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Application Note

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Continuous Sterilizing-Grade Filtration in Biopharmaceutical Manufacturing

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Abstract

Continuous manufacturing is a method for manufacturing pharmaceutical products from end-to-end on a single, uninterrupted production line and process. Drug supply chain challenges underscored by the COVID-19 pandemic and recent advances in manufacturing technology and process analytics have encouraged the pharmaceutical industry to evolve batch manufacturing processes into more efficient continuous manufacturing processes.[1] In 2023, the FDA published ICH guideline Q13 ("Continuous Manufacturing of Drug Substances and Drug Products"), which underlined the relevance of continuous manufacturing in supporting the pharmaceutical industry to improve quality management and reduce the risk of quality-related drug shortages.[2]

Continuous processing presents its own set of biosafety challenges for manufacturers. The extended processing time increases the risk of bacterial contamination of equipment and consumables, requiring the installation of close bioburden monitoring and microbial barriers. Maintaining an aseptic environment in those manufacturing processes is key to running continuous manufacturing processes efficiently and securely. Sterilizing-grade filters are an important tool in maintaining the sterility of the continuous manufacturing process and must prove their capability to retain microbials for those extended process times.

Sartorius provides a complete range of high-performance sterilizing-grade filters for all relevant applications in biopharmaceutical manufacturing. This study aimed to demonstrate the bacterial retention ability of Sartorius filters in continuous manufacturing with long processing times, employing process runtimes of ≥ 30 days.

Introduction

Regulatory agencies are proactively enabling the implementation of continuous manufacturing. This approach improves product quality and resolves underlying causes of drug shortages and recalls. Continuous biopharmaceutical manufacturing has the potential to achieve better product quality at lower costs and shorter time to market. Recent advances in fully integrated control systems and a dynamic market make continuous manufacturing highly relevant in the current pharmaceutical landscape.

One of the challenges in continuous manufacturing processes is bioburden control. By increasing process runtime, bioburden can accumulate in the process train. Controlling the microbial load is crucial to prevent it from surpassing set limits and to ensure successful retention by sterilizing-grade filtration. It is important to ensure sterilizing-grade filters maintain their sterilizing ability throughout the entire process.[3] Sartorius has a wide variety of sterilizing-grade filter products for normal flow filtration with different pore size combinations to ensure safe and highly economical filtration operations in biopharmaceutical manufacturing (Figure 1).

The aim of this study is to investigate the bacterial retention ability of specific Sartorius filters used in continuous manufacturing processes with a duration of 30 to 60 days. To mimic the conditions of continuous filtration, sterile reverse osmosis (RO) water was filtered at a 2,000 L/day flow rate in recirculation mode through the Sartorius sterilizing-grade filters. To test the bacterial retentive capabilities, filters were challenged with bacteria (*Brevundimonas diminuta*) at a concentration of 10^7 CFU/cm^2 over 30-60 days. The sterilizing-grade filtration capability of these filters was evaluated by sampling the filtrate and performing a microbial growth test. Bacterial retention ability and integrity of the test filters after the test runs were measured by performing a standard bacterial challenge test (BCT) according to ASTM 838-15a – "Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration"[4].

Figure 1: *Sterile Filters Across a Range of Sizes*

Materials & Methods

Three sterilizing-grade filters widely used for normal flow filtration in biopharmaceutical manufacturing processes were selected. The Sartopore® 2 0.2 µm (0.45 + 0.2 µm) is a heterogeneous double-layer membrane filter with polyethersulfone membrane used in many different sterile filtration applications in bioprocesses. The Sartopore® 2 XL* filters represent a class of sterilizing-grade filters that offer different pore size combinations to meet specific process needs. The Sartopore® 2 XLM (0.2 + 0.1 µm) and the Sartopore® 2 XLG (0.8 + 0.2 µm) were tested for their ability to filter cell culture media. The Sartopore® 2 XLM is a membrane microfilter with Mycoplasma retentive capability, while the Sartopore® 2 XLG is commonly used with its highly porous prefilter membrane when the medium to be filtered includes larger particles that may clog a standard filter prematurely. The details of the filters used and their test duration are given in Table 1.

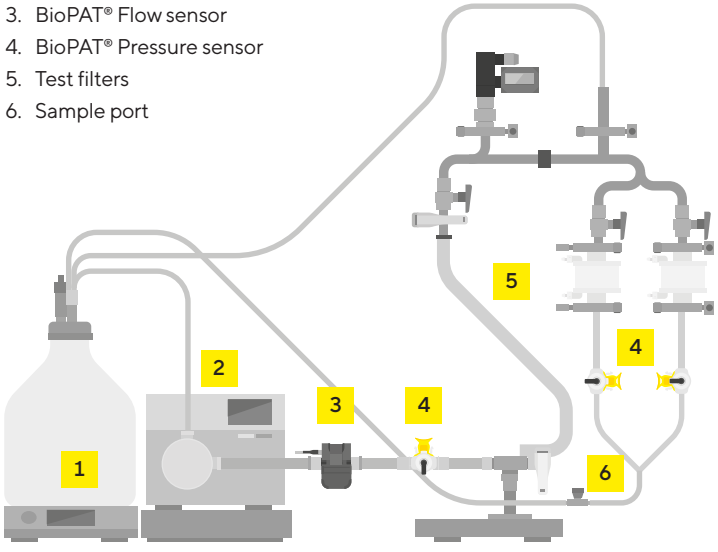
Table 1: *Filters Used in This Study*

Test Filters	Order Code	Lot Number	Pore Size [µm] (Prefilter + Final Filter Membrane)	Test Duration [days]
Sartopore® 2	5445307H7G-SS--A	2202006403	0.45 + 0.2	30
Sartopore® 2 XLM	5445358M7--SS--A	2145012703	0.2 + 0.1	45
Sartopore® 2 XLG	5445307G7--SS--A	2201005603	0.8 + 0.2	60

Continuous sterilizing-grade filtration was performed by circulating sterile RO water through the test filter for 30, 45, or 60 days at a flow rate of 60 LPH. Inlet flow and pressure were monitored using BioPAT® Flow and BioPAT® Pressure sensors. Filter integrity tests were performed before and after the continuous filtration process using the Sartocheck® integrity tester. The sterility of the test filters was evaluated by a microbial enumeration method. The test setup was assembled aseptically to avoid environmental contamination (Figure 2).

Figure 2: *Test Setup for Continuous Filtration*

1. Test solution
2. Pump
3. BioPAT® Flow sensor
4. BioPAT® Pressure sensor
5. Test filters
6. Sample port



The test skid was steam-sterilized at 121 °C for 30 min at 2 bar pressure, and test filters were installed aseptically. Four liters of sterile RO water were recirculated at 1 L/min (60 LPH) throughout the test duration. The filters were challenged every 2-4 days with *Brevundimonas diminuta* at 10^7 CFU/cm² filtration area by inoculation into the recirculation loop upstream of the filter. The test duration was increased from 30 days (Sartopore® 2) to 45 days (Sartopore® 2 XLM) and finally to 60 days (Sartopore® 2 XLG) to mimic the different process times and applications with different filters.

Filtrate sampling was performed via the sampling port downstream of the filter. The bacterial concentration of the test solution and the filtrate samples were analyzed quantitatively by microbial filtration method on tryptic soya agar (TSA). In addition, qualitative analysis was performed by inoculating 1 ml of the resulting sample into sterile tryptic soya broth (TSB). Filtrate samples, positive control (TSB with *B. diminuta*), and negative control (sterile TSB) were incubated at 30 °C for 5-7 days. All samples were measured in duplicates.

Bacteria addition and sample analysis were performed every alternative day. After completion of the continuous filtration run (30-60 days), a standard bacterial challenge test (BCT) was performed according to ASTM standard F838-83 to check the sterilizing-grade filtration capability at high test pressure after the process. During the BCT, the filter is challenged with 10^7 CFU/cm² of bacteria (*Brevundimonas diminuta*) at a test pressure of 1.0 bar for 60 min, and the filtrate is analyzed by a microbial filtration method on TSA. Sterile results after BCT indicate the bacterial retention capability of Sartorius filters after continuous filtration.

Results

Filtration Parameters

The pressure remained stable and did not exceed 0.25 bar throughout the test for all three filter types at a constant flow rate of 1 L/min (60 LPH). The stable pressure profile shows no significant filter blocking occurred throughout the process. Typically, a pressure limit of 1 bar is the criterion for aborting the filtration process due to blockage. The flow and pressure data for test filters are shown in Figure 3-5.

Figure 3: *Sartopore® 2 Flow & Pressure Data*

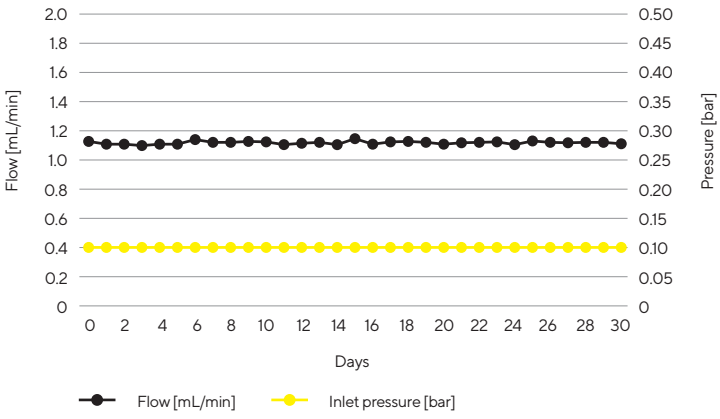


Figure 4: *Sartopore® 2 XLM Flow & Pressure Data*

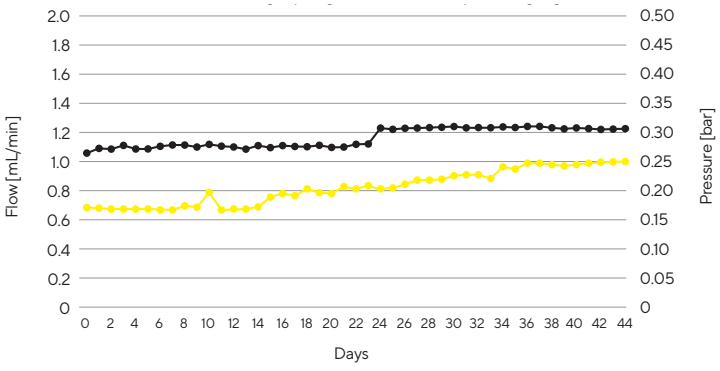
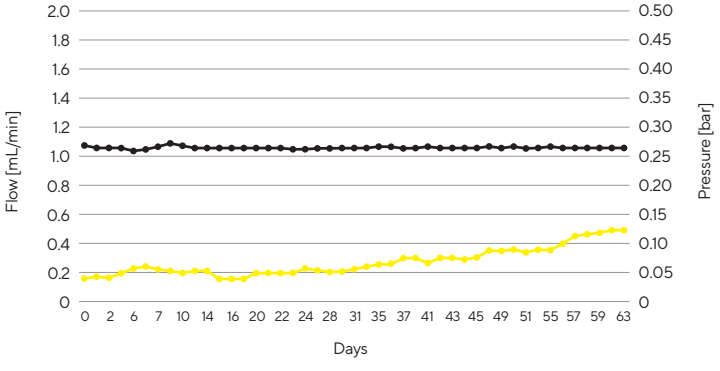


Figure 5: *Sartopore® 2 XLG Flow & Pressure Data*

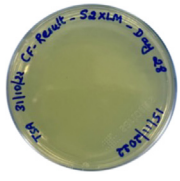


Microbial Evaluation

The bacterial load in the inoculum and challenge solution was determined by a growth promotion test. During the entire test duration, the bacterial load of the challenge solution was >1.0 × 10^7 CFU/cm^2.

Positive and negative controls were used to ensure the test system was prepared correctly: after incubation, turbid results were observed for the positive controls, while clear results were seen for the negative controls. Test results were evaluated every alternative day until the end of the specific test run. In all three filter types, sterility was observed throughout the individual test duration (Table 2).

Table 2: *Summary of Microbial Test Results*

Test Filters	T est Duration [days]	Number of Filtrate Samples	Bacterial Breakthrough Events	Sterile Result Pictures
Sartopore® 2	30	11	None	
Sartopore® 2 XLM	45	16	None	
Sartopore® 2 XLG	60	22	None	

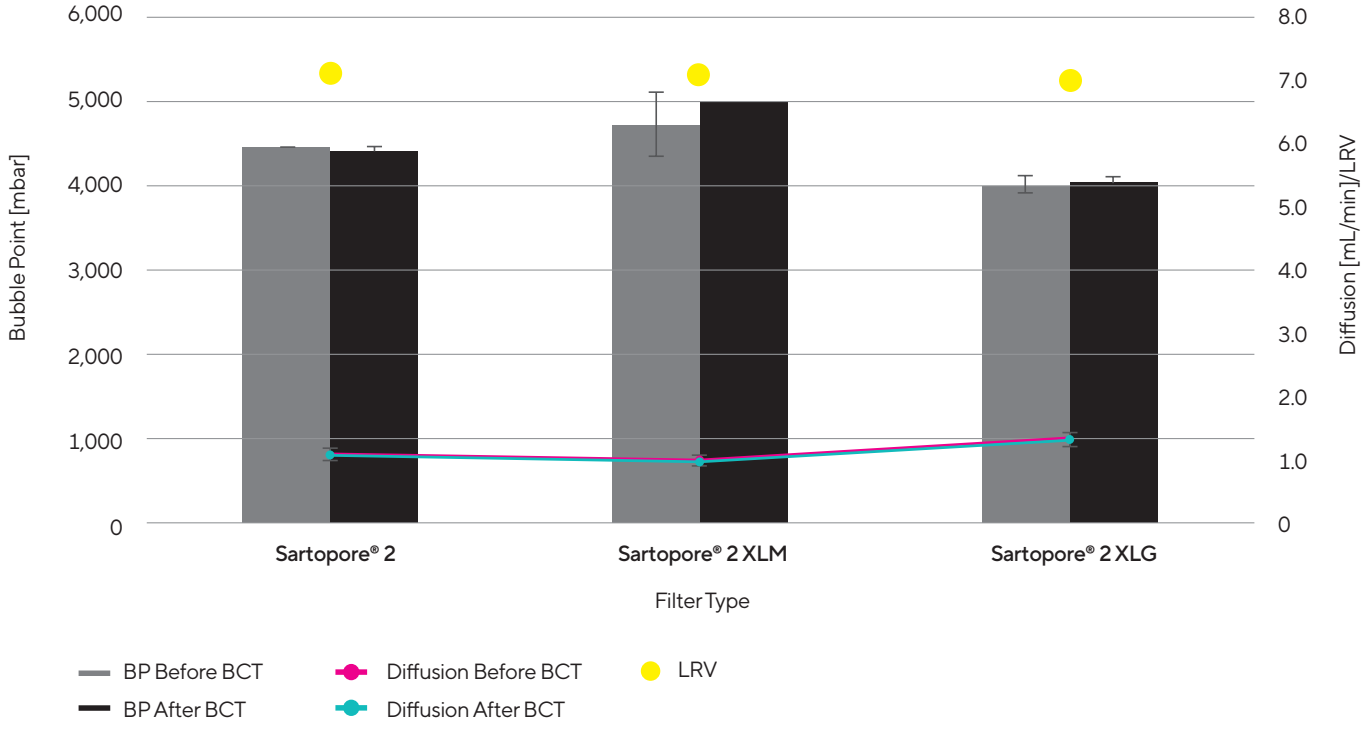
After completion of continuous filtration runs, the test filters were subjected to a standard bacterial challenge test. Sterile results were observed for all tested filter types, demonstrating that the filters successfully retained the bacteria load during the BCT, and as such, their integrity has been maintained.

Bacterial challenge test results are also expressed as log reduction value (LRV) to show the microbial removal efficiency of a filter. A filter with a LRV of 7 can reduce the organisms in the feed stream by seven orders of magnitude. BCT results, including integrity test results, colonies on an analytical filter, and LRV values, are presented in Table 3 and Figure 6.

Table 3: *Bacterial Challenge Test Results*

Test Filters	Bubble Point [mbar]		Diffusion [mL/min]		Colonies on Analytical Filter	LRV	Test Result
	Before BCT	After BCT	Before BCT	After BCT			
Sartopore® 2	4,467	4,417	1.1	1.1	0	7.1	Pass
Sartopore® 2 XLM	4,733	5,000	1.0	1.0	0	7.1	Pass
Sartopore® 2 XLG	4,019	4,043	1.3	1.3	0	7.0	Pass

Figure 6: *Standard BCT Results for Test Filters After Continuous Filtration Runs.*



BP = Bubble point, LRV = Log reduction value

Discussion & Conclusions

In this study, Sartorius sterilizing-grade filters were tested for their bacterial retention capability in continuous manufacturing processes. Three commonly used Sartorius filters were subjected to continuous filtration for 30-60 days in a worst-case scenario with repeated exposure of the filter to a bacterial load at a concentration of 10^7 CFU/cm² filtration area. To exclude premature clogging of filters by a feed with high particle or protein load, this study focused on the bacterial challenge aspects of continuous filtration in a water-based setup. Despite the high bacterial load throughout the test period, no significant filter blockage occurred during continuous filtration at a constant flow rate.

Importantly, sterile results were observed for all three filter types over the test period of up to 60 days, demonstrating the suitability of Sartorius sterilizing-grade filters in a continuous manufacturing process. Further, process-specific studies are needed to evaluate the impact of protein and particle-containing feed streams on filter blockage over long filtration times. Here, filter change strategies may be required to address long process times with specific feed compositions prone to cause blockages. Nevertheless, our proof-of-concept study demonstrates that Sartorius Sartopore® filters are capable of retaining bacteria over process durations lasting up to 60 days.

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