

Cell Culture Media Mixing and Microfiltration: Reducing Risk with Single-use Technology

Abstract

Cell culture media are complex mixtures of synthetic and biological components that provide the proper composition of nutrients for healthy cell propagation and high protein expression. Key concerns are to ensure product safety and purity. Dissolution of cell culture media can be facilitated by single-use mixing systems. Disposable mixers and filtration assemblies provide operational advantages by eliminating clean-in-place (CIP) and steam-in-place (SIP) process steps, reducing water usage and water for injection (WFI) consumption. In addition, the use of disposables can reduce the risk of microbial contamination. This case study presents our recommended methods for thoroughly mixing media, as well as reducing the risk of mycoplasma contamination through the use of 0.1 µm sterilizing-grade filters.

Introduction

Cell culture media is typically mixed in bulk and aseptically transferred to the bioreactor. Careful preparation of cell culture media is needed to ensure the sterility of the cell culture batch. Cell culture media span many media types and formulations to meet the needs of different cell lines. As a result, a wide variety of media constituents exist today at a wide range of concentrations.

Media and media additives must meet the following characteristics:

- Sterile/mycoplasma-free
- Regulatory-compliant
- Promote proper cell growth and expression
- Free of growth inhibitors and contaminants
 - Low in extractables
 - Low in endotoxins

Prefiltration is commonly used to remove the bulk of particulate and colloidal contaminants from media and to extend the service life of the downstream sterilizing-grade filters. At production scale, prefilters are often used to handle batch-to-batch media variability and to ensure that the sterile media fill into the bioreactor is completed successfully and on time.

Filtration of cell culture media through sterilizing-grade filters reduces the risk of bioreactor contamination from bacteria. Filtration through 0.1 µm filters further mitigates risk by removing potential mycoplasma contaminants.

Mycoplasma Contamination

A cell culture developer's objectives when preparing media for addition into the bioreactor are to ensure that the filtered medium provides proper cell growth and expression levels and that the bioreactor and downstream purification steps remain free from microbial contamination. Mycoplasma contamination is a tremendous concern for biopharmaceutical manufacturers, as these small, insidious bacteria thrive in the nutrient-rich environment of the bioreactor, are capable of passing through a 0.2 µm sterilizing-grade filter, and can induce changes to cell metabolism and expression levels. Mycoplasma can be brought into a cell culture process from plant- or animal-derived raw materials as well from humans. *Acholeplasma laidlawii*, *Mycoplasma arginini* and *M. hyorhinis* are isolated from hydrolysates, cell lines and sera. *M. fermentans*, *M. orale*, and *M. salivarium* are isolated from humans, animals and cell lines. The nutritive composition of cell culture media supplies the sterols, fatty acids, amino acids, salts, and carbohydrates necessary for mycoplasma species to thrive.

Mycoplasma can be difficult to detect in cell culture because many species do not produce turbidity or cytopathic effects. Due to their diminutive size and deformability, mycoplasma readily pass through 0.2 µm-rated devices. As a result, media preparation increasingly calls for the use of 0.1 µm sterilizing-grade filters to ensure sufficient log removal of mycoplasma.

Reducing the Risk of Mycoplasma Contamination

For bioreactor feed applications, membrane filter devices are designed for various performance characteristics, such as capacity, permeability, and mycoplasma removal. Capacity and microbial removal should be in balance to ensure a safe and efficient process.

Evaluating filter capacity by modeling filter throughput over time is a well-known method in the industry and fairly uniform in its application. However, methods for testing a filter's capability for mycoplasma removal are evolving. As a result, testing should be performed to verify the filter's mycoplasma removal properties, using the product under actual processing conditions.

Application requirements:

Process Development

- Filtration-sizing experiments
- Evaluation of sterility/mycoplasma clearance using validated test methods

Production

- Bioburden monitoring, mycoplasma detection
- Filter robustness/ease of handling evaluations

Mycoplasma retention and filter permeability were dependent on the test membranes. Equivalent retention was measured with Millipore Express® SHR and Manufacturer A's PES membrane filters but Millipore Express® SHR filters had more consistent retention and less variability between tests (Tukey post-test).

Permeability (LMH/psi) of Millipore Express® SHR filters was significantly higher than that of Manufacturer A or B (ANOVA, α 0.05, $P = 0.004$).

Considerations for selecting a mycoplasma clearance filter:

- Unit operation risk
- Process compatibility, filtration time
- Mycoplasma clearance
- Filtration flux and capacity
- Filter validation
- Cost of goods

Cell Culture Media Mixing With The Mobius® MIX 200 System

When dissolving cell culture media, consistent mixing is essential. Mobius® single-use mixing systems are designed to maintain uniformity as exhibited in this study. This technology delivers economic and operational efficiency, saving time and increasing operational flexibility. The Mobius® MIX 200 system uses a bottom-mounted, magnetically driven impeller inside a 3-D conical single-use process container made of Pureflex™ film. This high-purity, medical-grade film was designed to provide strength and flexibility, as well as inert contact and excellent gas barrier performance. As a result Mobius® mixing containers provide high performance when engaged with the motor and electronics. Mimicking the design of traditional stainless steel vessels, the Mobius® MIX 200 system minimizes foaming, particle generation, and vessel contamination.

Figure 2 shows the consistency of Mobius® MIX 200 system data when mixing serum-free media containing 5 g/L soy hydrolysate. Samples taken from the top and bottom ports of the Mobius® MIX 200 system and filtered through a single lot of Millipore Express® SHR membrane show similar results, demonstrating efficient mixing.

Figure 1 illustrates mycoplasma reduction and permeability performance among 0.1 μm -rated hydrophilic filters from three different manufacturers, using an animal component free medium. In this study, membrane samples were tested simultaneously in three separate experiments using three separate cultures of *A. laidlawii* ATCC® 23206. The average total challenge per membrane was 4.9×10^8 cfu/filter and the average challenge per membrane area was 3.5×10^7 cfu/cm².

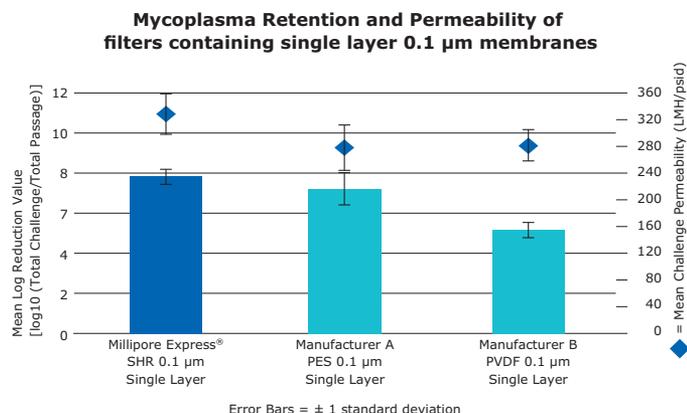


Figure 1. Mycoplasma reduction and permeability performance

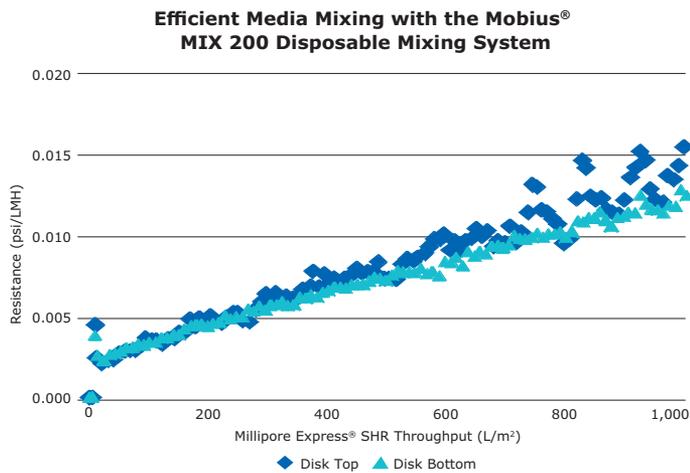


Figure 2. Efficient mixing of 5 g/L soy hydrolysate in water at 200 L scale.

Consistent Filtration Performance

In addition to reducing the risk of mycoplasma contamination, a cell culture developer must also ensure that the filtration train is robust and can handle variations in feed and batch media. To address this need, it is recommended to characterize filter variability and to size the filtration system appropriately.

Towards this objective, we initiated a case study to mimic the way a cell culture developer would assess filter lot variability with cell culture media. Throughput testing (to at least 80% of initial flow) was performed, using three samples each (n=3) of Millipore Express® SHR 0.1 µm membrane from three different, non-contiguous lots. Throughput testing was run at 10 psi constant pressure, using one batch of 5 g/L soy hydrolysate mixed with 10 g/L DMEM. Results, shown in **Figure 3**, demonstrate that Millipore Express® SHR 0.1 µm membranes have minimal lot-to-lot variation in filterability.

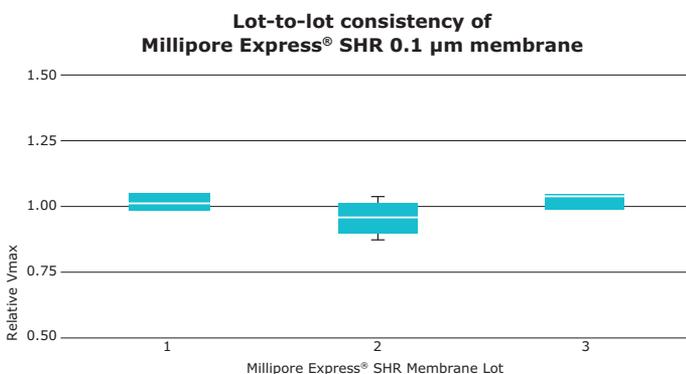


Figure 3. The study showed Millipore Express® SHR 0.1 µm membranes provided consistent performance with minimal variation in filterability across three non-consecutive filter lots.

Figure 4 shows Millipore Express® SHR 0.1 µm throughput performance on disks and pleated cartridge filters. Throughput testing performed with water and cell culture media on 47 mm disks and Opticap® XL3 devices showed similar results, demonstrating consistent and predictable filter performance between small and large scale device formats.

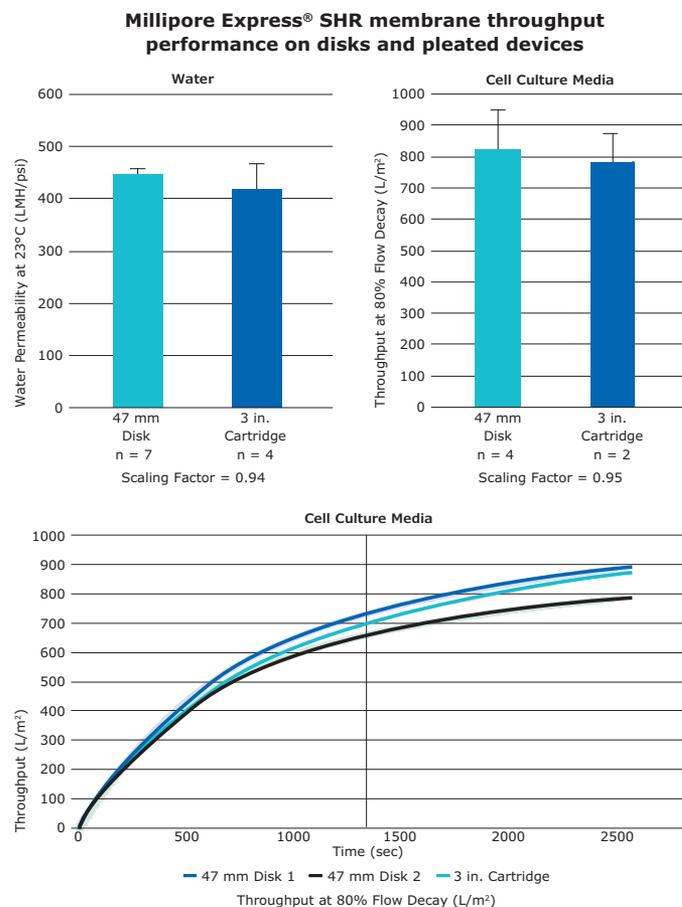


Figure 4. Predictable, scalable throughput; 47 mm disk testing predicts device performance within 20%.

Summary

This case study demonstrates the robustness and capability of the single-use Mobius® MIX 200 disposable mixing technology and sterilizing-grade, mycoplasma clearance filters with Millipore Express® SHR 0.1 µm membrane to meet a cell culture developer's needs for improved process efficiency and reduced risk of microbial contamination.

For additional information

[EMDMillipore.com](https://www.EMDMillipore.com)

