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Technical Note

Bioburden Reduction and Particulate Retention Using Milligard® PES Filters

Introduction

Membrane-based filters are used extensively in the production of biopharmaceutical products to protect the process fluid from microbiological and particulate contaminants. Milligard® PES filters contain two layers of asymmetric polyethersulfone (PES) membranes: a 1.2 µm upstream layer and a downstream layer offered in several pore sizes (Table 1). These filters can be used as prefilters upstream of sterilizing filters to increase their capacity or can be used stand-alone to reduce bioburden and turbidity of process streams. Downstream processing comprises multiple operations that require different levels of microbial control. For non-critical process steps, where sterile filtration may not be necessary, Milligard® PES filters offer an alternative to sterilizing-grade filters for bioburden risk reduction.

Table 1. Milligard® PES Membrane Pores Sizes.

Milligard® PES Filter	Typical Bioburden Reduction
1.2/0.2 µm nominal	≥6 logs of *Brevundimonas diminuta*
1.2/0.45 µm	≥6 logs of *Serratia marcescens*
1.2/0.8 µm	Not determined

The purpose of this application note is to describe the throughput and retention performance of Milligard® PES filters in various model streams when used stand-alone without a sterilizing filter downstream. In addition, we demonstrate the scalability of these filters from OptiScale® 25 screening tools to pilot and production-scale capsule and cartridge filters.

Materials and Methods

Membranes and Devices

The studies described in this document compare performance of Milligard® PES filters with commercially available filters of similar pore sizes and composition. Table 2 summarizes the characteristics of the filters tested. For throughput studies, membranes of competitive filters were removed from pleated cartridge filters and assembled into OptiScale® 25 capsules (3.5 cm^2); studies with Milligard® PES filters were performed with OptiScale® 25 capsules. Scalability of throughput performance was assessed using 10-inch cartridge and Opticap® XL10 capsule filters containing Milligard® PES membranes. Effective filtration areas of filters and smallest membrane pore size are listed in Table 2.

Table 2. Summary of filters tested.

Filter	Membrane Characteristics
Milligard® PES 1.2/0.2 µm nominal[1]	2-layer asymmetric PES with 0.2 µm nominal layer
Competitive filter A	3-layer asymmetric PES with 0.2 µm nominal layer
Milligard® PES 1.2/0.45 µm[1]	2-layer asymmetric PES with 0.45 µm layer
Competitive filter B	2-layer asymmetric PES with 0.2 µm nominal layer
Milligard® PES 1.2/0.8 µm[2]	2-layer asymmetric PES with 0.8 µm layer
Polysep™ II	2-layer borosilicate glass and mixed esters of 1.2 µm layer.

[1] Membrane areas: 10-inch cartridge filter, 0.60 m^2; Opticap® XL10 capsule filters, 0.60 m^2

[2] Membrane areas: 10-inch cartridge filter, 0.53 m^2; Opticap® XL10 capsule filters, 0.57 m^2

Challenge Streams

Four different model streams representing a range of particle size distributions were used for these studies, Figure 1. The CHO stream was prepared by clarifying a CHO harvest containing 1g/L monoclonal antibody through a Millistak+® D0HC depth filter, then diluting 1:100 in phosphate buffered saline and filtering through a 5 µm Durapore® membrane. The composition of the other streams has previously been described[1]. Streams were selected to represent the range of particle sizes that might be present in different process feeds. To minimize the volume of challenge solutions required, streams were formulated to achieve 90% flux decay at 500-1000 L/m².

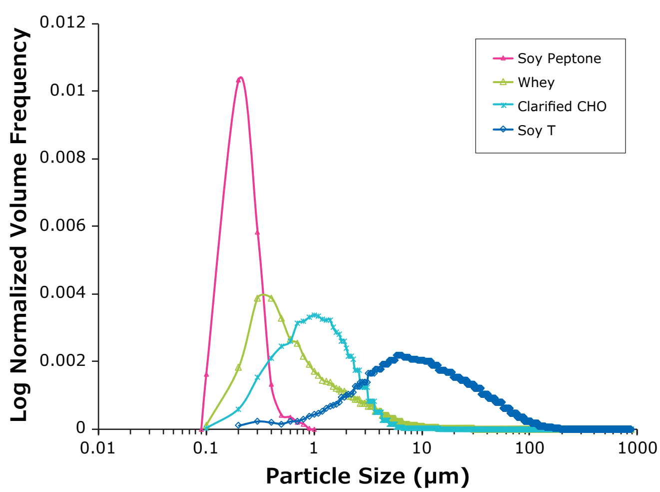

Figure 1. Particle size distributions of the challenge streams. Particle sizing was performed with Malvern MasterSizer and PMS Liquilaz.

Test Methods

Water Permeability and Throughput

Water permeability of Milligard® PES filters was measured at 10 psi and 21-25 °C. Filters were then challenged with the different model streams at 10 psi until permeability was reduced by 90% compared to the water permeability (90% flow decay). For all tests, temperature, pressure and filtrate volume data were collected as a function of time, using the experimental setup shown in Figure 2. Throughput performance of competitive filters in the different streams was normalized relative to the performance of the Milligard® PES filter in that stream.

Bioburden Reduction

Milligard® PES 1.2/0.2 µm nominal filters were challenged with soy peptone and whey solutions containing *Brevundimonas diminuta* at concentrations of at least 2.3 x 10^7 colony forming units (cfu)/mL. Milligard® PES 1.2/0.45 µm filters were similarly challenged with these same solutions spiked with *Serratia marcescens* at concentrations of at least 3.9 x 10^7 cfu/mL. In all tests, the spiked solutions provided a challenge greater than 10^7 cfu/cm² of filtration area. For each pore size, two membrane lots were each tested in the two streams with at least three replicate OptiScale® 25 capsules. Testing was performed at 2 psi and filtrate grab samples were collected during the runs and assayed for titer. When tests reached 90% flow decay, tests were stopped, and samples were collected from filtrate pools and assayed for titer. Log reduction values (LRV) for the intermediate samples were determined by comparing the log titer in the challenge solutions with the log titer of the intermediate filtrate samples. For the filtrate pools, LRVs were determined by comparing the log microbial load (concentration x volume) in the challenge solution with that of the filtrate pool.

Scalability

Scalability was assessed by comparing throughput performance of Milligard® PES membranes in the 10-inch cartridge or Opticap® XL10 capsule formats to that of the OptiScale® 25 capsules with one non-plugging (water) and two plugging streams at constant pressure of 5 psi. This pressure was selected to approximate the typical pressure a prefilter might experience in a plugging application. Scalability tests compared performance using filters containing the same membrane lot. All tests were stopped when permeability reached 90% flux decay relative to the initial water permeability of each filter (all OptiScale® 25 capsules exceeded 90% flux decay). Scaling factors represent the throughput of a 10-inch cartridge or capsule filter relative to the average throughput of five OptiScale® 25 capsules.

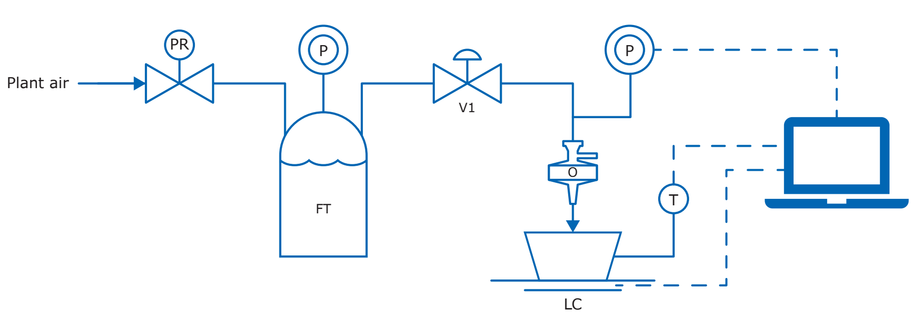

Figure 2. Test setup for throughput tests. Symbols: FT, feed tank; O, OptiScale® 25 filter; LC, load cell; P, pressure measurement; PR, pressure regulator; T, temperature measurement; V, valve. Temperature, pressure and filtrate volume were recorded by a data acquisition system.

Particle Removal

Particle removal capability of Milligard® PES filters was determined by comparing the particle concentration and size distribution of soy peptone before and after processing through OptiScale® 25 and Opticap® XL10 capsules containing Milligard® PES membranes at a constant pressure of 10 psi. Samples of challenge and filtrate solutions were analyzed using a Liquilaz Model SO2 particle analyzer.

Results and Discussion

Throughput

Throughput performance of Milligard® PES filters was benchmarked against comparable commercially available filters using four model streams containing particle sizes representative of bioprocessing fluid streams. Performance of competitive filters was normalized to Milligard® PES performance for each stream. Differences in throughput within 20% are not considered to be statistically significant.

Figure 3 shows that in most model streams, throughput of Milligard® PES 1.2/0.2 µm nominal filters is at least 20% higher than that of competitive filter A. The largest throughput advantage was shown with the soy peptone stream, which has the highest concentration of small particles in the streams tested and is most likely to challenge the internal structure of the Milligard® PES 1.2/0.2 µm nominal membrane. Milligard® PES filters exhibited lower throughput compared to the competitive filter in the soy T stream, the model stream containing the largest-sized particles. The competitive filter has a different membrane symmetry and pore structure to Milligard® PES filters and is more suited for processing feed streams containing relatively large particles. For these types of solutions, a coarser prefilter might be more appropriate.

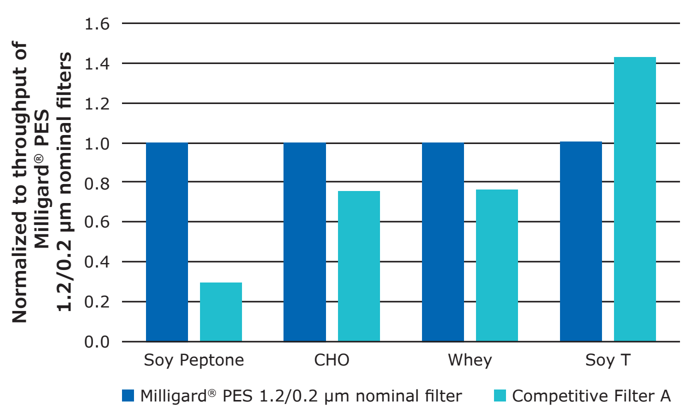

Figure 3. Throughput performance of Milligard® PES 1.2/0.2 µm nominal filters compared to competitive filter A. Each bar represents the average results of two OptiScale® 25 filters.

Figure 4 shows the results of similar throughput tests with Milligard® PES 1.2/0.45 µm filters. In the soy peptone stream, Milligard® PES 1.2/0.45 µm filters outperformed the competitive benchmark. In streams containing larger particles, whey and soy T, Milligard® PES 1.2/0.45 µm filters showed equivalent performance. However, for the CHO stream where most particles are closest to the filter pore rating, Milligard® PES 1.2/0.45 µm filters had lower throughput than competitive filter B.

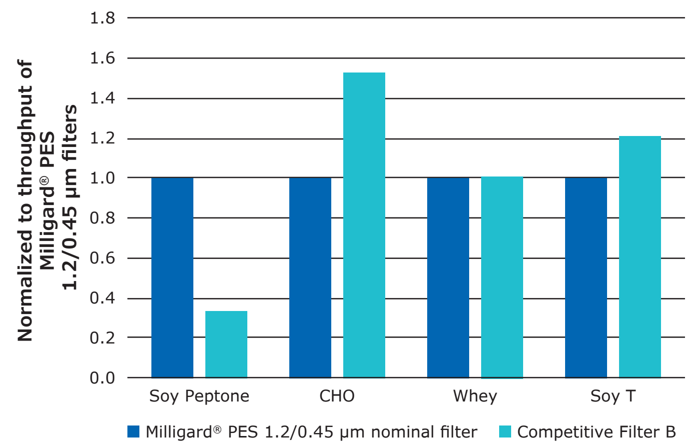

Figure 4. Throughput performance of Milligard® PES 1.2/0.45 µm filters compared to competitive filter B. Each bar represents the average results of two OptiScale® 25 filters.

No comparable commercially available filter was identified for benchmarking Milligard® PES 1.2/0.8 µm filter, therefore throughput performance was compared to Polysep™ II, our high capacity prefilter (Figure 5). Similar performance between the filters was shown in streams containing the highest concentrations of large and small particles, but in mid-particle size streams such as CHO and whey, Polysep™ II filters showed higher throughput than Milligard® PES 1.2/0.8 µm filters. This is likely due to higher particle adsorption on the borosilicate glass and mixed esters of cellulose membrane in Polysep™ II filters. However, in processes requiring filter compatibility with gamma irradiation or steaming in place (SIP) sterilization methods, Milligard® PES filters are an attractive option.

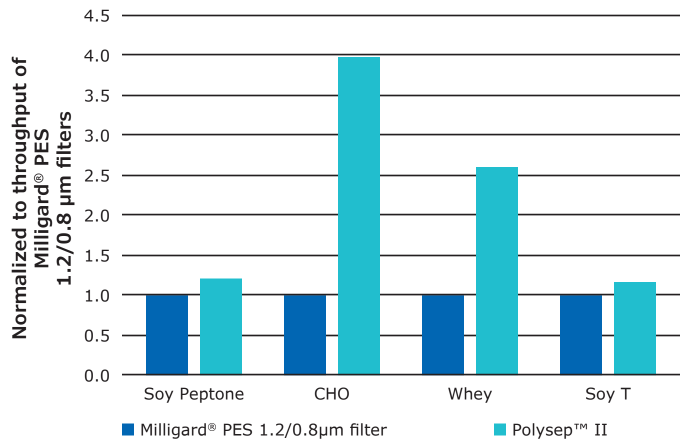

Figure 5. Throughput performance of Milligard® PES 1.2/0.8 µm filters as compared to Polysep™ II filters. Each bar represents the average results of two OptiScale® 25 filters.

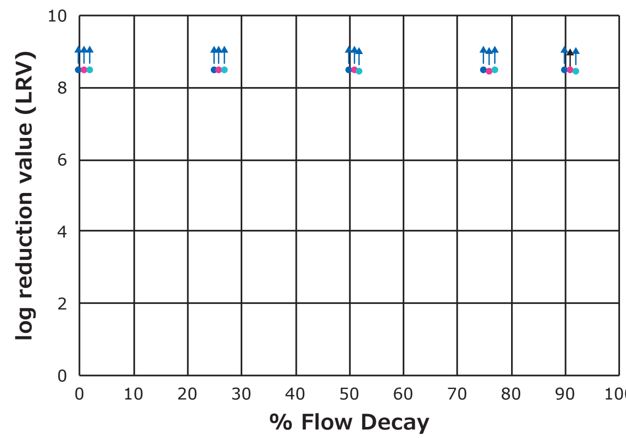

Figure 6. Retention of triplicate Milligard® PES 1.2/0.2 µm nominal filters challenged with soy peptone containing *B. diminuta*. Arrows indicate no bacteria was detected in the filtrate.

In summary, these results highlight the throughput capacity performance of Milligard® PES filters as compared to commercially available filters in four model streams of different particle size compositions. Each application and process fluid will have a different particle composition, which will affect the capacity of any given filter. However, throughput performance of Milligard® PES filters was favorable as compared to alternative commercially available filters in multiple challenge streams. In practice, we recommend evaluating process streams using Milligard® PES filters of different pore sizes to identify the preferred filter for maximizing throughput.

Bioburden Retention

Milligard® PES 1.2/0.2 µm nominal and 1.2/0.45 µm filters are designed to be used as stand-alone filters for bioburden control in non-critical process steps. Although these filters are not designed to deliver the same level of microbial protection as filters containing sterilizing-grade membrane, reliable microbial retention is a performance expectation, even when the membrane is highly fouled. Assessments of bioburden reduction performance of Milligard® PES 1.2/0.2 µm nominal and 1.2/0.45 µm filters was limited to the soy peptone and whey model streams which contained the highest concentrations of smaller particles. Although two membrane lots of each Milligard® PES membrane pore size were tested in each stream, all test results were similar, therefore a limited subset of results are shown.

Figure 6 shows the results of retention tests with Milligard® PES 1.2/0.2 µm nominal filters challenged with soy peptone stream containing *B. diminuta*. Bacteria were not detected in any filtrate grab samples, resulting in LRVs of at least 8, even out to 90% flow decay. A low level of bacteria was detected in one of the filtrate pools, but in all cases, LRVs exceeded 9.4, Table 3. Similar results were obtained when Milligard® PES 1.2/0.2 µm nominal filters were challenged with *B. diminuta* in the whey model stream (data not shown).

The test results of Milligard® PES 1.2/0.45 µm filters challenged with the soy peptone stream containing *S. marcescens* are shown in Figure 7. Bacteria were not detected in any filtrate grab samples, resulting in calculated bacterial retention of at least 8 LRV, even at 90% flow decay. Similar results were obtained when these filters were challenged with *S. marcescens* in the whey model stream (data not shown).

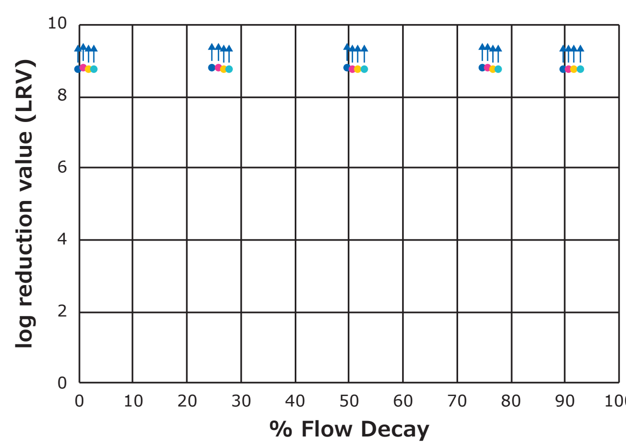

Figure 7. Retention of triplicate Milligard® PES 1.2/0.45 µm filters challenged with soy peptone containing *S. marcescens* at 10^7 cfu/cm^2. Arrows indicate no bacteria were detected in the filtrate.

Overall, robust bioburden retention was demonstrated with Milligard® PES filters in two model streams containing high concentrations of small to medium-sized particles. Importantly, retention performance of Milligard® PES filters is maintained under conditions that are typically challenging for membrane filters: where the membrane pores are highly fouled and permeability is 90% lower than the clean water permeability[2]. Table 3 summarizes LRVs in filtrate pools after processing model steams across Milligard® PES 1.2/0.2 µm nominal and Milligard® PES 1.2/0.45 µm filters.

Table 3. Milligard® PES Filter Final pool LRVs.
Values represent the lowest pool LRV.

		Challenge Fluid	
Microorganism	**Membrane**	**Soy peptone**	**Whey**
B. diminuta	Milligard® PES 1.2/0.2 µm nominal	9.3	≥ 9.4
S. marcescens	Milligard® PES 1.2/0.45 µm	≥ 9.5	≥ 9.4

Milligard® PES filters are not a substitute for sterilizing filters in final filtration, but offer an attractive option for bioburden risk reduction in non-critical process steps.

Scalability

Accurate estimation of filtration area requirements for large-scale processes relies on scaling factors that connect performance of small-scale sizing tools to larger filter formats. Scaling factors represent the ratio of performance of larger filters to small-scale sizing tools, normalized to membrane area, and ideally should be close to 1.0. These factors are influenced by filter design features such as pleat structure as well as the plugging characteristics of the process fluid. In practice, for filters containing sterilizing-grade membrane, scaling factors are commonly within about 20% of unity, whereas the range for filters used in non-critical process steps might be broadened. Figure 8 shows how the scaling factor can change with increasing filter fouling: as the filter fouls, the scaling factor tends to converge towards unity.

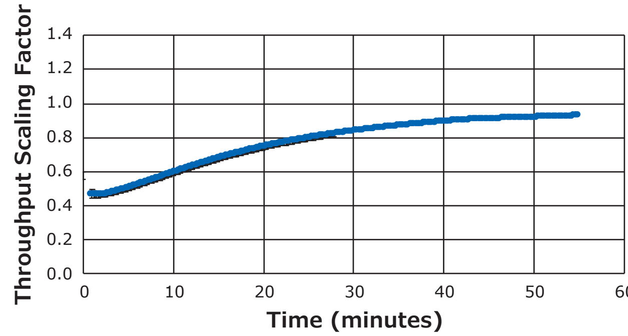

Figure 8. Throughput scaling factor as a function of filtration time for Milligard® PES 1.2/0.2 µm nominal cartridge filters in soy peptone. Error bars show the standard deviation of the mean.

Scalability of throughput in Milligard® PES 10-inch cartridge and capsule filters relative to OptiScale® 25 capsules was determined in water and two plugging model streams, Table 4. All throughput scaling factors were established at the time where the cartridge or capsule filters were 90% plugged. In a non-plugging stream such as water, scaling factors were in the range of 0.4-0.7. Similar low scaling factors have been reported for other filters and are a consequence of high membrane permeability and dense filter pleat structure which results in added flow resistance[3]. In these situations, safety factors typically included in filtration area sizing models could be increased to accommodate non-linear scaling from small to large-scale devices.

In plugging streams, scalability between the small-scale devices and larger filters was more linear. This is because as the membrane fouls it becomes the dominant resistance relative to resistances in other parts of the pleat structure.

Table 4. Milligard® PES filters scaling factors

	Non-plugging Stream	Plugging Stream	
Product	**Water**	**Soy peptone**	**Whey**
Milligard® PES 1.2/0.2 µm nominal cartridge	0.6	0.9	1.0
Milligard® PES 1.2/0.2 µm nominal capsule	0.7	0.9	0.9
Milligard® PES 1.2/0.45 µm cartridge	0.5	0.9	1.1
Milligard® PES 1.2/0.45 µm capsule	0.6	0.8	0.9
Milligard® PES 1.2/0.8 µm cartridge	0.6	1.0	1.1
Milligard® PES 1.2/0.8 µm capsule	0.4	0.7	1.0

Particle Reduction

Particle retention by Milligard® PES filters was quantified by measuring particle concentrations and size distributions in the soy peptone model stream before and after filtration through both OptiScale® 25 capsules and Opticap® XL10 capsules, Figure 9. Before testing, the Opticap® XL10 capsules were pre-sterilized by gamma irradiation.

The soy peptone challenge solution contains particles that are mostly smaller than 1 µm, with the highest concentration of particles in the 0.2-0.25 µm range. Milligard® PES 1.2/0.2 µm nominal filters retained over 90% of particles in the 0.2-0.25 µm range, more than the other membrane pore size offerings. All the filters removed greater than 80% of all particles larger than 0.2 µm.

This analysis highlights differences in performance related to membrane pore size. However, filter selection is generally guided by empirical results: throughput performance in combination with process needs for bioburden control and particulate removal.

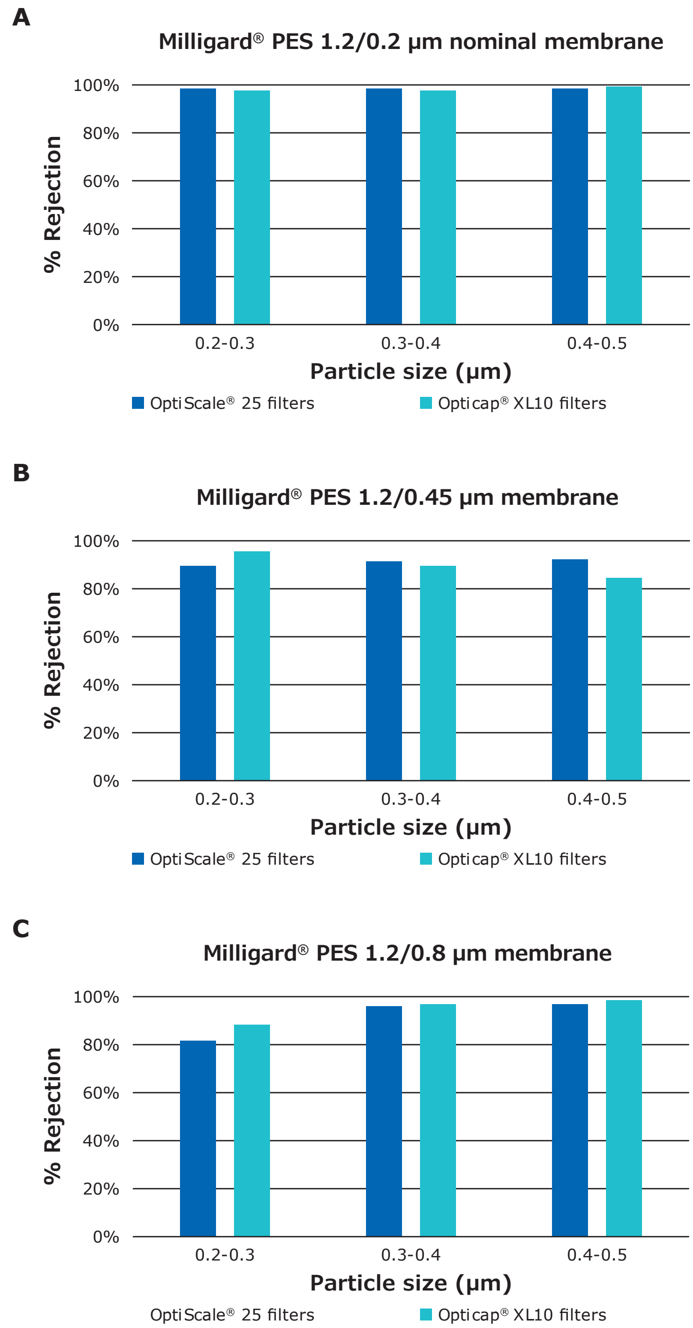

Figure 9. Particle removal from soy peptone stream following processing across OptiScale® 25 and Opticap® XL10 capsule filters containing Milligard® PES 1.2/0.2 µm nominal membrane (**A**), Milligard® PES 1.2/0.45 µm membrane (**B**), Milligard® PES 1.2/0.8 µm membrane (**C**).

Conclusions

Milligard® PES filters were demonstrated to provide high throughput capacity for several different streams representing wide ranges of particle size distributions. In model streams containing predominantly small to mid-sized particles (0.1-10 µm), Milligard® PES 1.2/0.2 µm nominal and 1.2/0.45 µm filters performed well against benchmark competitive filters. However, for streams containing predominantly larger particles (> 10 µm), Milligard® PES 1.2/0.2 µm nominal and 1.2/0.45 µm filters may not be the optimal choice. For this type of stream, coarser prefilters should be considered. Milligard® PES 1.2/0.8 µm filters show similar capacity to Polysep™ II filters in most, but not all, streams. However, a key advantage of Milligard® PES filters is their compatibility with gamma irradiation and thermal sanitization methods.

For non-critical process steps requiring bioburden control, Milligard® PES 1.2/0.2 µm nominal and 1.2/0.45 µm filters provide reliable bioburden removal, even under conditions where the filters are highly fouled. Greater than 6 log removal of *B. diminuta* and *S. marcescens* were demonstrated for Milligard® PES 1.2/0.2 µm nominal and 1.2/0.45 µm filters respectively.

Scalability testing of Milligard® PES filters indicated that as the membrane fouls, the scaling factor between OptiScale® 25 devices and 10-inch cartridges and capsules approaches unity, allowing for simple and reliable filter sizing. For low plugging streams, scaling factors are available that account for the effect of the high permeability and dense pleat structure of these filters.

In summary, these filters combine reliable particle retention, effective bioburden reduction and compatibility with thermal and gamma sterilization methods. For non-critical process steps, they are an attractive alternative to sterilizing filters for reducing bioburden and improving processing efficiency.

References

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