1. Introduction

In bioprocessing, process development scientists and engineers regularly seek improvements in filter performance to facilitate safe, cost-effective manufacture of drug and vaccine products. The latest developments in high-performance membrane filter technology are driven by ever-increasing industry demands for accelerated fluid processing, higher throughput capacity and more robust claims related to microbial contaminant removal, along with filter user expectations including low extractables, low product adsorption, broad fluid compatibility, wettability for integrity testing and resistance to sterilization by heat or gamma-irradiation. This white paper provides an overview of the main attributes of membrane filters used in bioprocessing, and highlights how advanced design and manufacturing innovations in filter design and construction can help filter users to meet such challenges.

2. Membrane Filters in Bioprocessing

The output of a pure, high quality biological drug or vaccine product is dependent on various factors including precisely-controlled cell growth, separation and purification stages which microbially-rated filters are either integral or accessory to. Microbially-rated membrane filters for fluid sterilization, bioburden control and prefiltration are commonly used for the filtration of growth media, cell harvest material, intermediate and final bulk product, buffers and reagents and the filling of drug formulations into dosage containers. Depending on the type of process fluid and degree of optimization that the user has been able to achieve in process development, microbially-rated membrane filters may be oriented within a process either as standalone units, or as within multi-stage filter systems.

3. Common Attributes of Microbially-Rated Filters

The majority of commercially-available membrane filters designed for the filtration of biopharmaceutical fluid streams utilize hydrophilic membranes comprised of polymers, including:

- Polyethersulfone (PES)
- Polyvinylidene fluoride (PVDF)
- Nylon 6-6
- Cellulose Ester

A typical membrane filter assembly comprises a filter membrane layer (or layers) paired with upstream and downstream support and drainage materials pleated around a latticed central core, sealed at the top and bottom by end caps, and surrounded by an outer cage. An assembly will also feature an adaptor at one end to allow for installation in a stainless-steel filter housing, or for its containment within a polymeric filter capsule ‘shell’. The end caps, core and cage components, support and drainage layers, adaptor materials and polymeric capsules (if applicable) form the filter cartridge’s ‘hardware’. Combined PES/PVDF membranes with polypropylene hardware components tend to be the materials of choice in the latest membrane filter developments for reasons of compatibility, low adsorption, low extractables, high flow/low pressure drop, throughput capacity, wettability for integrity testing and tolerance to sterilization procedures.
4. **Overview of Main Attributes for Membrane Filters Used in Bioprocessing**

**Fluid Compatibility**

In upstream through to downstream stages in bioproduction where a diversity of fluids across a broad pH range may be processed (e.g. feeds, reagents, chromatography and diafiltration buffers), filters that demonstrate broad fluid compatibility by maintaining claimed performance specifications while under extremes of pH, are essential. Due to enhanced chemical compatibility properties, filters comprising PVDF and PES filter membranes are reliable options for a range of microbial filtration applications in bioprocessing; PES is particularly well-suited for filtration of both strong acids and bases.

**Low Active-Ingredient Adsorption**

As it is typically the objective of a drug or vaccine manufacturer to maximize the output of their end product, when filtering process streams containing drug substances, vaccines or other process-critical components it is favourable to use a filter membrane that does not diminish the concentration of any active ingredients to the detriment of yield. This is of particular significance in biotechnology, where variable concentrations of highly-potent monoclonal antibodies, proteinaceous active ingredients and other components that serve to influence product efficacy will be presented to a filter membrane during downstream processing. Hydrophilic, modified PVDF and PES membranes are recognised as having low protein-binding properties, and the use of a filter incorporating either of these membranes helps to minimize the risk of active ingredients or stabilizers being adsorbed from a process stream.

**Low Extractables**

The passage of fluid through a filter may encourage leaching of small quantities of the filter’s materials of construction into a process stream, which could have significant implications on product quality or efficacy.

An indicator of a filter’s propensity to leach materials can be linked to the ‘extractables’ claim within its overall specification, typically expressed as the mass of non-volatile-residues (NVR) drawn from a filter following exposure to an extraction fluid (usually water, or to simulate particularly aggressive conditions, an organic solvent such as ethanol) for a fixed time period. This type of quantitative analysis can also be complemented by qualitative analysis, where both the organic and inorganic content of extracted matter can be identified through Fourier Transform Infrared Spectroscopy (FTIR), Inductively Coupled Plasma Spectroscopy (ICP), High Temperature Gas-Liquid Chromatography (HTGLC) or other analytical methods.
The degree to which a filter’s materials of construction leach is affected by many variables, including chemical composition of the process stream, the duration contact time with process fluid and conditions of operating temperature, pressure and flow rate. The significance of the quantitative and qualitative composition of any leachables caused by a filter in the user’s process will depend on what stage in the process the filter is being used, and for what kind of fluid it has been chosen to filter. The impact of leachables from any equipment tends to become more significant as a process approaches its endpoint. In the filtration process, leachables are most keenly observed in product-containing process streams that have been filter-sterilized during formulation into final dosage containers.

Ease of Filter Integrity Testing

The integrity testing of sterilizing-grade filters in pharmaceutical or vaccine manufacturing by either forward flow or bubble point procedures is commonplace. Where filters are used to sterilize a final dosage formulation, it is a universal regulatory requirement that a post-use filter integrity test is carried out following filtration. To confirm that a filter will perform its expected function before use, a pre-use integrity test is also widely recommended. As an accurate integrity test measurement depends upon a fully-wet filter membrane (partially-wet membranes can incur false failures), the ease of wettability of a microbially-rated filter with water or an aqueous process fluid is a prerequisite. PVDF and PES membranes are inherently hydrophobic, however they can be rendered hydrophilic (and easily wettable) through chemical modification at some stage during the membrane manufacturing process. These proprietary surface modification techniques differ among filter manufacturers, as do membrane morphologies, making membranes using PVDF or PES as their base polymers less similar than often assumed.

Sterilizable by Gamma Irradiation, Autoclave and Steam-In-Place (SIP) Procedures

Pall’s microbially-rated filter assemblies with PES and PVDF membranes demonstrate maintenance of bacterial challenge performance and integrity following exposure to doses of gamma irradiation up to 50 kGy, and in-line steam and autoclave cycles at >121 ºC. A filter that retains its key performance claims following gamma irradiation assures its suitability for inclusion in single-use bioprocessing systems that are not capable of tolerating forms of steam sterilization, and instead require radiation doses typically >25 kGy (to a maximum of 50 kGy) to ensure that they can be supplied fully sterilized, and safe for use.

Despite the trend towards the adoption of single-use-systems incorporating filter capsules, many drug manufacturers continue to use membrane filter cartridges fitted in stainless steel housing configurations, or introduce standalone single use membrane filter capsule assemblies into hard-piped stainless steel installations, either in ‘legacy’ manufacturing platforms or in new plant construction. For this reason, it is also important that new filter membranes continue to show equivalent compatibility with steam and autoclave sterilization methods to the filters they have been designed to succeed.

Clean Water Flow (Flow Rate versus Differential Pressure) and Throughput Capacity

A common driver for the selection of microbially-rated filters is performance efficiency, which is usually linked to two performance parameters:

- How quickly fluid may flow through a filter at a given differential pressure, and what capacity a filter may have for a process stream until blockage, or
- A point at which the flow of the effluent has deteriorated such that it cannot serve a downstream system that depends on its input at an expected minimum flow rate

These parameters are influenced mainly by the type of membrane layer or layers incorporated into a filter (which will have varied pore symmetry to effect the manufacturer's target specifications of throughput capacity and flow rate performance), combined with the nature of the cross-sectional pleating construction incorporated within the filter, the overall effective filtration area of membrane and to a degree, the type of support and drainage layers used in the filter. PVDF, and particularly PES membranes have been developed by filter manufacturers for the reason that their porosity can be carefully developed and controlled during the manufacturing process and because they are durable enough to accommodate novel pleating configurations. PVDF and PES membrane filters can produce markedly-improved flow rate and throughput performance over microbially-rated filters using nylon or cellulose acetate membranes.
5. **Categorization of Microbial Removal Requirements in Bioprocessing: Sterilizing-Grade Filtration, Bioburden Control/Pre-Filtration, Mycoplasma Control Filtration**

**Sterilizing-Grade Filtration**

The FDA Guidance for Industry (Sterile Drug Products Produced by Aseptic Processing) 2004 defines a sterilizing-grade filter as “…a filter that, when appropriately validated, will remove all microorganisms from a fluid stream, producing a sterile effluent”. The document further states that when qualifying a sterilizing-grade filter, “…a bacterial challenge concentration of at least $10^7$ organisms per cm$^2$ of effective filtration area should generally be used, resulting in no passage of the challenge microorganism”. It is typical for filter manufacturers to qualify a filter as ‘sterilizing-grade’ using the organism *B. diminuta* (*Brevundimonas diminuta*) at the described challenge conditions, due to ease of culture in the laboratory and its ability to penetrate filter membranes rated at 0.45 microns.

**Bioburden Control/Pre-Filtration**

Despite the prevalence of sterilizing-grade membrane filters in biological manufacturing, they may be surplus to requirements in certain process stages where the removal of microbial contaminants is a requirement yet a complete absence of bacteria is not to be expected. For example, sterilizing-grade filters (due to the frequency of their use in neighbouring process steps) are often chosen to remove microorganisms from fluid streams which enter systems that cannot be operated under sterile conditions, such as chromatography or tangential flow filtration systems. In such cases bioburden control filters, often developed with higher flow rate and throughput performance than their sterilizing grade counterparts, may be satisfactory. A bioburden control filter validated with a titer reduction claim (rather than a sterilizing-grade claim) for a diminutive microorganism such as *B. diminuta*, can still reduce microbial contaminants to a minimum level (effectively zero) without compromising process safety. High-flowrate/high-throughput membrane filters with a bioburden control titer reduction claim can also be used for prefiltration, protecting sterilizing-grade filters in order to extend their service life and lower their influent microbial load.

**Mycoplasma Control Filtration**

Alternatively, in other applications which necessitate a sterilizing-grade filter it may be desirable to use not only a sterilizing-grade filter qualified as above, but one with additional claims associated with the removal of other known problematic microbial contaminants such as mycoplasma species. In bioproduction, filters designed for sterile filtration with mycoplasma control are typically required for the filtration of cell culture growth media (mycoplasma are adventitious contaminants of animal and plant-derived growth media ingredients and can also be introduced into growth media by humans during preparation), and in water systems and other applications where a bioburden isolate, known to penetrate 0.2 micron-rated sterilizing-grade filters under process conditions, may present a contamination risk. Such filters, possessing an even tighter porosity than 0.2 micron-rated filters, and referred to 0.1 micron-rated filters, may show a typical titer reduction of in excess of 8 log reduction value (LRV), and as high as >10 or 11 LRV for *Acholeplasma laidlawii* per 10-inch filter. This type of performance claim is indicative of an even higher level of safety than that offered by a 0.2 micron-rated sterilizing-grade filter.

Whether the end user requires sterilizing-grade, bioburden control or mycoplasma control filtration at different stages throughout a bioprocess, fluid streams will vary in chemical and biological composition, viscosity, contaminant load and volume. Operating conditions of temperature, pressure and flow rate are also unlikely to be consistent from one stage to another. Membrane filters must be precisely developed and adapted to manage the highly-differentiated uses for sterilizing-grade filters. Users need to be appropriately informed about what characteristics confer the level of performance they need so that they can make the best decisions when it comes to qualifying a microbi ally-rated filter in their process.
6. Innovations in Membrane Development and Filter Design for Advanced Performance

As previously discussed, today's commercially-available microbially-rated filters possess many common properties in design, materials of construction and mechanical/operational performance that allow their use in bioprocess applications. Consequently, it can be challenging for the user to differentiate between the many different filters that are available when making a selection for a given application. Though many microbially-rated filters are able to satisfy an end user’s main objective of achieving quality and process safety, they may not always deliver adequate efficiency, and may result in costly filter systems. Microbially-rated filters that deliver an optimum balance of both process safety and process economy do exist, and can be identified through certain design features and adaptations.

7. The Current Industry Standard in Filter Design

The most common filter configuration used by pharmaceutical manufacturers is a single open-ended cartridge comprised of single-to-multiple units of 10-inch filter sub-assemblies, installed for use in permanent stainless-steel housings. In the last decade there has been an increasing trend to use these ‘industry standard’ configurations pre-installed in polymeric ‘single-use’ filter capsules. These can offer benefits over stainless steel housing/filter installations in certain applications, through ease of operation and handling and notably, minimizing the need for cleaning, sterilization and associated validation. Due to the long-standing market acceptance of both filter cartridge and filter capsule formats derived from 10-inch sub-assemblies, and well established production platforms that deliver these products, filter manufacturers have had to deal with the challenge of finding novel ways to maximize throughput capacity per filter.

8. Meeting The Challenge

Pall has adopted an innovative approach to enhancing the performance of filter cartridges and capsules by focusing on three key areas: adapting membrane morphology, modifying pleat geometry and adjustment of sub-assembly internal core dimensions.

Adapting Membrane Morphology

In recent years, the development of highly-asymmetric microporous membranes with controlled pore size gradients (Figure 2) has contributed to a tangible increase in throughput performance per unit of filter membrane area. A filter membrane constructed with an asymmetric profile removes larger particles within the upstream thickness of the membrane layer while smaller, sub-micron contaminants (such as host cell proteins, nucleic acids and membrane aggregates and microorganisms in bioproduction) are retained by the narrower portion of the filter membrane within the mid-side and downstream-side thickness. For biological process streams comprised of contaminants with a broad particle size distribution, a highly-asymmetric membrane will ensure that the filter membrane captures particles throughout its entire depth, showing superior throughput performance when compared to a membrane of equivalent mean pore size, but a more symmetric thickness profile.
Modifying Filter Pleat Geometry

Filters assembled such that the pleated filter media pack in cross-section shows a conventional fan or ‘star pleat’ construction have been superseded by Pall filters whose cross-section displays a laid-over ‘crescent pleat’ construction (Figure 3). These crescent-shaped pleats, densely packed into 10-inch filter sub-assemblies result in finished configurations with increased effective filtration areas and correspondingly-higher flow rates and better throughput capacity (Table 1). This technology not only improves flow rate, throughput and thus efficiency, it also produces a very robust and mechanically-stable filter.

Adjustment of Sub-Assembly Internal Core Dimensions

A more mechanically-stable filter pack afforded by a laid-over pleat construction can allow for further modifications to improve filter performance, such as adjustment of a filter’s core dimension. A narrower core design (Figure 4) provides for an additional increase in effective filtration area, further extending the fluid path and effecting even higher ‘flow versus differential pressure’ performance for the filter cartridge, as well as increasing the capacity available for unwanted particles.

A filter combining a laid-over-pleat construction with a narrow core dimension may now contain 1 m² of membrane, in some cases up to double the effective filter area of more conventionally-designed 10-inch filters.
The combined effects of the three enhancements described enables users that utilize 10-inch filters to:

- Dramatically downsize existing stainless steel-housed multi-round/multi-high filter systems
- Benefit from the most streamlined filter set-ups in new manufacturing facilities
- Convert smaller stainless steel filter housing installations into single-use-system-friendly encapsulated configurations

Furthermore, the recent introduction of 5-inch filters with these design innovations now enables users accustomed to single-stage 10-inch filters that incorporate a more traditional design, to cut system sizes by 50 %, saving costs and increasing process speed (Figure 5).

**Figure 4**
*(Left) Standard Core Cross-Section and (Right) Narrow Core Cross-Section*

**Figure 5**
*(Left) 5-inch Filter Cartridge and (Right) 5-inch Filter Capsules Allow for More Compact Filter Installations, In Place or Instead of Systems Traditionally Using Single 10-inch Filters*
Innovative Microbial Control Filtration Solutions for Biopharmaceutical Production

Table 1 shows a list of current Pall filter membranes suitable for the diverse range of microbial removal requirements in bioprocessing, incorporating the advanced membrane technology and design features described in this article.

Table 1
Filter Selection for Bioburden Control, Sterility and Mycoplasma Control

<table>
<thead>
<tr>
<th>Bioburden Control</th>
<th>Sterilizing-Grade</th>
<th>Sterilizing-Grade</th>
<th>Sterilizing-Grade</th>
<th>Mycoplasma Control</th>
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<tr>
<td>Membrane Grade (Code)</td>
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<td>Supor EX</td>
<td>Fluorodyne® EX</td>
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<td>Material</td>
<td>Polyethersulfone</td>
<td>Polyethersulfone</td>
<td>Polyethersulfone/ Polyvinylidenedi fluoride hybrid</td>
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<tr>
<td>Removal Rating (µm)</td>
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<td>0.2</td>
<td>0.2</td>
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<td>Effective Filtration Area Per 10-inch Filter (m²)</td>
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<td>0.6</td>
<td>1.04</td>
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<tr>
<td>Clean Water Flow (Liters Per Minute)</td>
<td>20</td>
<td>13</td>
<td>17</td>
<td>10</td>
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<tr>
<td>Microbial Removal Claim</td>
<td>B. diminuta: Titer Reduction &gt; 6 LRV</td>
<td>B. diminuta 10⁷/cm²: STERILE EFFLUENT</td>
<td>B. diminuta 10⁷/cm²: STERILE EFFLUENT</td>
<td>B. diminuta 10⁷/cm²: STERILE EFFLUENT</td>
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<td>Laid-Over</td>
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<td>Highly-Asymmetric</td>
<td>Highly-Asymmetric</td>
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9. Summary

The biopharmaceutical industry continues to search for economical solutions for improved process safety, whilst maintaining high yields of premium quality drug products. As illustrated here, the use of modern, high-performance filters for the microbial control filtration of biological fluids can contribute to the achievement of these objectives. Advanced filtration technology developed by Pall can offer significant process advantages by providing high levels of safety, reliability, shorter process times, smaller filter systems, improved ease of use and reduced overall filter expenditure.