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Application Note

Scalability Studies for Millistak+® HC and Millistak+® HC Pro Micro 20 Devices

Scale-up and Scale-down Primary and Secondary Clarification with C0HC, D0HC, X0HC, C0SP, D0SP, and X0SP Media Grades

Depth filtration tests were conducted to evaluate the scalability performance of the Micro 20 device format for Millistak+® HC and Millistak+® HC Pro depth filters. These tests included primary clarification of Chinese Hamster Ovary (CHO) harvest feed streams using C0HC, D0HC, C0SP, and D0SP and secondary clarification of CHO centrate feed streams using X0HC and X0SP media grades. Several best practices including depth filter tubing selection, cell culture feed stream loading operation, and the impact of process flux on depth filter device performance are also described. A good agreement in depth filtration capacities is reported for all Micro 20 depth filter media grades versus the corresponding 0.11 m^2 Process-Scale Pod devices. The new Micro 20 device format provides a more accurate depth filter sizing estimate for process-scale harvest and secondary clarification operations.

Introduction.

Recent improvements in upstream cell culture processes have resulted in significantly increased monoclonal antibody (mAb) product titers (>5 g/L) (Shukla & Thömmes, 2010). These titer improvements are accompanied with corresponding increases in the cell density and soluble impurities (Nejatishahidein, Borujeni, Roush, & Zydney, 2020). The increased cell density and solids removal requirements are challenging for current process template clarification methods (Singh, et al., 2016). The capacity of the depth filters used in harvest clarification may also be limited for high cell density feed streams and reductions in depth filtration capacity may pose significant challenges in manufacturing facilities with hardware constraints limiting the maximum depth filter area that can be installed (Felo, Christensen, & Higgins, 2013). In addition, many depth filtration methods often employ large safety factors to mitigate risks associated with upstream and clarification process variabilities (Lutz, et al., 2009). Small-area depth filtration devices with good scalability performance are critical for accurately assessing filter sizing requirements, robust process characterization, and troubleshooting during manufacturing operations.

As a result of these industry trends, better methods are needed to determine depth filter area requirements for high density cell culture harvests (Lutz, et al., 2015). The filtration capacity of depth filter devices is often determined by means of constant flow filtration testing using scale-down devices with reduced filtration areas (Lutz, et al., 2009). This is also beneficial when there is limited availability of feed stream volumes for the filter sizing tests.

Millistak+® HC and Millistak+® HC Pro filter devices.

A summary of available laboratory, pilot, and process-scale devices for Millistak+®, Millistak+® HC, and Millistak+® HC Pro depth filter product families are provided in **Table 1**. Previously, filter media screening studies for Millistak+®, Millistak+® HC, and Millistak+® HC Pro were conducted using the 23 cm^2 µPod® filter device followed by filter sizing studies using Laboratory-Scale Pod devices ranging in filter area from 135 cm^2 to 540 cm^2. Final sizing confirmation studies could then be conducted at pilot scale using 0.11 to 1.1 m^2 Process-Scale Pods in a pilot holder.

Shown in **Figure 2**, the new Millistak+® Micro 20 device presents a filtration area of 20 cm^2 and its internal construction closely duplicates the critical filter media dimensions that are used in larger process-scale Pod devices.

This Technical Brief demonstrates the performance of this device using relevant CHO feed streams and includes several best practice recommendations to help obtain the most accurate sizing for depth filtration processes.

Materials and methods.

Cell culture preparation.

CHO cells derived from CHO-S cell lines producing a monoclonal antibody (mAb02, mAb05, or mAb09) or a recombinant fusion protein were grown in Mobius® 50 L and 200 L bioreactors (MilliporeSigma) to a total cell density of 10 - 21 × 10^6 cells/mL and harvested at 60 - 90% viability.

For primary clarification tests using Millistak+® HC Pro D0SP, C0SP or Millistak+® HC D0HC, C0HC depth filters, the raw cell culture harvest was used immediately after removal from the bioreactor. For secondary clarification tests using Millistak+® HC Pro X0SP or Millistak+® HC X0HC filters, cell culture centrate (170 – 210 NTU) was prepared using a Thermo Scientific™ Sorvall LYNX 6000 Superspeed Centrifuge.

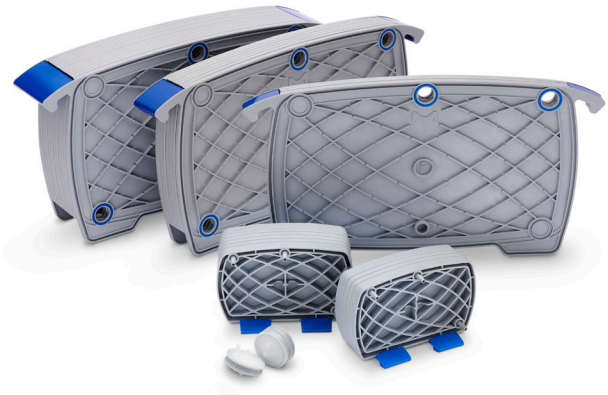

Figure 1. Millistak+® HC Pro Pod filter devices. Process-Scale Pod (0.11, 0.55, and 1.1 m^2), Laboratory-Scale Pod (135, 270 cm^2), and Micro 20 (20 cm^2).

Figure 2. Millistak+® Micro 20 depth filter devices. These 20 cm^2 depth filter devices are suitable for scale-down depth filter screening and sizing studies.

Table 1. Summary of Millistak+®, Millistak+® HC, and Millistak+® HC Pro depth filter device scales and formats.

Device Format	Effective Filtration Area	Intended Use	Available filter grades
Micro 20	20 cm^2	Filter media screening, Filter sizing	Millistak+® HC C0HC, D0HC, X0HC Millistak+® HC Pro C0SP, D0SP, X0SP
µPod®	23 cm^2	Filter media screening, Filter sizing	Millistak+® CE, DE Millistak+® HC A0HC, A1HC, B1HC, F0HC
Laboratory-scale Pod	135 cm^2 to 540 cm^2	Filter sizing	Millistak+® CE, DE Millistak+® HC A0HC, A1HC, B1HC, C0HC, D0HC, F0HC, X0HC Millistak+® HC Pro C0SP, D0SP, X0SP
Process-scale Pod	Single-layer goes up to 1.4 m^2 10 per rack x 3 racks = 42 m^2	Pilot to production scale filtration applications	Millistak+® CE, DE Millistak+® HC A0HC, A1HC, B1HC, C0HC, D0HC, F0HC, X0HC Millistak+® HC Pro C0SP, D0SP, X0SP

Depth filtration testing.

Depth filtration experiments were conducted using Millistak+® HC or Millistak+® HC Pro depth filters in Micro 20 format (20 cm^2) and Process-Scale Pod device format (0.11 m^2) (MilliporeSigma). Prior to filtration, Millistak+® HC devices were flushed with 100 L/m^2 water at 600 LMH to fully wet the filter media. Millistak+® HC Pro devices were flushed with 50 L/m^2 water at 300 LMH. The capacities of the depth filters were evaluated using the Pmax™ testing method at a constant filtrate flux of 150 LMH (MilliporeSigma, 2021). During filtration, the differential pressure was recorded as a function of time and the depth filtration capacity was defined as the throughput achieved at a maximum pressure drop of 15-20 psi. Final filtrate volume and filtrate turbidity samples were recorded at the end of each experiment.

Results and discussion.

Feed stream tubing selection.

In the primary clarification of high-density cell culture feed streams, significant particle settling may be observed within the feed tubing leading from the cell culture harvest tank to the depth filtration devices (**Figure 3**). Care should be taken to avoid particle settling within the feed tubing lines, as this material does not reach the depth filter and may result in under-sizing of the depth filtration system. Also, the particle settling observed in benchtop tests is not easily replicated at the process scale, resulting in different filter fouling behavior. The amount of particle settling within the feed tubing lines is directly related to the residence time within the tubing set and the settling distance (i.e., tubing diameter). To investigate the effect of tubing residence time on particle settling, tests were conducted using a CHO harvest feed stream (mAb05, 9.7 x 10^6 TC/mL, 62% viability) with three different pump tubing sets of varying internal diameter (i.d.) at three different flow rates, as shown in **Table 2** below. In all cases, the feed tubing length was 230 cm; this tubing length was chosen to represent a worst-case tubing length for a typical benchtop filter evaluation.

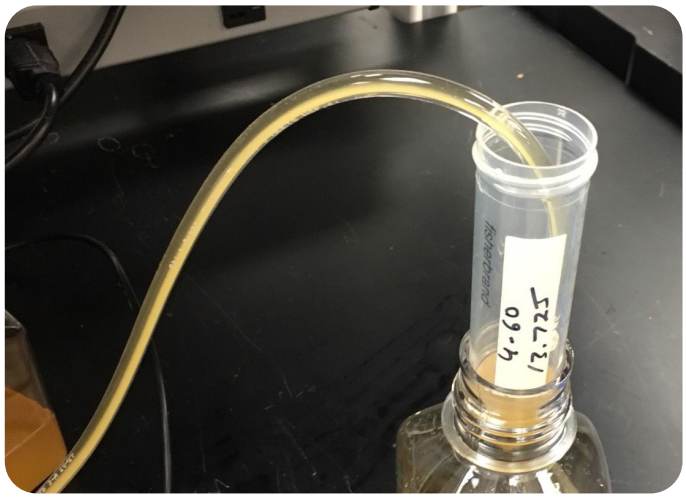

Figure 3. Settling of cell culture in tubing. Feed stream: CHO cell culture harvest (mAb05, 9.7 x 10^6 TC/mL, 62% viability). A 1/8 in i.d. pump tubing at a flowrate of 2.5 mL and total runtime of 60 minutes is shown (system residence time: 7 minutes).

Table 2. Flow rate and flux settings for tubing residence time study.

Flow rate (mL/min)	Flux (LMH)	Residence time (minutes)		
		1/16th in, i.d.	1/8th in, i.d.	1/4th in, i.d.
2.5	75	1.8	7.2	29
5.0	150	0.9	3.6	15
10	300	0.5	2.0	8.0

For each condition, the biomass in the cell culture fluid exiting the tubing set was measured and compared to the starting feed stream. These measurements were conducted at three timepoints to represent the particle challenge reaching a depth filter early (20 minutes), mid (40 minutes), and late (60 minutes) into a clarification trial. These timepoints were selected to account for feed stream dilution due to the system hold-up volume early in the run, while the longer timepoints were expected to allow the system to reach a steady state. All tests were conducted in duplicate.

In these studies, no residence time corrections were made to account for the observed tubing diameter constriction due to particle settling within the tubing set (**Figure 4**). A significant biomass reduction was observed for 1/8-inch i.d. tubing when run at a flow rate corresponding to a 7-minute residence time. In contrast, when 1/16-inch i.d. tubing is used, the residence time is decreased by a factor of 4 and only a minimal biomass loss was observed under these conditions. As an extreme case, 1/4-inch i.d. tubing was also evaluated at flow rates corresponding to 8—29-minute residence times, and a significant biomass loss was observed over this residence time range. Based on these results, if the tubing residence time is below 1 minute, the CHO feed stream will not settle within the feed tubing lines.

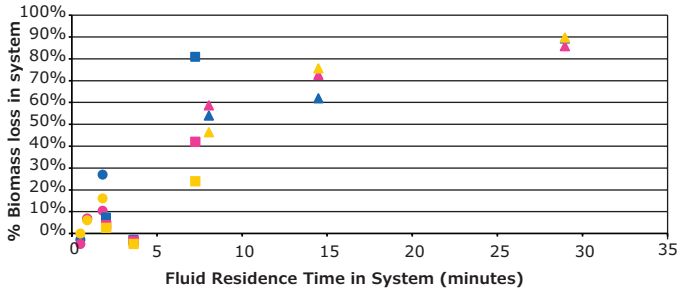

Figure 4. Biomass loss vs. fluid residence time in the pump tubing upstream of a depth filtration device at varying process timepoints (Circle: 1/16-in i.d., Square: 1/8-in i.d., and Triangle: 1/4-in i.d.). Early run (20 minutes, blue markers), mid run (40 minutes, red markers), and late run (60 minutes, green markers). Feed stream: CHO cell culture harvest (mAb05, 9.7 x 10^6 TC/mL, 62% viability).

Maximum recommended tubing lengths for Millistak+® HC and Millistak+® HC Pro Micro 20 devices for a range of flux values using 1/16-inch and 1/8-inch i.d. tubing sets are provided in **Table 3**, below. Based on the typical operating flux, 1/4-inch i.d. tubing is not recommended for Millistak+® HC and Millistak+® HC Pro Micro 20 devices. These options will achieve the maximum residence time of 1 minute over a flux range of 50 to 300 LMH while maintaining a calculated tubing pressure drop of less than 1.0 psi.

For larger clarification devices and systems, residence time calculations upstream of the clarification filter should be completed on a case-by-case basis to ensure minimal residence time while maintaining low flow resistance upstream of the clarification filter. The residence time may be determined by dividing the tubing hold-up volume in mL by the volumetric flow rate in mL/min.

Table 3. Recommended maximum tubing lengths for a Micro 20 device at the specified process flux.

	1/16-in. I.D.		1/8-in. I.D.	
Flux (LMH)	Max Length (cm)	Pressure Drop @ Max Length (psi)	Max Length (cm)	Pressure Drop @ Max Length (psi)
37.5	63	0.01	16	0.00
75	126	0.05	32	0.00
150	253	0.20	63	0.00
300	505	0.78	126	0.01
600	322	1.00	253	0.05

Pressure drop data are calculated values. Values are calculated for 1-minute residence time and < 1.0 psi pressure drop or < 1-minute residence time and 1.0 psi pressure drop)

Depth filter device loading with cell culture feed stream.

Depth filters often present significant device hold-up volumes. Since the ratio of depth filter area to device hold-up volume will vary from bench/laboratory to process scale, it is recommended that the user fill the upstream portion of the depth filter device with process fluid prior to the start of the clarification test. One method to accomplish this is to close the device outlet line and collect one half of the known device hold-up volume from an open device vent port. Estimated device hold-up volumes for Micro 20 devices are provided in **Table 4**. The corresponding upstream hold-up (filter headspace) values for larger Pod devices may be determined by consulting the available product literature such as the Pod Depth Filters User Guide (UG4697EN00). The described operation will ensure that the concentration of the cell culture feed stream is consistent for all device sizes at the beginning of the filter scalability test.

Table 4. Depth filter hold-up volumes for Micro 20 devices (20 cm²).

Depth filter type	Filter grade	Total filter hold-up volume (mL)
Millistak+® HC	D0HC	23
	C0HC	21
	X0HC	27
Millistak+® HC Pro	D0SP	45
	C0SP	40
	X0SP	29

Filter process flux studies.

Depth filter sizing trials are typically conducted at a single process flux (ex. 150 LMH) to conserve cell culture feed stream volumes and to minimize the trial duration. Optimization of the trial flux by studying the filtration behavior at multiple flux values may be beneficial in situations where the cell culture feed stream is suspected of exhibiting a flux-dependent fouling based on previous studies. In this study, depth filtration tests were conducted at various process fluxes to span the range of possible values for a given manufacturing process. To illustrate the importance of flux optimization, Millistak+® HC Pro D0SP Micro 20 filters were evaluated using a dairy whey feed stream (40 g/L dairy whey in PBS buffer) that demonstrates a flux-independent fouling behavior (**Figure 5a, c**).

The Millistak+® HC Pro D0SP Micro 20 filters were also evaluated using a CHO feed stream (mAb02, 18.1 x10^6 TC/mL, 68%) that demonstrates a flux-dependent fouling behavior (**Figure 5b, d**). The Millistak+® HC Pro D0SP Micro 20 filters were evaluated by measuring the filter resistance versus throughput and filtrate turbidity vs. throughput at 75, 150, and 300 LMH process fluxes. For the tests using the CHO harvest, the filtration capacities were observed to increase with increasing flux values that could not have been predicted using a single trial flux. Given these observations, if the scaled-up process flux, based on batch time, volume, and maximum pressure constraints, is calculated to be less than half of the scaled-down trial flux, it is recommended to re-run the trial at the process flux to confirm the filtration performance. This will mitigate the risk of under-sizing the system if the stream exhibits flux-dependent fouling. Similarly, if the scaled-up process flux is much higher than the trial flux, the system may be unnecessarily oversized. While this situation may not pose a capacity risk for the process, it may have a negative economic impact.

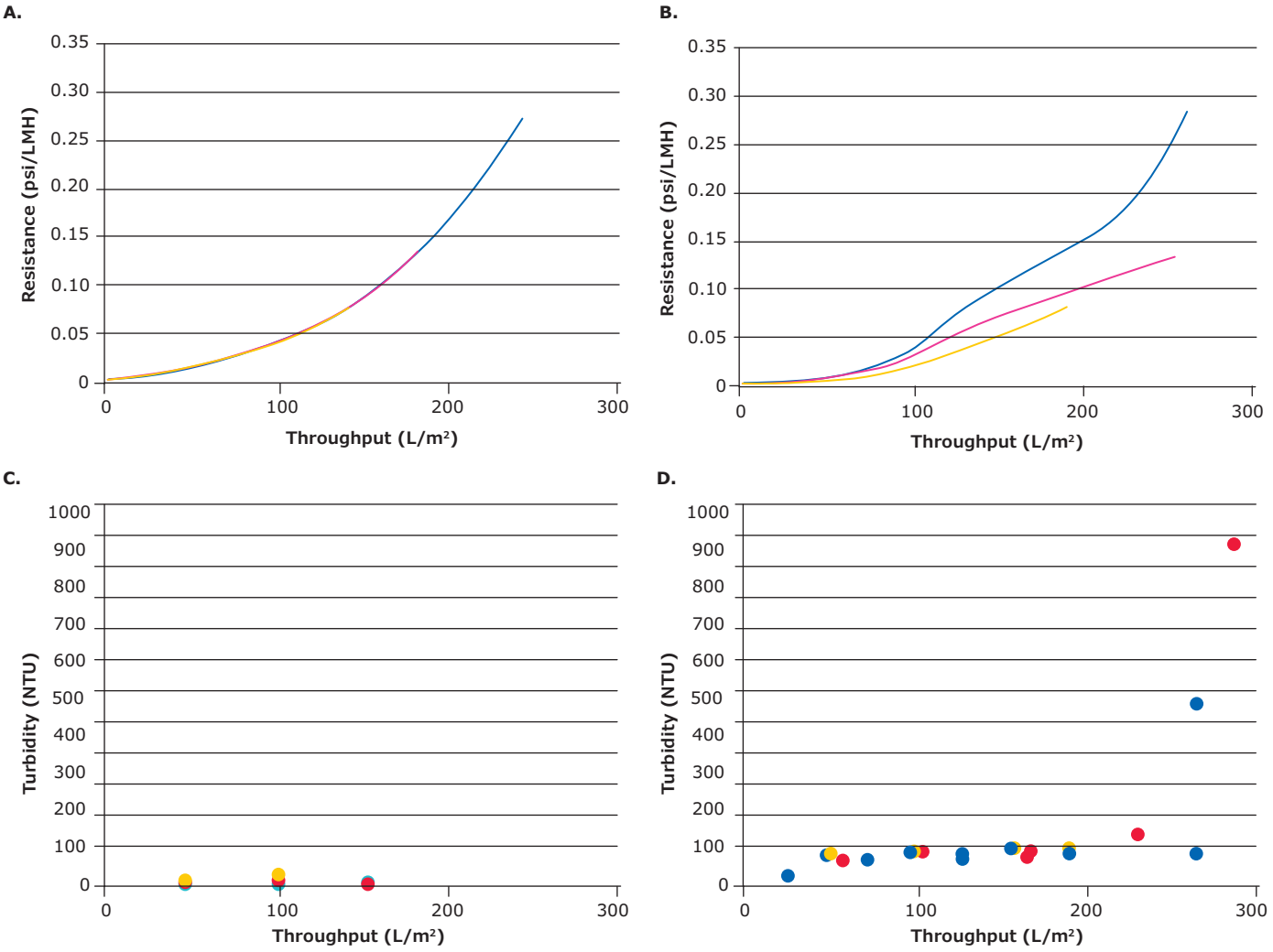

Figure 5. Resistance vs. throughput and turbidity vs. throughput profiles for Millistak+® HC Pro D0SP Micro 20 devices using a flux-independent model feed stream, 40 g/L dairy whey in PBS buffer (A, C) and a flux-dependent CHO feed stream (mAb02, 18.1 x10^6 TC/mL, 68%) (B, D) at three different fluxes: 75 LMH (blue), 150 LMH (red), and 300 LMH (yellow). (Average ± Range/2, n=2)

Filter performance examples.

Filter scalability test results for Millistak+® HC Pro D0SP depth filter devices using a CHO feed stream (mAb02, 20.5 x 10^6 TC/mL, 92% viable) are shown in **Figure 6a**. The Millistak+® HC Pro D0SP Micro 20 and 0.11 m^2 Process-Scale Pod (PSP) devices had similar resistance curves, and the devices were all stopped at a throughput of approximately 175 L/m^2 due to turbidity breakthrough. The filtration capacities of the Micro 20 devices were all within 10% of the filtration capacity of the Process-Scale Pod device.

Figure 6b summarizes the filter scalability test results for Millistak+® HC Pro C0SP depth filter devices using a CHO feed stream (recombinant fusion protein, 19.8 x 10^6 TC/mL, 79% viable). In this example, the Micro 20 devices had similar resistance profiles to the Process-Scale Pod devices; however, the Process-Scale Pod devices demonstrated a turbidity breakthrough at 125 L/m^2 while the Micro 20 devices showed no turbidity breakthrough and reached their terminal pressure at a throughput of approximately 150 L/m^2. The filtration capacities of the Micro 20 devices were within 20% of the filtration capacity of the Process-Scale Pod devices.

Filter scalability test results for Millistak+® HC Pro X0SP depth filter devices using a CHO centrate feed stream (mAb02, 173 NTU, original harvest 16.9 x 10^6 TC/mL, 93% viable) are shown in **Figure 6c**. The Micro 20 and Process-Scale Pod devices all reached a terminal pressure of 20 psi at 150 LMH (0.13 psi/LMH) with no turbidity breakthrough. The filtration capacities of the Micro 20 devices were all within 10% of the filtration capacities of the Process-Scale Pod devices.

All Millistak+® HC Pro D0SP, C0SP, and X0SP scalability tests were evaluated according to the best practices recommendations described above.

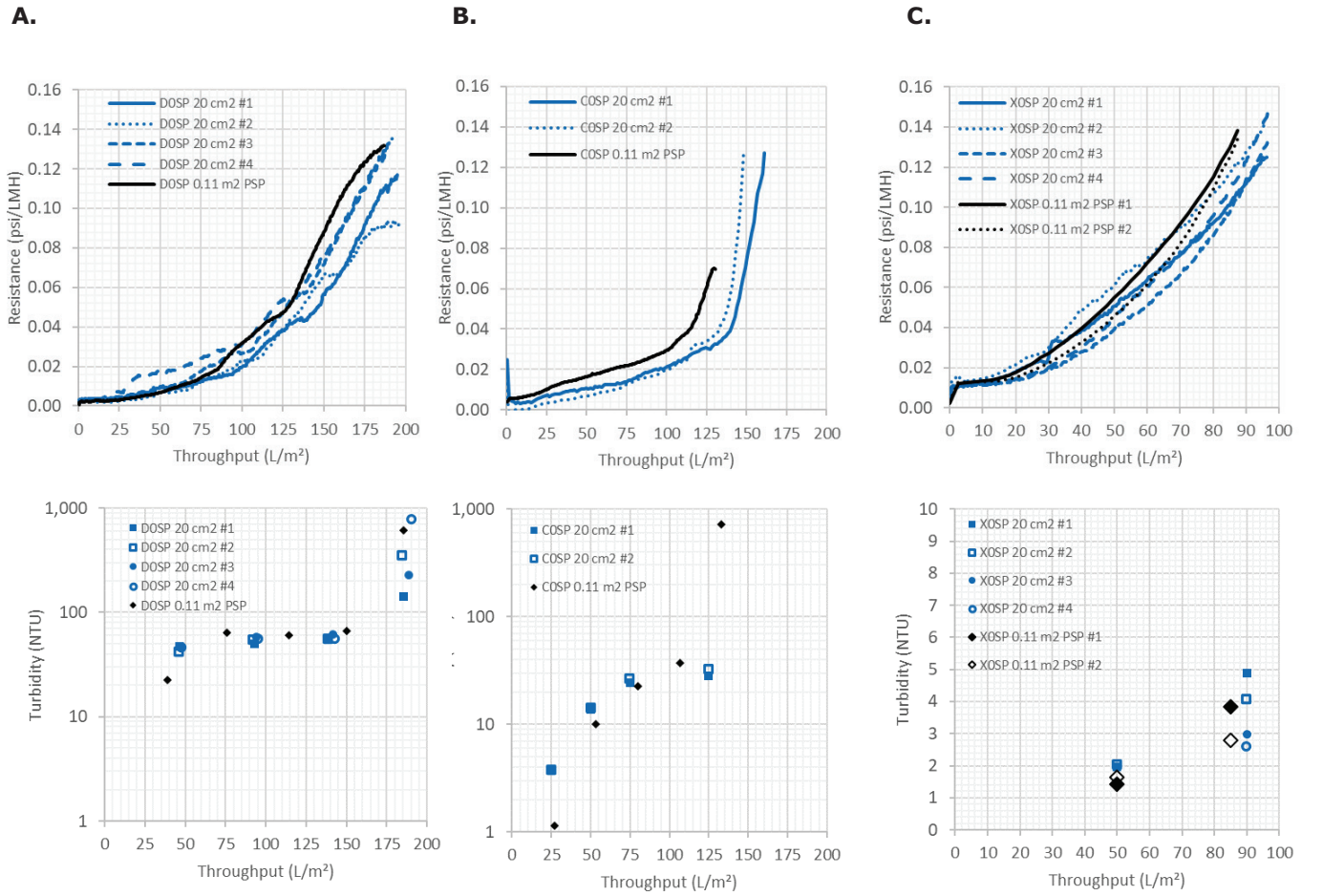

Figure 6. Filter scalability test results for Millistak+® HC Pro depth filter devices using selected CHO feed streams.

(a) Millistak+® HC Pro D0SP filter resistance and turbidity breakthrough versus throughput for the primary clarification of a CHO feed stream (mAb02, 20.5 x 10^6 TC/mL, 92% viable). **(b)** Millistak+® HC Pro C0SP filter resistance and turbidity breakthrough versus throughput for the primary clarification of a CHO feed stream (recombinant fusion protein, 19.8 x 10^6 TC/mL, 79% viable). **(c)** Millistak+® HC Pro X0SP filter resistance and turbidity breakthrough versus throughput for the secondary clarification of a CHO centrate feed stream (mAb02, 173 NTU, cell culture harvest details: 16.9 x 10^6 TC/mL, 93% viable). Filter resistance profiles are shown in the top panel and filter turbidity breakthrough curves are shown in the lower panel. Millistak+® HC Pro Micro 20 (20 cm^2) and 0.11 m^2 Process-Scale Pod filter devices are shown as lines and markers, as indicated.

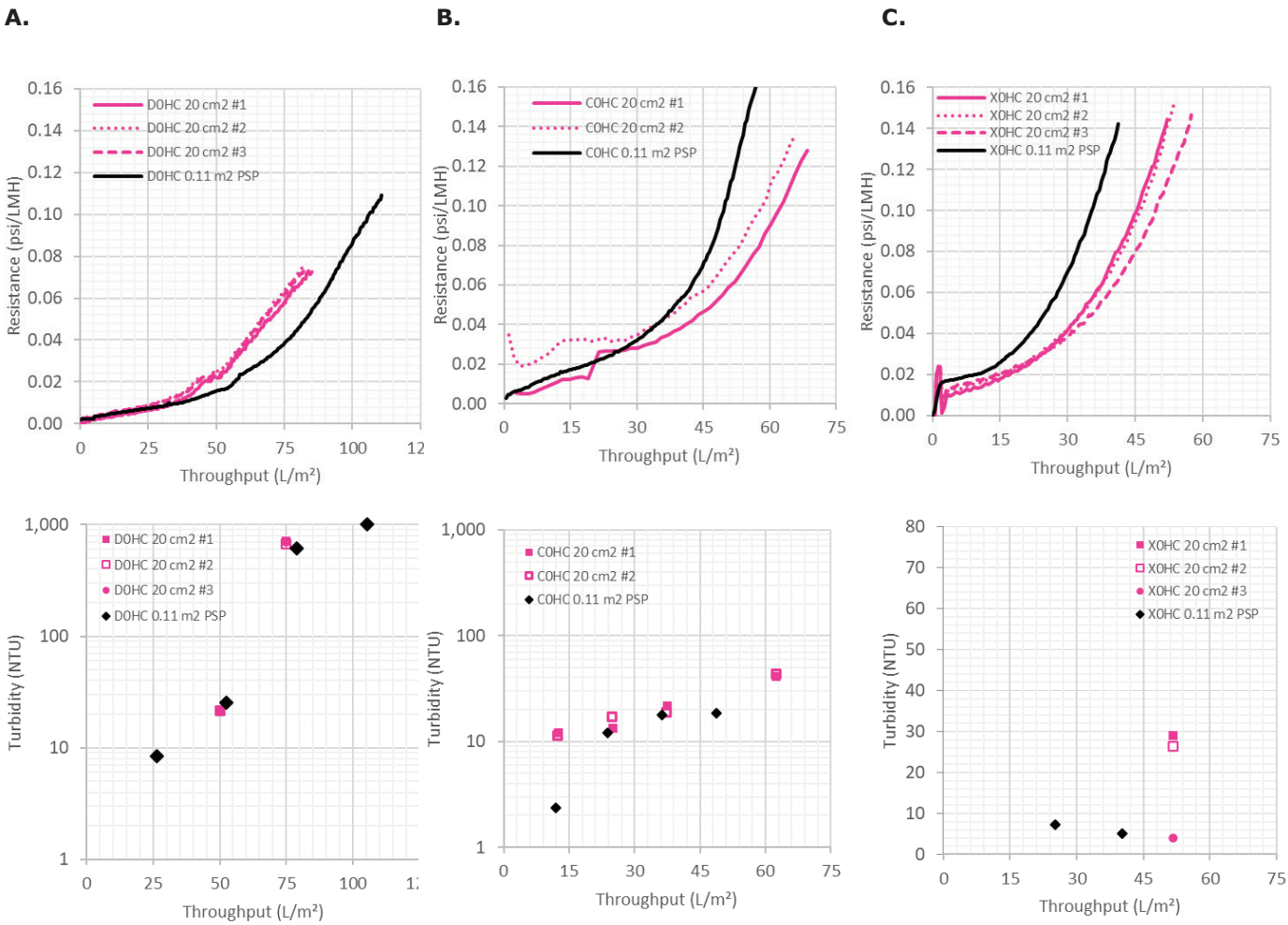

Figure 7. Filter scalability test results for Millistak+® HC depth filter devices using selected CHO feed streams.

(a) Millistak+® HC D0HC filter resistance and turbidity breakthrough versus throughput for the primary clarification of a CHO feed stream (mAb09, 14.9 x 10^6 TC/mL, 90% viable). **(b)** Millistak+® HC C0HC filter resistance and turbidity breakthrough versus throughput for the primary clarification of a CHO feed stream (recombinant fusion protein, 19.8 x 10^6 TC/mL, 79% viable). **(c)** Millistak+® HC X0HC filter resistance and turbidity breakthrough versus throughput for the secondary clarification of a CHO centrate feed stream (mAb09, 218 NTU, cell culture harvest details: 14.9 x 10^6 TC/mL, 90% viable). Filter resistance profiles are shown in the top panel and filter turbidity breakthrough curves are shown in the lower panel. Millistak+® HC Micro 20 (20 cm^2) and 0.11 m^2 Process-Scale Pod filter devices are shown a lines and markers, as indicated.

Figure 7a shows the filter scalability test results for Millistak+® HC D0HC depth filter devices using a CHO feed stream (mAb09, 14.9 x 10^6 TC/mL, 90% viable). The Process-Scale Pod and Micro 20 devices all exhibited a turbidity breakthrough at approximately the same throughput, and the filtration capacity of the Micro 20 devices were within 10% of the filtration capacity of the Process-Scale Pod device (75 L/m^2). While the resistance profile in **Figure 7a** shows the Millistak+® HC D0HC Process-Scale Pod device continuing to build resistance past 75 L/m^2, a significant turbidity breakthrough had already occurred by this point.

The filter scalability test results for Millistak+® HC C0HC depth filter devices using a CHO feed stream (recombinant fusion protein, 19.8 x 10^6 TC/mL, 79% viable) are summarized in **Figure 7b.** The Millistak+® HC C0HC devices had similar resistance profiles and the filter capacities of both the Micro 20 devices and the Process-Scale Pod devices were within 20%. Significant turbidity breakthrough was not observed with any of these devices prior to the filtration endpoint.

Figure 7c presents the filter scalability test results for Millistak+® HC X0HC depth filter devices using a CHO feed stream (mAb09, 218 NTU, original harvest 14.9 x 10^6 TC/mL, 90% viable). The filtration capacity of the Micro 20 devices was within 30% of the filtration capacity of the Process-Scale Pod device (40 L/m^2). No turbidity breakthrough was observed for any of the Millistak+® HC X0HC devices evaluated.

All Millistak+® HC D0HC, C0HC, and X0HC scalability tests were evaluated according to the best practices recommendations described above.

Conclusion.

In this Application Note, a set of recommended best practices for performing depth filtration sizing trials using commercial depth filtration devices, including the new Micro 20 depth filtration device are provided. The Micro 20 device has been optimized for scalability to the corresponding Millistak+® HC and Millistak+® HC Pro Process-Scale Pod depth filters. In order to minimize observed cell settling within process tubing lines during primary clarification tests, specific recommendations for filter tubing selection to minimize the system residence time are provided. Likewise, process flux excursion studies and a pre-use cell culture device loading recommendation are provided to understand the feed stream fouling behavior and to minimize feed stream dilution or filter headspace effects. These recommendations can help to ensure a more consistent device performance in depth filter scalability tests.

Depth filtration tests were also conducted to evaluate the scalability performance of the Micro 20 device format for Millistak+® HC and Millistak+® HC Pro depth filter media grades. A good agreement in depth filtration capacities was reported for all device formats versus their corresponding 0.11 m^2 Process-Scale Pod devices.

The new Micro 20 depth filter device is expected to provide a great scalability performance and accurate depth filter sizing estimates for our customer's harvest and secondary clarification processes.

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