workable approach in the industry. This method can overcome the challenges of loss virus infectivity over time because fresh virus can be continuously introduced. Inline spiking involves a complex setup and equipment, however.

Numerous other challenges for validation of continuous virus filtration must be addressed, such as the use of an inline pre-filter and potential filter blockage by the feedstream and/or virus itself with increasing volume.

Process implementation

One possible process implementation for virus filtration in continuous processing is to use a set-up with two filtration lines that can be operated independently of each other in a preparation mode or operation mode (2, 6), as shown in **Figure 4**. Each line has a pump, flow and pressure sensor, adsorptive pre-filter, virus filter, and buffer and water-for-injection supply. Steps such as flushing, equilibration, filtration, buffer flush, wetting for integrity tests (IT), and IT are performed in preparation mode, whereas the product filtration is performed in operation mode. Ideally all valves would be fully automated, and implementation would be achieved in a sterile manner to avoid the need for steam-in-place and clean-in-place operations.

Passed IT of virus filters are essential in order to release a batch, which is a challenge in continuous processing (7). Risk assessments have to be performed in order to minimize the risk of failed post-use IT in production. Some potential approaches like conducting pre-use, post-sterilization IT (PUPSIT) on all filters are currently discussed in the industry to mitigating the risk. This approach could potentially be incorporated into an end-to-end, integrated continuous process from bioreactor to fill/finish.

Conclusion

In this study, the design space for continuous virus filtration was defined with respect to filtration parameters, and parameters such as low flow rates, long filtration times, and increased pressure releases showed no impact on the filter tested. Commercially available virus filters can be run in continuous mode. Although some challenges for validation of continuous virus filtration must still be addressed, parallel filtration lines that allow in-line filter testing are one concept for allowing implementation of continuous virus filtration in commercial manufacturing.

Acknowledgements

The authors would like to thank Magnus Warnke and the bacteriophage team from Sartorius for conducting the studies.

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