Summer 2012 • Volume 11 / Issue 2 • ISSN 1538-8786

BioProcessing JOURNAL

Trends & Developments in BioProcess Technology

A Production of BioProcess Technology Network

BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Process Development Toward a Robust and Economically-Sized Virus Filtration Process

By WILLEM KOOLS

Mammalian cell culture processes used in the production of monoclonal and recombinant proteins require orthogonal steps in the downstream process to clear virus. Virus filtration is a common, size exclusion-based method in these processes. Initially, tangential flow products were used, but as a result of new-generation virus filtration products, most operations are now constant-pressure, normal-flow operations that can be executed in a single shift operation. The virus filtration step toward the end of the downstream process is now yielding increased protein concentrations due to:

- Newer chromatography resins with higher binding capacities and reduced elution volumes resulting in elution pools at higher concentration
- New flow-through chromatography products that no longer require dilution in the polishing step

Beyond the protein higher concentration, we see increasingly higher mass throughputs in the downstream process due to increasing bioreactor titers. These trends are making it important to select a robust virus filter that can handle those increased protein titers in the purification process.

Understanding virus filtration in its entirety is critical in designing a robust and economical process. Economic analysis of virus filtration is frequently based only on filter sizing followed by a simple calculation for filter cost per area of the selected filter. A more comprehensive approach includes an analysis of economically-rationalized safety factors such as feed, process, and membrane variability as well variable costs associated with setup, processing, and product recovery.

This article discusses several aspects of virus filter performance: optimization direction, possible artifacts, and variability characterization leading to economical yet robust virus filtration.

Vmax Methodology

Virus filtration is a normal-flow filter operation that can be well described by a partial pore blockage model. EMD Millipore's Vmax model is a way to identify the initial flux and total capacity that can be processed through the membrane—two critical parameters that define proper sizing.

Figure 1A shows a graph that depicts what is measured, the volume per surface area as a function of time; while Figure 1B is a rearranged depiction often used to define total capacity (V_{max}) (inversely proportional to slope)

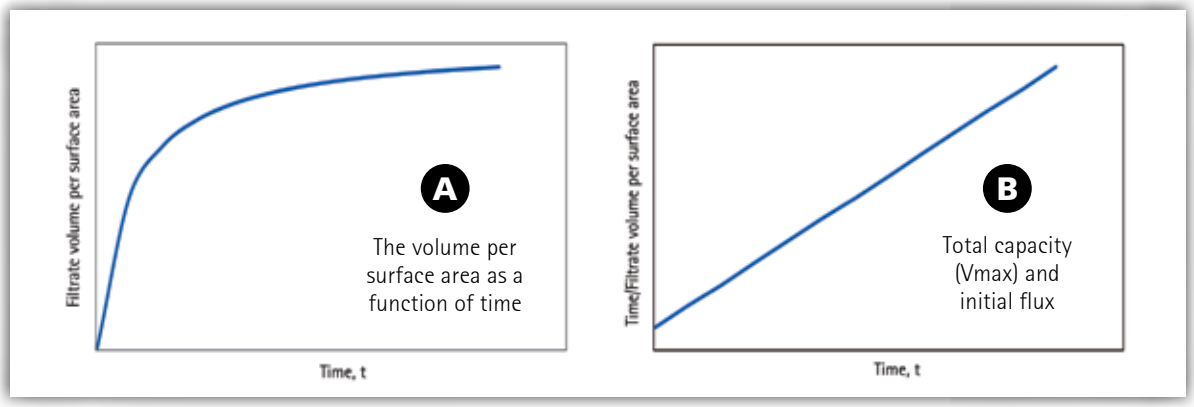

FIGURE 1. **Vmax methodology.**

ABOUT THE AUTHOR

Willem Kools, PhD (Willem_Kools@merckgroup.com) is Director, Biomanufacturing Science Network—North America, EMD Millipore, 900 Middlesex Turnpike, Billerica, Massachusetts 01821 USA; Phone: +1 781-533-2068

and initial flux (Q_i) (inversely proportional to intercept). Extrapolation of volume filtered with maximum time (∞) provides the maximum volume that can be processed through that filter. In the actual experiment, a protein solution is pressurized at constant pressure and filtered through the test filter. The resulting curve will reveal an initial large flux, Q_i, which slowly reduces. In combination with design process time (t_B) and design volume (V_B), the equation below can be used to define the minimum area, A_{min}, required for the intended process:

$$A_{\min} = \frac{V_B}{V_{\max}} + \frac{V_B}{Q_i \times t_B}$$

Typical performance of virus filters can be depicted on the Vmax nomograph (Figure 2).[1] The x-axis is defined by the capacity-driven limitations on processing performance while the y-axis is the initial flux × the process time. Initial flux is defined by permeability of the membrane, the operating pressure over the membrane, and the viscosity of the fluid. Lines of equal area requirements are provided in order to compare different membrane products with distinct different characteristics in terms of initial flux and capacity. Process optimization leads to the smallest installation needed to process a batch robustly, as shown in the upper right corner of the nomograph (Figure 2). This can be achieved by increasing permeability (filter selection), lowering viscosity (feed modification), higher operating pressures, or longer process times (y-axis). Another way to optimize this process is to look at a prior purification or do a prefiltration (x-axis).

Two major types of virus filtration technologies are commercially available. One represents the fairly low-flux (LF) membrane devices, some of which might have high calculated capacities. The other type is the high-performance (HP) filter, with significantly greater permeabilities and the possibility of lower capacities.

For the LF classification of virus filters, optimization is performed by extending process times and then operating at higher pressures or lowering viscosity. LF-type virus filters are often flux-constrained. For HP virus filters, optimization can be performed through flux and capacity. Higher loadings will improve overall economics of the process. HP type filter sizing is often based on a capacity component as well as a flux component.

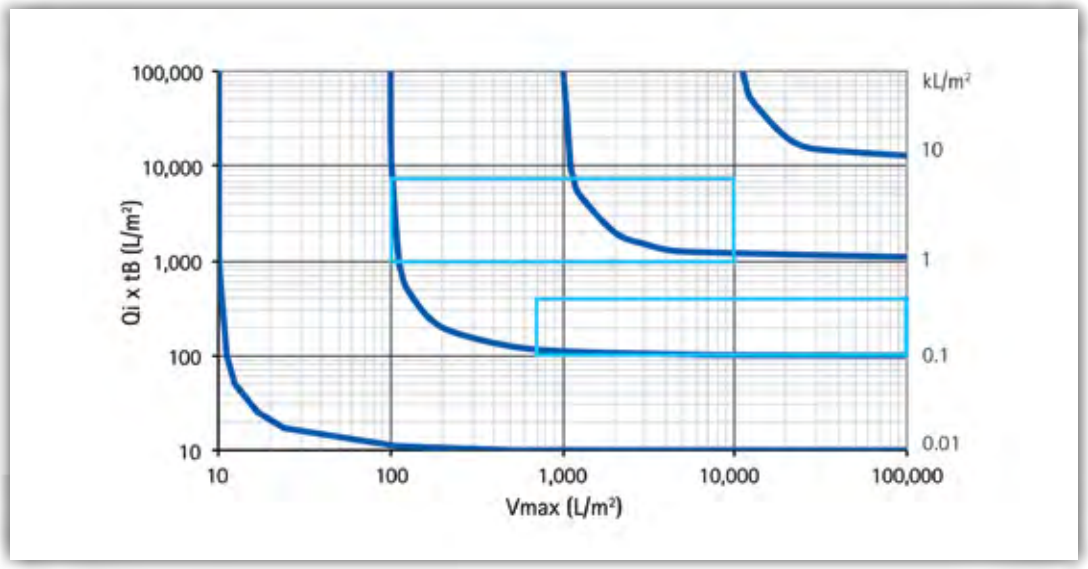

FIGURE 2. **With the Vmax method, the typical performance of virus filters can be depicted.** The x-axis is defined by the capacity-driven limitations on processing performance while the y-axis shows the initial flux × the process time. Process improvements can shift filter performance to the right upper corner of the graph.

Creating a Robust and Economical Process

Robustness is tied to performance variability. Understanding your process variability is important. Variability can come from: (a) the feed material; (b) process set-point choices; and (c) membrane device choices. Once these fundamentals are understood, certain aspects of variability can often be controlled, while others can be characterized. Care must be taken to avoid artifacts that cause deviations from process performance in small-scale testing.

As virus filtration is often done at the end of the

purification train, possible root causes for performance differences related to the feed material are product-specific foulants like aggregate levels and viscosity. Aggregate levels and viscosity might be influenced by high concentrations, choices used in process buffer components (stabilizers, conductivity, *etc.*) and pH. Since protein mass trends high, it may be advantageous to look at final mass loading (kg/m^2) instead of dilution which could lead to lower viscosities.

Known artifacts in virus filtration performance deviations following standard manufacturing-scale process outcomes are typically: (a) capacity reduction due to the effects of age or freeze-thaw; (b) aggregation due to excessive air contact (*i.e.*, splashing of liquid in handling of feed); and (c) the impact of foulants in virus preps during viral validation.

By being familiar with the root causes of variability and artifacts in the feed, mitigation strategies can be created (*e.g.*, for aggregate levels, purification control of prior unit ops to lower aggregate levels, implementing an adsorptive prefilter,[2,3] and selecting the right buffer components and solution conditions [for conductivity and pH]). An adsorptive prefiltration has also been used to remove artifacts due to freeze-thaw, excessive air contact, or age of protein solutions in small-scale experiments. Another mitigation strategy includes using recently commercialized clean virus preps at various contract testing organizations to avoid the excessive flow decay artifact due to foulants in the virus prep.

Once critical parameters in feed are controlled, the remaining variability can be characterized in terms of required area associated with potential feed variations.

Example Feed Variability: pH and Conductivity Mapping

Figure 3 shows the effects of pH and conductivity, as well as prefiltration. Figure 3A is an example of Viresolve® Pro (EMD Millipore) with a very high aggregate level containing polyclonal IgG solution. The performance is very uniform across the board while the overall performance is very poor. Purification levels can be boosted by using an adsorptive prefilter. By doing so, one can identify an optimal area requirement at a pH of 5.5 and a conductivity of 10 mS/cm for the Viresolve Pro+ solution (Figure 3B).

Figure 4 demonstrates one example of the impact that pH and conductivity can have on loadings with lower aggregate-levels in monoclonal antibody solutions. In this case, again an optimized process window can be defined where the capacity is about 8–10 kg/m^2.

Some companies have process templates in place, for which not only the virus filter should work for a specific molecule but for a whole range of molecules. For a template process, it is useful to have various molecules

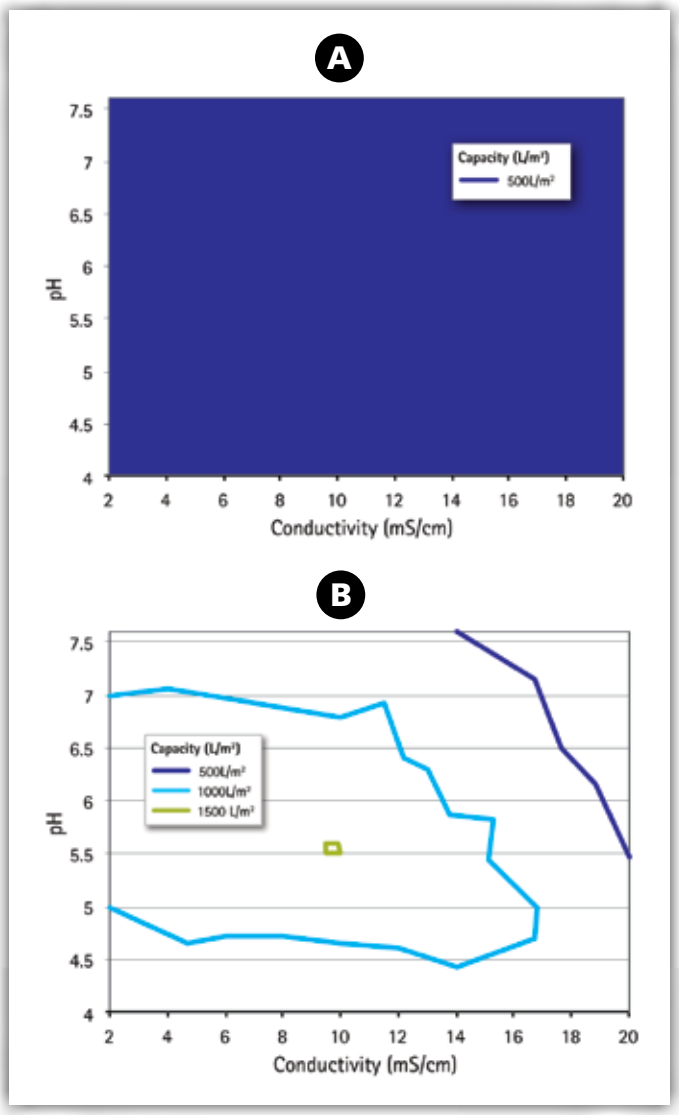

FIGURE 3. **Effects of pH, conductivity, and prefiltration.** (A) performance of Viresolve Pro solution only; and (B) performance boosted by the use of an adsorptive prefilter (Viresolve Pro+ solution).

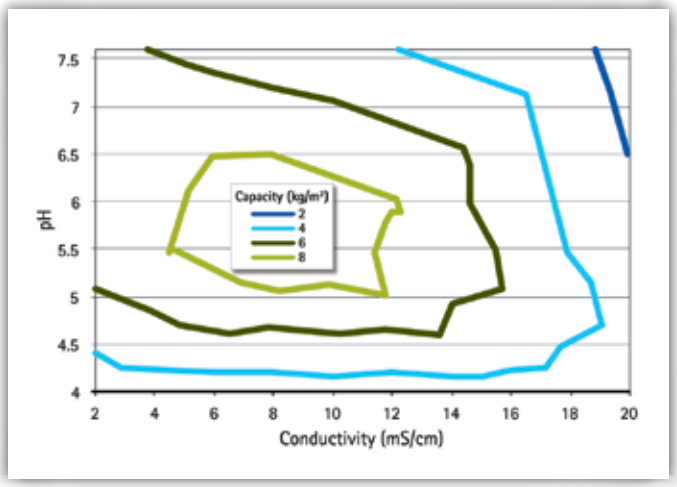

FIGURE 4. **Effects of pH, conductivity, and prefiltration** for a lower aggregate-level monoclonal antibody solution.

screened to assess the typical performance of the virus filter. Figure 5 shows a distribution of performance based on over 400 data points with a variety of proteins, protein concentrations (concentration ranging from 1.8–50 g/L with an average of 12.6 g/L), and positions in the downstream process including experimental results based on processing artifacts. Without specific optimization or exclusion of artifacts, we observed a capacity in excess of 5 kg/m^2 for most cases over a very wide range of proteins and different process steps. In most template processes however, optimized conditions and positions in the purification train are used, leading to the expected outcome of improved distribution of performance.

Example Feed Variability: Artifacts

Inevitably, artifacts are going to occur. Figure 6 shows an example of the freeze-thaw effect on capacity with a 10 g/L monoclonal antibody feed. Without adsorptive prefiltration, 50% of the capacity was lost. With adsorptive prefiltration, a more robust performance, in terms of capacity, was achieved and no significant artifact effect was noticed on area requirements. It is clear that feed artifacts need to be minimized as much as possible during process development as they do have a significant impact on predicted area requirements.

Process Variability

In terms of process variability, one can consider design vs. actual volume, possible pressure fluctuations or pressure limitations (*e.g.*, maximum pressure), and any other manipulations at large scale (*e.g.*, mixing). Inconsistencies in these process parameters can often be explored at small scale. If differences exist at small and large scale, the material representing large scale is used. Preconditioning steps are used for small-scale experiments that are similar to the large-scale experiments. Variations in the parameters stated previously will usually lead to inconsistent performance. With a solid understanding of the upstream process, downstream variability can be mitigated by setting the proper process controls and selecting the appropriate system. With proper selection of virus filter and system, variability due to process effects is often small.

Membrane Device Variability

With optimization of feed and process, the proper membrane selection comes into play. Variability will depend on the design of that membrane and module by the virus filter vendor and product-specific variability can be assessed and included in a safety factor analysis.

Table 1 briefly summarizes the results from one of the few published studies comparing different membrane devices. They were characterized with a BSA model stream to determine just how much variation there was.[4] The five different membrane products varied in performance

TABLE 1. **Robustness:** variability due to membrane/device.*

Membrane Type	Variability in Throughput (L/m^2)		
	Within Lot	Between Lots	Total
Membrane 1	Medium	High	High: (40%)
Membrane 2	Low	Medium	Low–Medium: (15%)
Membrane 3	High	Very High	Very High: (50%)
Membrane 4	Low	High	Medium: (20%)
Membrane 5	High	Very High	Very High: (60%)

Low: 0–10%; Medium: 10–15%; High: 15–40%; Very High: >40%

Minimal public information

*Adapted from: Biopharmaceutica Production Week (Carlsbad, CA), *Systematic evaluation of virus removal by parvovirus nanofilters*, Dayue Chen, Lilly Research Laboratories, Eli Lilly & Co. (BSA as model stream).

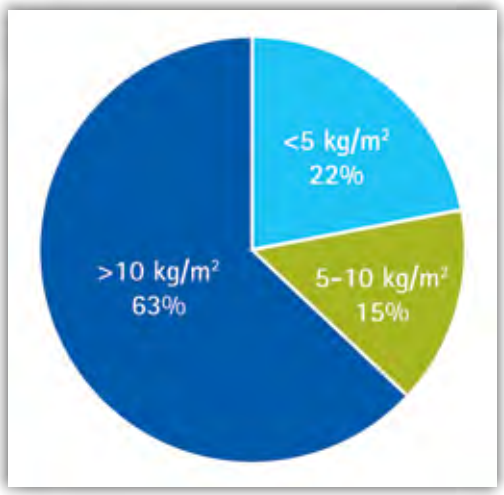

FIGURE 5. **Template "friendliness"** for a range of proteins and process steps.

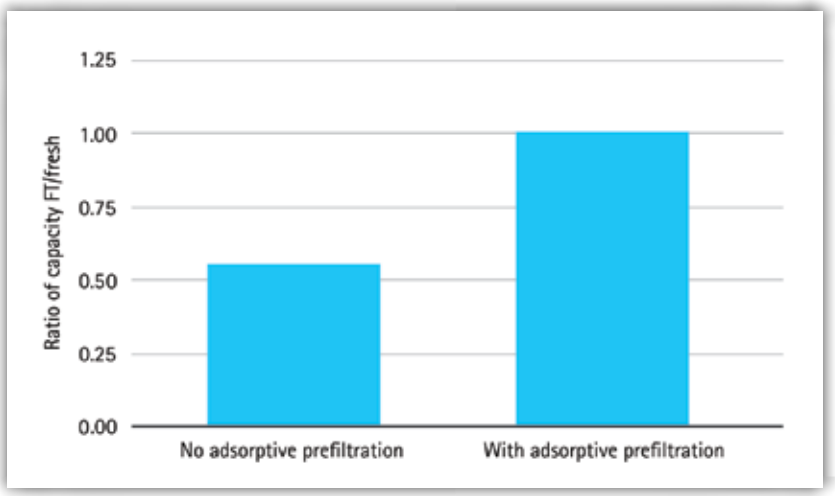

FIGURE 6. **Effect of freeze-thaw** on capacity with a 10 g/L monoclonal antibody feed.

from between 15–60% of the feed coming through the membranes and devices. Selecting the appropriate filter module design and vendor will help mitigate the range of variability in any comparable downstream process.

Figure 7 illustrates how limited variability in permeability and capacity can be achieved through selective layering with a two-layer virus filtration device.[5] Figure 7A shows two low-performing permeability device layers in a two-layer device with two high-permeability layers in the device. Without a layering approach, a significant variation can occur. With a selectively-layered strategy of using various combinations of high- and low-performance membrane layers, a preferred range of averaged performance can be engineered into the product. A similar phenomenon is achieved for capacity (Figure 7B) where a variation in capacity for 75% flow decay is shown to be reduced from about 40% for non-selectively layered devices to about 10% for selectively-layered devices.

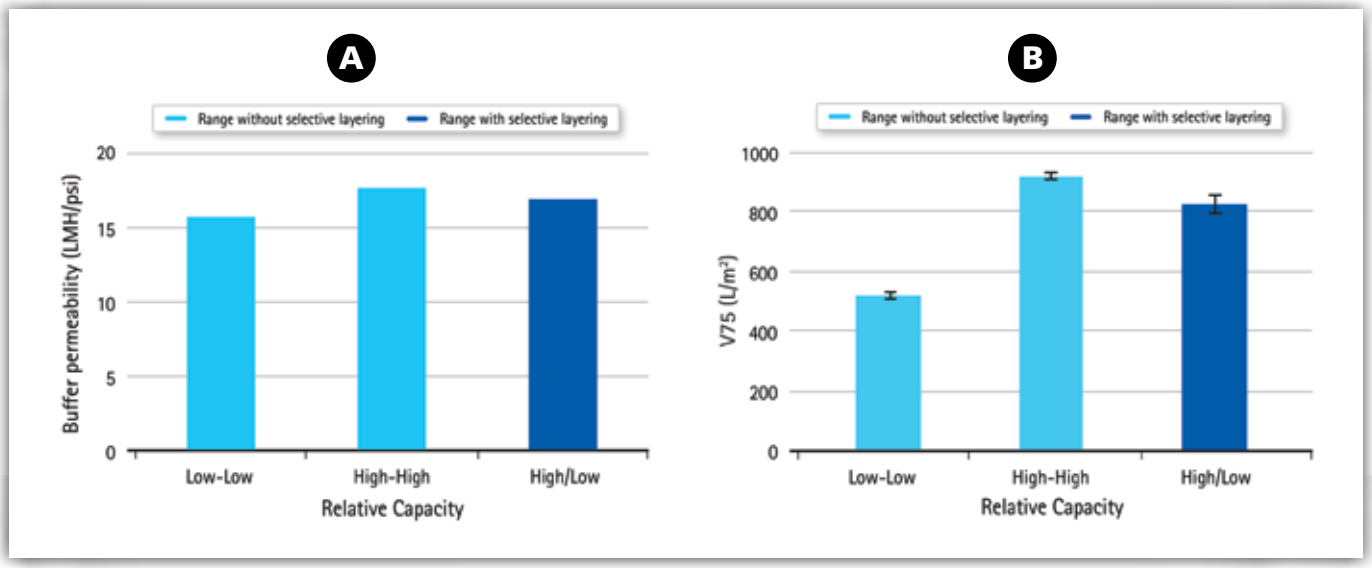

FIGURE 7. **Layering of membranes** to reduce variability in permeability and capacity.

Safety Factors

The variability of required surface area due to feed, process, and membrane performance can readily be assessed by performing Vmax experiments and then quantified in a coefficient of variation (COV) around required membrane surface area.

One could include this variation directly into a safety factor and significantly oversize the filter installation. This will in turn increase the operational-variable costs by necessitating additional: (a) virus filters; (b) preconditioning buffers; and possibly (c) integrity test materials. If however, insufficient membrane surface area is installed, a portion of the batch might be lost as it cannot be processed within the validated range or, in case of leading to a nonconformance, the entire batch might be lost. This can become very expensive in terms of the cost of work-in-progress and associated quality control strategies.

Cost-ratio (CR), an important parameter, is the balance between cost of failure and total variable cost associated with additional membrane area:

$$CR = \frac{C_{failure}}{C_{variable}\overline{A}}$$

Using Lutz' analysis towards economically-rationalized safety factors,[6] the variability and its impact on economics can be expressed readily. The safety factor (SF) will be the ratio between installed surface area (A´) and the average minimum required surface area ($\overline{A}$) to process a batch volume in a certain batch time:

$$SF = \frac{A'}{\overline{A}} = 1 + COV\sqrt{2\ln\left(\frac{CR}{\sqrt{2\Pi}COV}\right)}$$

As the virus filtration process is often operated towards the end of the downstream process, the typical CR for virus filtration is relatively high (5–90).

Using the variabilities described in Table 1, Figure 8 shows that for the LF-type filter (with 20% variability) and "membrane 2" HP-type filter (with 15% and 60% variability), performance safety factors of about 1.6, 1.5, and 2.5 (respectively) are derived. These significant differences in SF might trump the differences seen in average performance and hence could be taken into account for filter selection.

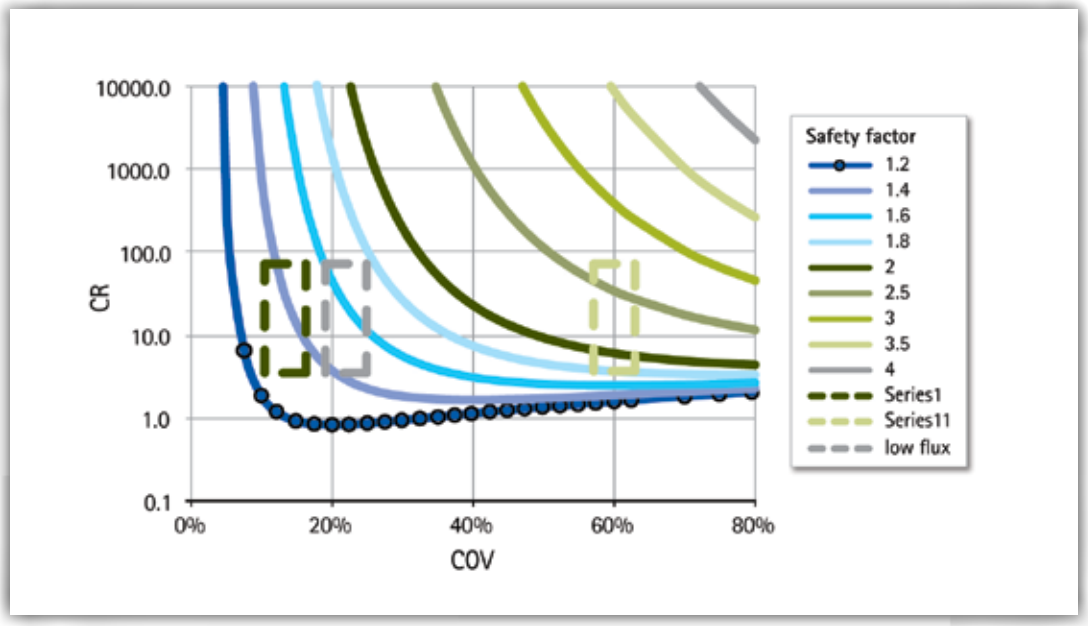

FIGURE 8. **Resulting safety factors** for combinations of COV and CR.

Conclusion

Determining the best selection of virus filters takes into account:

- Defining the average filter performance (initial flux and capacity) and how it can be improved (*i.e.*, position of filtration step in downstream process, adsorptive prefiltration, high membrane permeability)
- Understanding the variability in the process (feed, process, and membrane device)
- Avoiding artifacts in process development (fouling virus preps, freeze-thaw effects) that could influence accurate sizing

Understanding virus filtration in all these aspects will lead to robust and economical processes.

REFERENCES

[1] Raghunath B *et al.* The Vmax Nomograph: A Quick Reference Tool to Estimate Filter Sizing. *BioProcess International 2006* 4(7): 56–57.

[2] US Patent 7,118,675B2, Siwak M *et al.* Process for removing protein aggregates and virus from a protein solution.

[3] US Patent 7,465,397B2, Siwak M *et al.* Process for removing protein aggregates and virus from a protein solution.

[4] Chen D. Systematic evaluation of virus removal by parvovirus nanofilters. *BioPharmaceutical Development & Production Week,* March 1-2, 2010.

[5] US20090277835 A1, Giglia *et al.* Method for reducing performance variability of multi-layer filters.

[6] Lutz H. Rationally defined safety factors for filter sizing. *Journal of Membrane Science* 2009 341: 268–278.