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Performance Guide

Process Recommendations and Scalability Strategies for Optimization of Perfusion Cell Culture with the Cellicon® Cell Retention Solution

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Outline

Introduction

In biopharmaceutical manufacturing, the primary objective of the upstream process is to produce as much high-quality therapeutic protein as possible in an efficient and cost-effective manner. Conventional strategies utilize fed-batch processes to grow cells that are engineered to produce the protein of interest in a bioreactor. The cells are maintained in a controlled environment and periodically provided feeds that contain nutrients to encourage cell growth, productivity, and high viability for as long as possible before harvesting and purifying the product. To further increase upstream process efficiency, perfusion processes can be implemented. Here, spent cell culture media is continuously removed, while fresh media is added. Since nutrients are continuously supplied and cell waste products are removed, perfusion creates a more optimal environment for cell growth, viability, and protein expression. Compared to fed-batch processes, perfusion can achieve up to 10x higher cell densities, stable process durations over 30 days, and greater total protein yield. Furthermore, these benefits can be amplified with continuous or intensified downstream processing technologies.

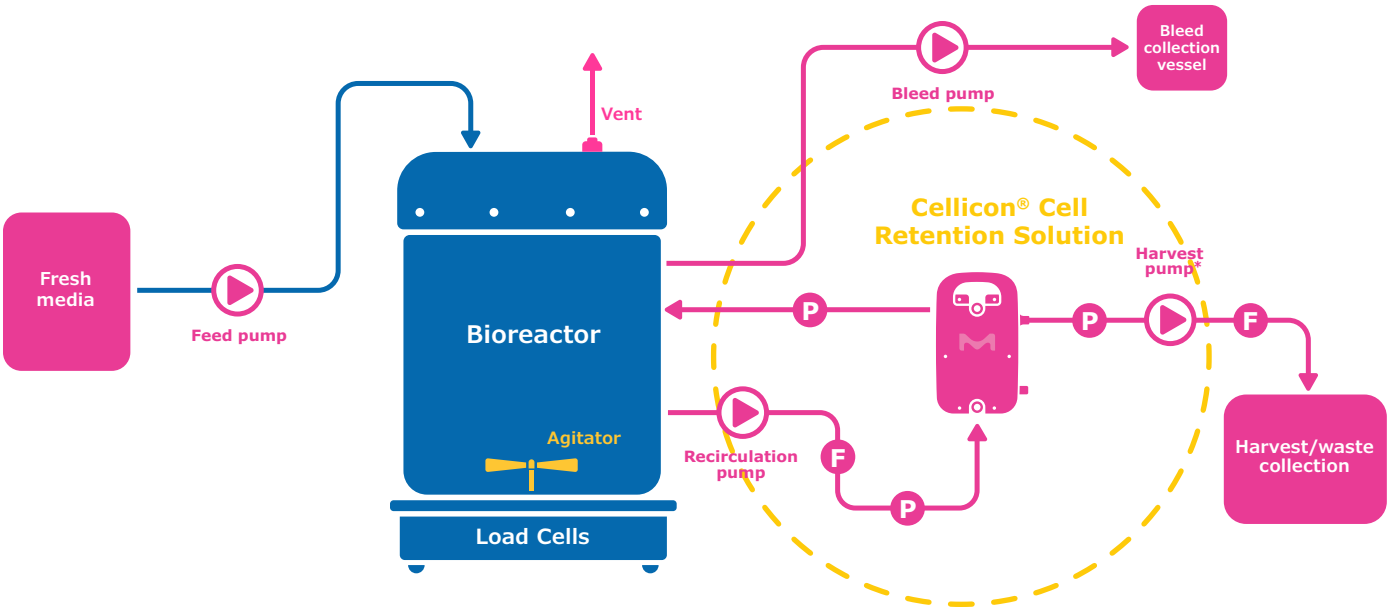

Figure 1. Schematic of perfusion bioreactor, including the cell retention device, circled in yellow. *harvest pump not included in lab scale Cellicon® Cell Retention Solution

The implementation of perfusion relies on a cell retention device to remove spent media and waste products while also retaining the cells within the bioreactor. Cell retention devices (CRDs) commonly utilize a membrane filter in tangential flow mode for separation of these elements, keeping cells inside the system and allowing spent media containing the protein of interest to pass through. Membrane filtration is an optimal strategy for this application due to the efficiency of separation, the effectiveness and fit with continuous processes, and the ability to perform scale-down studies prior to full-scale implementation.

This document is intended to provide detailed operational recommendations and scalability strategies for the Cellicon® Cell Retention Solution, a technology designed to simplify perfusion processing.

Background on Cellicon® Cell Retention Solution

The Cellicon® Cell Retention Solution is designed for simplified process intensification by enabling perfusion with the Cellicon® Filter Assembly and the Mobius® Cell Retention System. The filter sizes are intended to span the range of single-use bioreactors across lab scale (3 L) and process scale (50 – 2000 L). The gamma-irradiated, single-use Cellicon® Filter Assembly (Figure 3) consists of a flat sheet tangential flow filter (TFF) with a 5-micron Durapore® membrane, levitating centrifugal pump head, pressure sensors, perfusate flow sensor (process scale only) and CPC Aseptiquik® connectors (process scale only). The Cellicon® Filter Assembly is easily installed on the Cellicon® Perfusion Controller (lab scale) and the Mobius® Cell Retention System (process scale) within minutes. (Figure 4). The CPC Aseptiquik® connectors and thermo-weld compatible tubing allow for flexibility of aseptic connection to any bioreactor. The Mobius® Cell Retention System consists of a Levitronix® centrifugal pump drive (feed), Watson-Marlow peristaltic pump (perfusate), non-invasive Levitronix® feed flow meter, LEVIFLOW® converter for measuring perfusate flow, and PendoTech single-use pressure sensor cables. The Mobius® Cell Retention System is provided with Bio4C ACE™ application control software that is optimized for perfusion cell culture with real time process monitoring and control.

Figure 2. Cellicon® Cell Retention Filters. Pictured are the 3 L, 50 L, 200 L, 500 L, and 1000 L sizes.

Figure 3. Cellicon® Cell Retention Filter assemblies. Pictured are the 3 L and 50 L versions.

Figure 4. Lab-scale Cellicon® Cell Retention Solution and rendering of the Cellicon® Filter Assemblies installed on the Mobius® Cell Retention Systems. Pictured are the 3 L, 50 L, 200 L, 500 L, 1000 L, and 2000 L versions. For the Mobius® Cell Retention System 2000 L, 2 x 1000 L Cellicon® Filter Assemblies are used.

Critical Process Parameters

Critical process parameters are factors that significantly influence the performance of the Cellicon® Cell Retention Filter and can be adjusted to impact the results. This section will describe how the crossflow rate, shear rate, filter flux, cell density, and viability impact filter performance.

Crossflow Rate

The crossflow rate is the liquid flow being taken from and returned to the bioreactor vessel after passing through the cell retention filter, as facilitated by the centrifugal feed pump (Figure 5). The tubing line that connects the bioreactor to the fluid entrance of the cell retention filter is described as the “feed line”, and the tubing line that connects the fluid exit of the cell retention device to the bioreactor is described as the “retentate line”. The cell culture fluid will continuously recirculate through this loop over the duration of the process at a set crossflow rate.

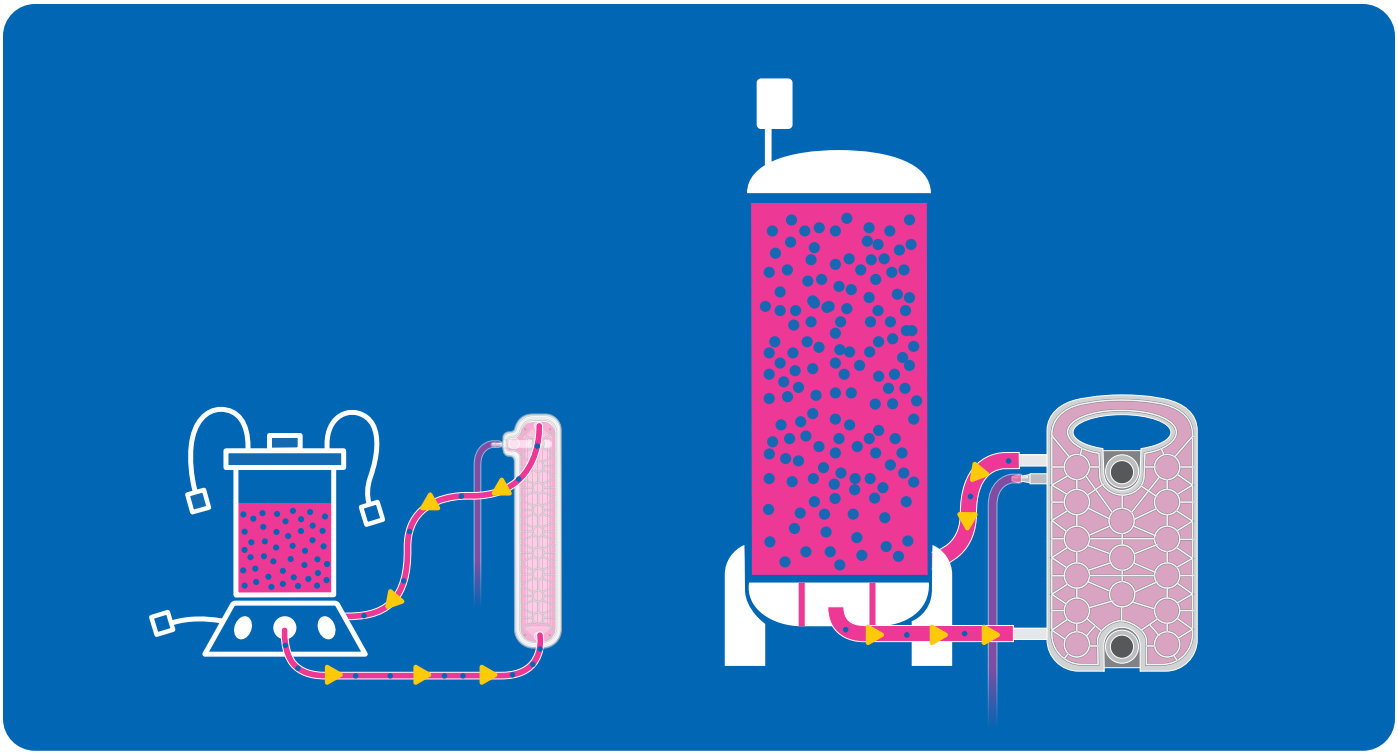

Figure 5. Drawing of cell culture fluid passing through the lab scale (left) and process scale (right) Cellicon® Cell Retention Filters, with yellow arrows indicating the direction of flow.

The crossflow rate is a critical parameter for tangential flow filtration as fluid flowing parallel to the membrane provides continuous sweeping of the membrane surface preventing particle accumulation (Figure 6). If the crossflow rate is not high enough, the larger particles within the cell culture can get stuck on the membrane surface and cause fouling through the formation of a cake layer. Therefore, it is important to select a crossflow rate that can provide sufficient sweeping of the membrane surface to prevent premature cake fouling.

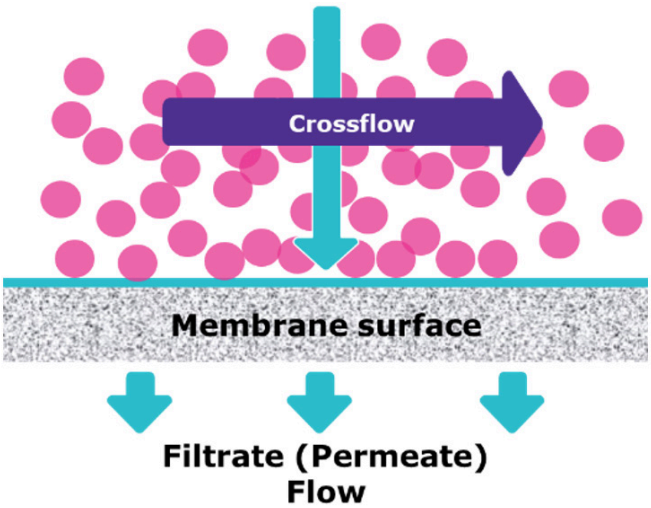

Figure 6. Representation of the fluid passing over the membrane surface with cells (pink) retained, while spent media and waste products pass through the membrane pores.

Shear Rate

Shear stress within the cell retention filter is a mechanical force experienced by the cell membrane from the friction of liquid within the feed channel due to the velocity gradient perpendicular to the membrane surface.[1] This force is highest at the surface of the membrane, or the "wall" of the filter, and should be maintained as one of the critical process parameters. While a minimum threshold crossflow rate is needed to prevent cake fouling on the membrane surface, a maximum threshold also exists as the crossflow creates a continual shear stress at the membrane surface that could potentially have negative effects on cell health. Shear rate in the Cellicon® Filter is a function of channel height, channel width, and crossflow rate. The Cellicon® Filter targets a shear rate of 2500 s^{-1} to provide adequate sweeping and prevent membrane fouling while remaining safe for cells. The targeted shear rate of 2500 s^{-1} is experimentally within a "safe" range for most cell lines, but there is variability across cell lines regarding shear tolerance and this parameter can be tuned accordingly. Excess shear on the cells can affect cell growth, viability, productivity, or product quality within the process and reduce filter performance.

Device	Plate and Frame (slit flow) half slit height x=0 to B
Shear	$\frac{3(v)x}{B^2}$
Wall Shear	$\frac{3(v)}{B}$

Table 1. Wall shear calculation in terms of flow velocity, v, and channel height, B, within a flat sheet cell retention device.[1]

Because mechanical shear forces can also be imparted by other areas of the system, the Cellicon® filter and assembly were designed to minimize these effects. For example, the inner diameter of the tubing through the feed channel was selected to maintain a shear rate through the tube of less than the wall shear at the membrane surface. Therefore, care should be taken to avoid attaching the assembly to external tubing that has an inner diameter smaller than that provided on the Cellicon® filter assembly.

The mechanical action of pumps can also introduce shear on cells. To minimize this effect, a Levitronix® centrifugal pump is utilized in the filter assembly to provide low shear pumping through the feed channel. Centrifugal pumps provide lower cell shear than alternative pump types, such as peristaltic pumps.[2] Higher pump speeds will result in higher shear rates, therefore monitoring RPM throughout a run is recommended. The pump sizes for each scale were chosen to achieve the target flow rates while minimizing pump shear by keeping speeds within the lower range of the pump's capacity.

Flux

Flux is defined as the normalized flow rate of liquid passing through an area of membrane. In the Cellicon® filter, the flux is calculated using the flow rate of the cell-free perfusate flowing through the total area of membrane in each filter size.

$$\text{Flux[LMH]} = \frac{\text{Flow Rate } \left[\frac{\text{L}}{\text{hr}}\right]}{\text{Filter Area } [\text{m}^2]}$$

Flux is a critical process parameter because it impacts the rate of filter fouling. At lower fluxes, the filter will foul slower than at higher fluxes. To optimize performance, care should be taken to select a perfusate flow rate that is as low as possible and less than the maximum recommended flux for the filter.

Typically, since spent medium is removed through the perfusate, the perfusate flow rate also correlates with the rate of addition of fresh medium to the bioreactor. Therefore, the filter flux impacts the rate of nutrient addition as well as the removal of the waste and protein product. Reducing the perfusate flow rate to the minimum amount to support the needs of the cells, reduces media consumption and extends the filter performance.

One common approach to selecting a perfusate flow rate is to choose a minimum or target cell specific perfusion rate (CSPR). The CSPR is the volume of fresh media that is provided to each cell within the culture over a period of time and is commonly reported in units of pL/cell/day. Once a target CSPR is defined, it can then be used to select an appropriate perfusate flow rate, either through a control loop or through manual setpoint changes over the duration of the run. As described above, the flux on the cell retention device should be monitored to ensure it does not exceed the recommendations provided to reduce filter fouling.

$$\text{CSPR}\left[\frac{\text{pL}}{\text{cell} \bullet \text{day}}\right] = \frac{\text{VVD } [\text{days}^{-1}]}{\text{VCD } [\frac{\text{cells}}{\text{mL}}]} \bullet 1\text{E9}\,[\frac{\text{pL}}{\text{mL}}]$$

VCD: Viable Cell Density; VVD: Vessel Volumes per Day

Cell Density & Viability

Another critical process parameter that will influence filter performance is the cell density of the culture, defined as the number of cells per unit volume. At higher cell densities, filtration will be more challenging since there is a greater mass of cells and debris that must be separated by the membrane. For this reason, processes running at lower cell densities will typically demonstrate a longer filter lifetime than those running at higher cell densities. Additionally, lower viabilities can also correlate with more debris present in the culture and reduce filter performance. These results vary considerably due to factors such as cell line, clone, and medium, but when these factors are held constant, the target cell density of a process can be used as a lever to affect the performance of the cell retention device.

In the experimental data shown below, the lab-scale Cellicon® Cell Retention Solution was used to evaluate the effect of cell density on filter performance. In the experiment, one bioreactor was used to grow cells up to a density of 60E6 cells/mL and then a cell bleed was started to maintain the cell density at steady state while another bioreactor was used to grow cells to a higher cell density of 160E6 cells/mL. Both bioreactors had the same working volume and used filters with the same crossflow and flux conditions. The results show that at higher cell density, the filter fouling was faster than at the lower cell density (Figure 7). Therefore, the target cell density should be considered when developing a process since increased cell density could affect filter fouling and run duration.

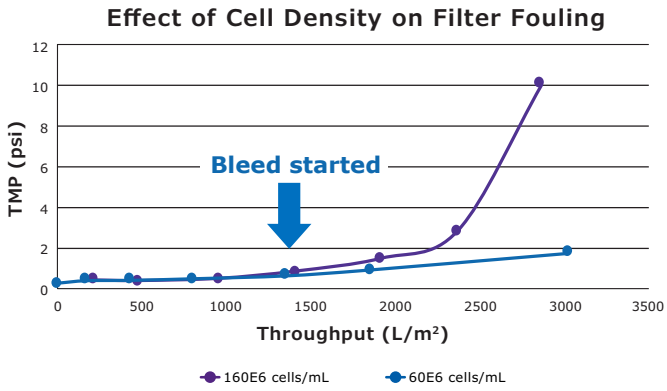

Figure 7. Evaluation of lab-scale Cellicon® Filters at low (blue) and high (purple) cell densities. Increased pressure (TMP) indicates filter fouling.

Key Performance Criteria

Key performance criteria are the parameters that should be measured and monitored to evaluate the performance of the Cellicon® Cell Retention Solution and assist with troubleshooting. This section will describe the transmembrane pressure (TMP) and pressure drop, filter throughput, and cell growth.

TMP and Pressure Drop

TMP and pressure drop are the primary measurements used to monitor fouling of the membrane within the filter. They are calculated using the formulas below:

$$TMP = \frac{P1+P2}{2} - P3 = \frac{P_{feed}+P_{retentate}}{2} - P_{perfusate}$$

Pressure drop (dP) = P1 - P2 =
Feed pressure - Retentate pressure

In the TMP calculation, the pressure from the feed line (P1) and retentate line (P2) are averaged and then the pressure from the perfusate (P3) is subtracted, resulting in a pressure value for TMP. TMP is a measurement of the resistance of flow through the filter, representing the health of the filter, with low TMP values indicating a clean filter while rising TMP values indicate fouling of the filter.

The pressure drop is another parameter that helps monitor for openness of the feed channel. It is calculated by subtracting the retentate pressure from the feed pressure. When there is clogging within the feed channel, the feed pressure will rise while the retentate pressure remains low, resulting in an increased pressure drop. If both the feed pressure and the retentate pressure rise at the same time, the pressure drop within the filter will not rise. This could indicate a possible blockage within the recirculation loop, in which case the line should be checked for a possible kink or other blockage. Alternatively, it could be caused by pressurization of the vessel, so the bioreactor pressure should also be checked.

When clogging occurs, it can be observed through two different mechanisms. The first mechanism is when the feed channel pressure drop within the filter rises. If the feed channel pressure drop increases over the course of the run, this is indicative of clogging occurring within the feed channel of the filter and will often be accompanied by a rise in the speed of the recirculation pump. The second and most common mechanism for filter clogging is seen when the permeate pressure drops below zero and causes an increase in TMP. As the pores of the membrane begin to fill with debris the resistance to flow through the membrane increases and the perfusate pump can no longer pull liquid through the membrane as effectively. The filter is considered fully clogged or fouled when the TMP reaches 5 psi, at which point the run should be ended or the filter replaced to extend the duration of the run.

Filter Throughput

Throughput is a key performance measurement that is used to compare performance from run to run, and to evaluate scalability. It is calculated based on the total volume of spent media that has passed through the filter, in units of volume per unit of area.

$$\text{Throughput } [\frac{L}{m^2}] = \frac{\text{Total volume processed by filter [L]}}{\text{Filter Area [m}^2\text{]}}$$

When the same process is performed multiple times, the throughput can be compared from run to run to assess reproducibility of the cell retention filter performance. Typically, a graph of throughput (x-axis) vs. TMP (y-axis) will be used to visualize the fouling of the filter based on the volume of media it has processed. Since throughput is normalized by the membrane area, when a process is scaled linearly, the throughput can be expected to match within a safety factor of +/- 20%. Therefore, a process can be designed and tested at lab-scale and the resulting throughput will help determine the expected performance at pilot or process scale. Confirmation runs across scales should yield a similar output for throughput vs. TMP trends.

Cell Growth

Cell growth can be indirectly affected by the cell retention filter, and should be monitored as an indicator of run performance. When the cell retention filter is operating as intended, it will effectively replace spent media with fresh media, maintaining an optimal environment for cell growth. At times, a problem with the cell retention filter can be indicated by an impact on cell growth. For example, excess shear can be detected through cell growth when the crossflow rate is too high, or the speed of the recirculation pump rises due to clogging of the feed channel. Cell growth is monitored by calculating the rate at which a population of cells doubles, known as the doubling time. This value should remain constant when the cells are in exponential growth phase but may change as the cells shift into production phase.

$$VCD_f = VCD_i \bullet e^{\text{growth rate} \bullet \text{time}}$$

$$\text{Growth Rate [days}^{-1}\text{]} = \frac{\ln\left(\frac{VCD_f}{VCD_i}\right)}{\text{time elapsed [days]}}$$

$$\text{Doubling Time [hours]} = \frac{\ln(2)}{\text{growth rate [days}^{-1}\text{]}/24\left[\frac{\text{hours}}{\text{day}}\right]}$$

Scalability Strategy

The Cellicon® filter was designed for scalability from lab to production. The following parameters were designed to scale-up linearly with increasing filter size.

Dimensions

The Cellicon® filter uses a flat sheet format, ensuring linearity of design and optimal scalability. The channel height and length remain consistent across all sizes, while the channel width expands from lab to process-scale. The augmentation of membrane area continues within process scale devices by increasing the number of feed channels. With the dimensions of each channel held constant, the fluid flow velocity over the membrane surface remains the same as crossflow rate and the number of channels are increased by scale. The perfusate channels from each internal plate join at a single perfusate port outlet.

Membrane Area

The membrane area of each filter was selected to maintain as close as possible to a constant ratio of bioreactor working volume to membrane area. As seen in Table 2, across all scales, there is a ratio of 236-265 liters of bioreactor working volume for each square meter of membrane area. With this strategy, reproducible performance can be expected from one size to another because the membrane area within the cell retention filter is appropriately adjusted to match the scale.

Max Working Volume	2.7 L	50 L	200 L	500 L	1000 L	2000 L
Membrane Area	0.01 m^2	0.2 m^2	0.8 m^2	1.9 m^2	3.8 m^2	7.6 m^2
Volume/ Area	262 L/ m^2	236 L/ m^2	260 L/ m^2	265 L/ m^2	265 L/ m^2	263 L/ m^2

Table 2. Comparison of membrane area and volume/area.

When designing scaling experiments, this ratio should be considered. As an example, when designing a lab-scale process to represent a production process with a 1500 L working volume and the 7.6 m^2 filter, a volume to area ratio of 197 L/m^2 would be obtained. Therefore, a scale-down process could be designed at 1.97 L (197 L/m^2 * 0.01 m^2) with the 0.01 m^2 filter. The results at lab-scale would be expected to predict the performance at the production-scale targets, assuming all other scalability factors are maintained.

Crossflow/Shear Rate

The recommended crossflow rate for each filter size was selected to maintain a wall shear at the membrane surface of 2500 s^{-1}, as described in the "Shear Rate" section, above. By maintaining the shear rate across scales, uniform sweeping of the membrane surface will be provided. The recommended crossflow rates are provided as a starting point but can be adjusted up or down as desired to increase or decrease the shear on the membrane surface (Table 3).

Device Size	0.01 m^2	0.2 m^2	0.8 m^2	1.9 m^2	3.8 m^2	7.6 m^2
Recommended Crossflow Rate	80 mL/ min	1.57 L/ min	5.72 L/ min	14 L/ min	28 L/ min	56 L/ min
Levitronix Pump Size	i30	i100	i600	i600	2000	2 x 2000

Table 3. Recommended crossflow rates.

The Levitronix® pump sizes were selected to deliver the target crossflow rates while minimizing pump shear by maintaining a speed in the low range of the pump capacity. If crossflow rates are increased, it is important to realize that pump speeds will also increase and therefore the effect on cell shear should be evaluated across all scales.

In the experimental data shown in Figure 8, the lab-scale Cellicon® filter was used to evaluate the effect of three different crossflow rates on performance. In each experiment, all other parameters were held constant including the flux across the membrane and the cell density. The results indicate that an increased crossflow leads to a greater membrane throughput before reaching the recommended TMP cutoff of 5 psi, as well as a longer run duration. Based on this information, if a greater membrane throughput or longer run duration are desired, then increasing the crossflow rate may achieve this result provided the cells can support the additional shear.

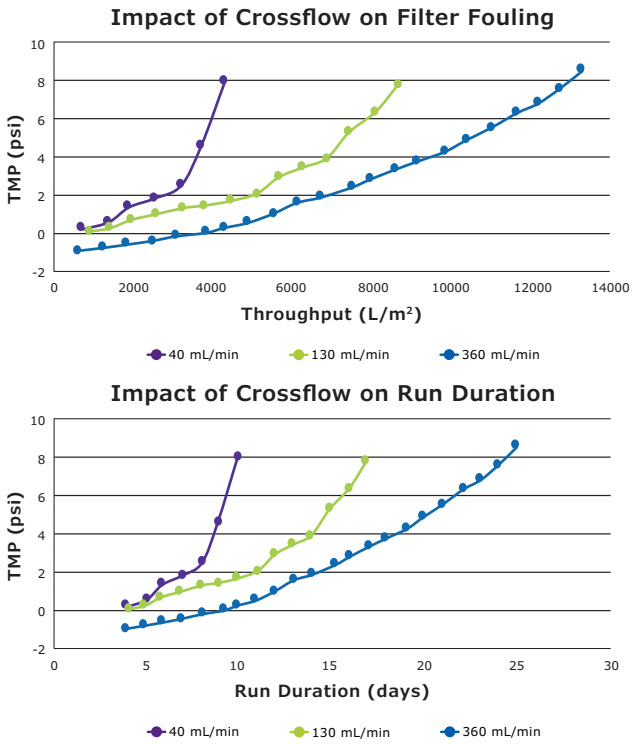

Figure 8. Lab-scale Cellicon® filters were tested at various crossflow rates in a steady state process at 60E6 cells/mL. Results show that an increased crossflow rate can lead to improved filter throughput prior to fouling (top), and increased run duration (bottom).

Perfusion Rate

The Cellicon® Filter was designed to deliver the required perfusion rates for media exchange. Since the membrane area is scaled linearly with bioreactor volume, the flux across the membrane can also be scaled linearly. It is recommended that the target perfusate flux be maintained at or below 22 L/m²*hr, or LMH. This value was selected based on success across many different experiments with different process parameters and cell lines, though some processes may be able to accommodate a higher flux. A maximum flux of 22 LMH results in a filtration rate of approximately 2 VVD for the maximum working volume at each scale, as seen in Table 4.

Device Size	0.01 m²	0.2 m²	0.8 m²	1.9 m²	3.8 m²	7.6 m²
Max Recommended Working Volume	2.7 L	50 L	200 L	500 L	1000 L	2000 L
Max Recommended Flux	22 LMH	22 LMH	22 LMH	22 LMH	22 LMH	22 LMH
Max Perfusate Flow Rate	3.78 mL/ min	77.7 mL/ min	282 mL/ min	692 mL/ min	1.38 L/ min	2.77 L/ min
Max Filtration Volume/Day	5.44 L	112 L	407 L	996 L	1992 L	3984 L
Max VVDs	2.0	2.2	2.0	2.0	2.0	2.0

Table 4. Perfusion rates for media exchange.

In the experimental data shown in Figure 9, the lab-scale Cellicon® filter was used to evaluate the effect of flux on filter performance. In the experiment, two crossflow rates (low of 40 mL/min and high of 150 mL/min) were tested at high and low flux values across the membrane (13 and 26 LMH) in steady-state perfusion cultures at a cell density of 60E6 cells/mL. The results indicate that at a low flux, the duration of the run will be extended compared to the high flux condition, but overall throughput is not significantly affected. Based on this information, if a longer duration run is desired, then a lower flux may provide additional days to the process before filter fouling. However, a critical flux exists at which the device can experience quicker fouling and decreased performance, which is the reason for the recommended flux being ≤ 22 LMH.

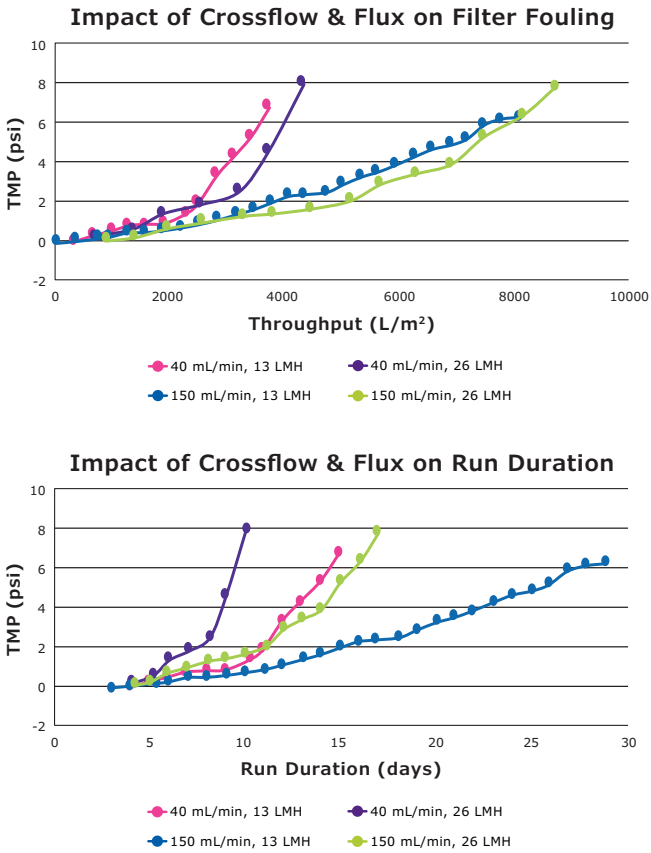

Figure 9. Lab-scale Cellicon® Filters were tested at various fluxes and crossflow rates in a steady state process at 60E6 cells/mL. Results show that at two different crossflow rates, decreased flux can lead to longer run duration but has no significant impact on filter throughput.

Scalability Experiments

Assessment of Filter Performance Across Scales

In order to assess the filter performance across scales and gain confidence that the lab-scale and process-scale filter performance were comparable, several scalability studies were performed. To do so, experiments were designed in which two different filter assemblies of varying size were run from the same cell culture vessel, therefore eliminating any process variability. This was accomplished by designing a "T" in the tubing assembly of a process-scale filter to create a secondary loop for the lab-scale filter assembly, as seen in Figure 10.

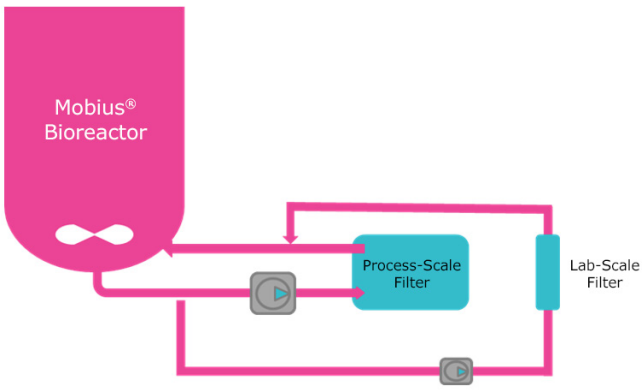

Figure 10. Experimental setup for scalability studies comparing process-scale filters to lab-scale filters.

The cell culture process was executed at the target working volume for the process-scale filter, with the process parameters described in Table 5. The shear rate was maintained at 2500 s^{-1} for both filter sizes and the perfusate flow rate was split between the two filters at a ratio that would provide an even flux to each filter. The cells were grown exponentially by increasing the perfusion rate based on vessel volumes per day to support the cell density within the bioreactor.

Process Variable	Value	
Study Conducted	3 L vs. 50 L	3 L vs. 200 L
Working Volume	50 L	200 L
Bioreactor Type	200 L Mobius® Single-Use Bioreactor	
Cell Line	CH0ZN® & UCOE® Combined Platform	
Recombinant Protein	IgG1 mAb	
Cell Culture Media	EX-CELL® Advanced HD Perfusion Media	
Temperature	36.5 °C	
pH	7.0 +/- 0.05	
DO target	50%	

Table 5. Process parameters used for scalability experiments to compare process-scale and lab-scale filter performance within the same cell culture process.

Results showed that across three scalability experiments, two with the 50 L filter and a third with the 200 L filter, the larger filters fouled at the same rate as the lab-scale filter (Figure 11). Very little fouling was observed up to a throughput of 1500 L/m^2, as the cells reached high cell densities of 89-112E6 cells/mL with viability >98%. Data from these studies indicated that the results observed with the lab-scale filter were directly scalable to the process-scale filters when scalability factors such as crossflow, shear rate, flux, and cell density are held constant.

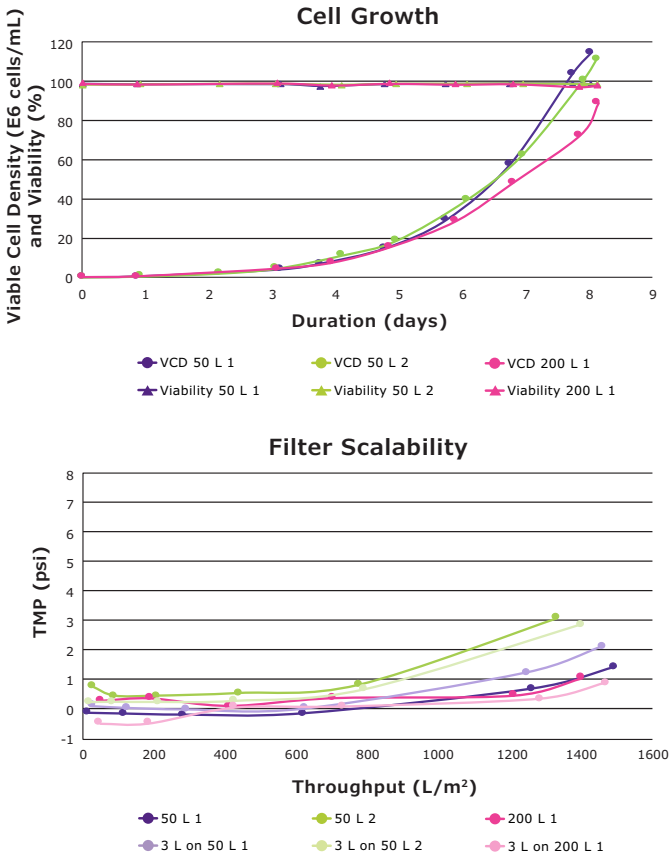

Figure 11. Results of scalability studies comparing lab-scale and process-scale filter performance. Each of the three processes reached high cell densities with high viability (top), and showed consistent filter performance from lab to process scale (bottom).

Design of Scale-Down Experiments

When designing scale-down experiments with the Cellicon® filter, the large-scale process will dictate the required setup for the target scale-down process. The lab-scale Cellicon® filter was designed to provide ideal sizing for scale-down studies such that performance at bench scale will accurately predict performance at the lab-scale.

The bioreactor process parameters to control the cell culture will also need to be scaled appropriately, in addition to the filter parameters described here.

Three example processes below illustrate how to design scale-down studies.

	Process 1	Process 2	Process 3
Mode	N-1 Perfusion	Steady State N Perfusion	Dynamic N Perfusion
Target VCD	60E6 cells/mL	40E6 cells/mL	120E6 cells/mL
Working Volume	1000 L	600 L	1800 L
Media Requirements	CSPR of 25 pL/cell/day	2 VVD	1 VVD

Table 6. Process parameters for three example processes.

Process-scale Cellicon® Filter sizes were developed for maximum working volumes of 50 L, 200 L, 500 L, and 1000 L. The filter size being used for the process should be determined by starting with the filter that will most closely match the final process scale and then rounding up. The filter size can be adjusted up or down to increase or decrease the capacity, respectively.

	Process 1	Process 2	Process 3
Cellicon® filter area	3.8 m^2	3.8 m^2	7.6 m^2

Table 7. Appropriate filter selection for each example process.

Once the process-scale filter size is selected, the desired volume of the bioreactor and area of the cell retention device for the scale-down experiments should be identified (Table 8). Typically, the smallest representative scale of experiments is used to minimize costs such as media, equipment, and personnel. Therefore, the 0.01 m^2 Cellicon® Filter was selected to scale down to a 3 L bioreactor. The ideal scale-down working volume should be calculated by dividing the at-scale working volume by the at-scale membrane area. This result is then multiplied by membrane area of the scale-down filter to achieve the proper bioreactor volume at small scale.

	Process 1	Process 2	Process 3
Scale-down working volume	1000 L/3.8 m^2 = 263 L/m^2 263 L/m^2*0.01 m^2 = **2.6 L**	600 L/3.8 m^2 = 158 L/m^2 158 L/m^2*0.01 m^2 = **1.6 L**	1800 L/7.6 m^2 = 237 L/m^2 237 L/m^2*0.01 m^2 = **2.4 L**

Table 8. Calculation of scale-down working volumes for the three example processes.

The next step in the study is to identify the operating parameters for the Cellicon® Filter at both scales. The recommended crossflow rate for each filter was selected based on maintaining a membrane shear rate of 2500 s^{-1} for each filter. If the resulting fouling profile is unsatisfactory, the crossflow rate can be increased to improve performance. If findings indicate that the cells are experiencing the effects of shear, the crossflow rate can be decreased to reduce these effects.

The perfusion rate is based on the media requirement of the cells and related to the daily protein harvest targets. If the perfusion rate is defined by a CSPR, such as in example Process 1, the maximum VVDs can be calculated using the maximum cell density. Once the maximum VVDs are obtained, this can be converted into a maximum required flow rate for each scale using the predetermined working volumes (Table 9). The flow rates can then be converted into maximum flux values using the membrane area of each filter. If the calculations are performed correctly, the flux values should match across scales (minor differences in the chart are due to rounding error). The maximum flux is recommended to stay below 22 LMH.

	Process 1	Process 2	Process 3
Maximum VVDs	(25 pL/cell/day * 60E6 cells/mL) / 1000 = 1.5 VVD [day^{-1}]	2 VVD [day^{-1}]	1 VVD [day^{-1}]
Flow rate at process scale	1.5 VVD [day^{-1}] * 1000 [L] / 1440 [min/day] = **1.04 L/min**	2 VVD [day^{-1}] * 600 [L] / 1440 [min/day] = **0.83 L/min**	1 VVD [day^{-1}] * 1800 [L] / 1440 [min/day] = **1.25 L/min**
Flow rate at lab-scale	1.5 VVD [day^{-1}] * 2.6 [L] * 1000 [mL/L] / 1440 [min/day] = **2.71 mL/min**	2 VVD [day^{-1}] * 1.6 [L] * 1000 [mL/L] / 1440 [min/day] = **2.22 mL/min**	1 VVD [day^{-1}] * 2.4 [L] * 1000 [mL/L] / 1440 [min/day] = **1.67 mL/min**
Process-scale filter flux	1.04 L/min * 60 [min/hr] / 3.8 [m^2] = **16.4 LMH**	0.83 L/min * 60 [min/hr] / 3.8 [m^2] = **13.1 LMH**	1.25 L/min * 60 [min/hr] / 7.6 [m^2] = **9.9 LMH**
Lab-scale filter flux	2.71 mL/min * 60 [min/hr] * .001 [L/mL] / 0.01 [m^2] = **16.3 LMH**	2.22 mL/min * 60 [min/hr] * .001 [L/mL] / 0.01 [m^2] = **13.3 LMH**	1.67 mL/min * 60 [min/hr] * .001 [L/mL] / 0.01 [m^2] = **10.0 LMH**

Table 9. Calculation of appropriate flow rates and filter flux based on media requirements parameters for each example process.

Scale-Down Case Study: Intensified Seed Train

A scale-down case study was designed at lab-scale to understand the performance of the Cellicon® Filter in an N-1 intensified seed train application. This process was performed in a 200 L single-use bioreactor where the cells would be transferred to inoculate a 20,000 L stainless steel vessel. The goal of this experiment was to determine the maximum cell density that could be obtained in the N-1 process using the 0.8 m^2 Cellicon® Filter and at a 200 L working volume.

The scale-down study was performed using the 0.01 m^2 Cellicon® Filter in an Applikon 3 L glass bioreactor. The working volume was 2.5 L using the calculation provided previously (200 L / 0.8 m^2 = 250 L/m^2 * 0.01 m^2 = 2.5 L). The cell line was a clone from the CHOZN® & UCOE® Combined Platform producing an IgG1, and the media was EX-CELL® Advanced HD Perfusion Medium. The cells were previously determined to have a required media consumption rate of 20 pL/cell/day. This value was used to adjust the perfusion rate on a daily basis as the cells grew exponentially. The process was maintained until devices fully fouled indicated by a TMP of more than 5 psi. Figure 12 summarizes the experimental results.

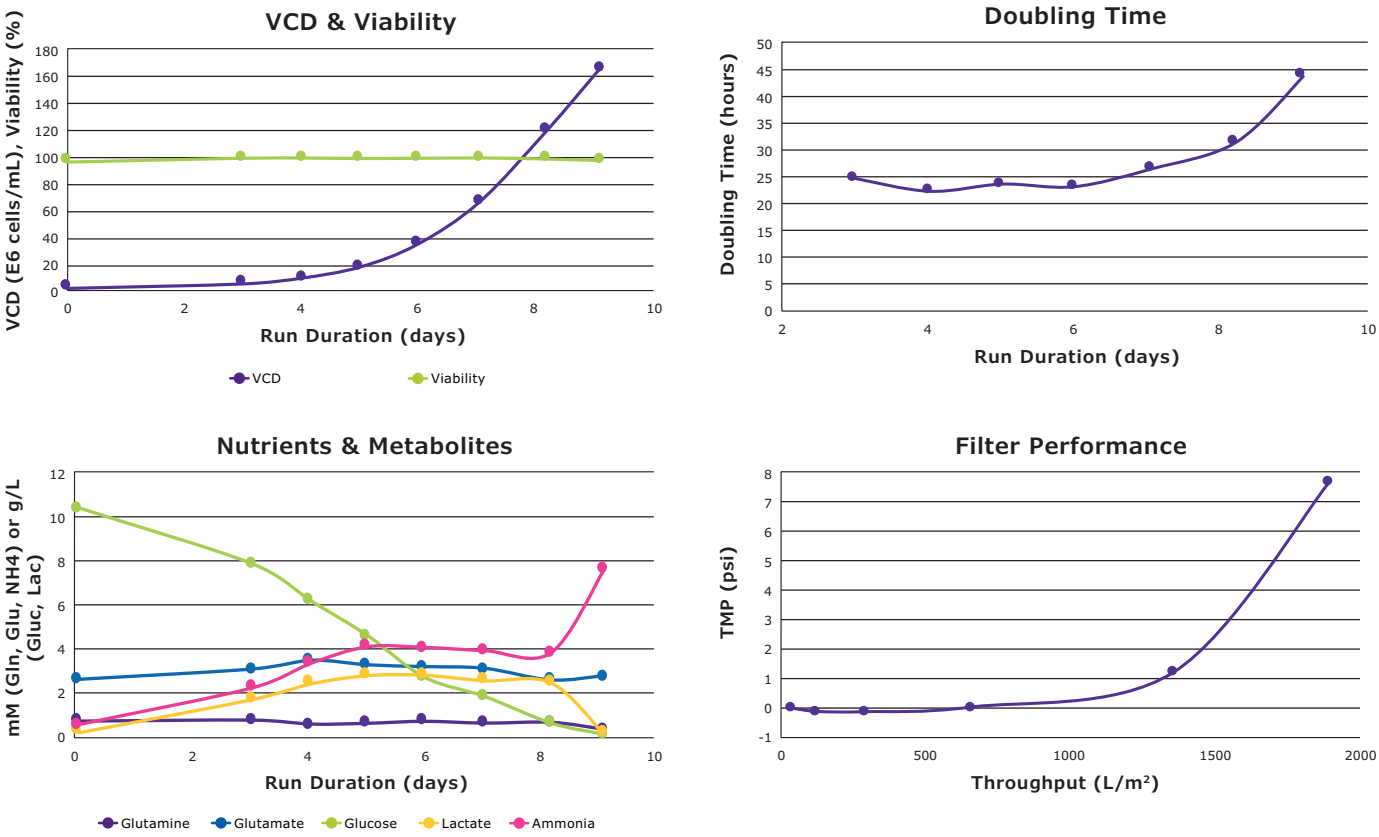

Figure 12. Results of a scale-down intensified seed train process. Data shows cell density and viability (top left), doubling time (top right), nutrients and metabolites (bottom left), and filter throughput versus TMP (bottom right).

The cells were able to grow to 165E6 cells/mL with a high viability before the filter experienced fouling with a TMP over 5 psi on day 9. The doubling time remained relatively stable until day 7 when it began to rise slightly as the cell density increased. Except for the glucose concentration which dropped throughout the process, the nutrients and metabolites remained relatively stable until day 8. The data indicated that the cells would be healthy enough to use to inoculate the production bioreactor up until day 8, at a cell density of 120E6 cells/mL. For an added safety factor and improved cell health during the transfer, the N-1 process may be ended and cells transferred on day 7 at 60E6 cells/mL.

Lab-scale data is expected to yield comparable cell retention filter performance in the intended process at 200 L scale when the process parameters are scaled accordingly. Therefore, the same cell densities and filter fouling profile would be expected. With this insight, the process can end on day 7 at 60E6 cells/mL which would yield enough cells to inoculate the 20,000 L production bioreactor at 0.6E6 cells/mL (60E6 cells/mL * 200 L / 20,000 L = 0.6E6 cells/mL).

Scale-Down Case Study: Steady State Perfusion

A second scale-down case study was designed at lab scale for a steady state perfusion production process with the goal of scaling to a 2000 L bioreactor with a 1500 L working volume. In this application, the protein was harvested from the perfusate in order to understand the productivity.

This scale-down study was performed using the 0.01 m^2 Cellicon® Filter in an Applikon 3 L glass bioreactor, and a working volume of 2 L (1500 L/7.6 m^2 * 0.01 m^2 = 2.0 L). A cell line generated with the CHOZN® & UCOE® Combined Platform producing a different IgG molecule from the previous study was used in a perfusion process operated at a CSPR of 20 pL/cell/day. Once the cells reached 60E6 cells/mL, a cell bleed was initiated to maintain steady state. At this point, the feed VVDs were approximately 1.5 and the harvest VVDs were approximately 1.2, with the difference being sent to waste in a cell bleed. This resulted in a maximum flux on the filter of 10 LMH. The filter had a relatively slow rate of fouling, reaching a throughput of almost 3000 L/m^2 before the run was ended, at which time the TMP was only at 1.5 psi. Measurements of the perfusate titer were collected over the duration of the run (Figure 13).

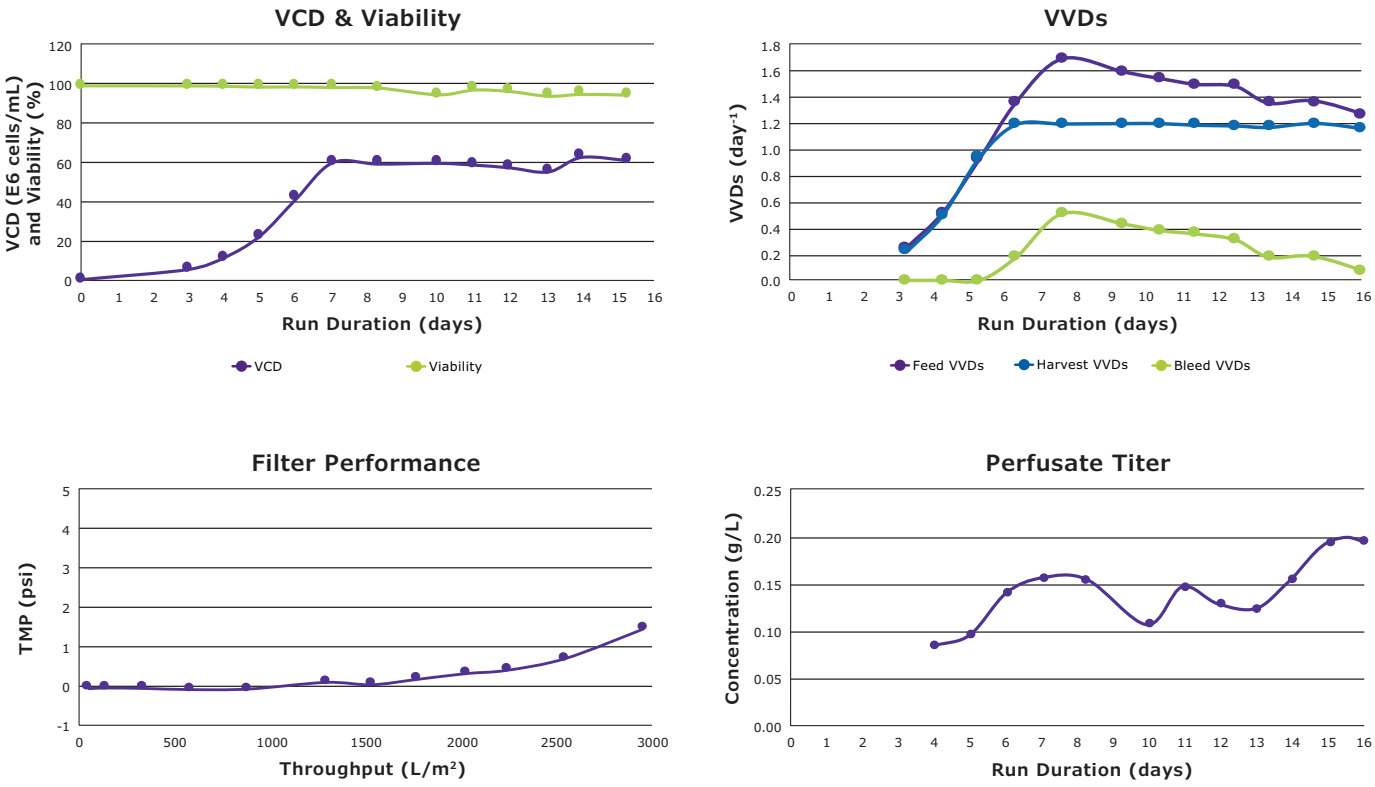

Figure 13. Results of a scale-down steady state perfusion process. Data shows cell density and viability (top left), VVDs (top right), filter throughput versus TMP (bottom left), and perfusate titer (bottom right).

Based on these results, equivalent cell retention filter performance can be expected up to 2000 L, and if the cell culture and bioreactor process is scaled effectively, the same results can be expected for cell growth, viability, and productivity. Over the duration of this process, 4.3 g of protein was harvested, that translated to 2.15 g per L of cell culture. Therefore a 1500 L process with the same output should produce 3225 g of protein.

Conclusion

The Cellicon® Cell Retention Solution was designed to simplify perfusion. It was built to deliver seamless scalability that provides linear scale-up from lab to production. By understanding the critical process parameters, users can develop processes to achieve their targets while gaining insights from the key performance criteria. This solution makes designing and interpreting cell retention filter performance and scaling experiments both easy and effective, increasing confidence in the ability to adopt perfusion for reliable, at-scale implementation of intensified upstream biomanufacturing.

References

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Note

Customer is responsible for and must independently determine suitability of our products for customer's products, intended use and processes, including the non-infringement of any third parties´ intellectual property rights.

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