

Contamination Costs

Anil Kumar, Stefan Egli, Jerold Martin, Charles Golightly and Ralf Kuriyel at Pall Life Sciences discuss the current trends in process economics for mycoplasma-retentive filtration of cell culture media

Recent trends in the biopharmaceutical industry involve using large volumes of cell cultures with high expression levels to accommodate an increasing demand for biopharmaceutical drugs. These trends have contributed to an increase in the value of bioreactor batches, as well as an increase in the cost of a possible contamination event. In addition, the pressure to reduce process costs demands an improvement in biopharmaceutical process developments, which include minimising the risk of mycoplasma contamination inherent in processing large volumes of cell culture media, and improving process economics while minimising operational complexity. Risk of mycoplasma contamination must also be considered when using filter-sterilised culture media for validation of aseptic filling. This article highlights the various factors governing the filter selection for culture media filtration, where high mycoplasma retention and improved process economics are desired.

In the last few decades, several instances of mycoplasma contamination in cell culture have been reported in biopharmaceutical processing (1-3). If the presence of mycoplasma is detected within a fermentor, the entire fermentor batch has to be discarded, as most treatment options are expensive and often mycoplasma strain-specific. Similarly, the presence of mycoplasma contamination in aseptic fill validation culture media can have costly consequences. Mycoplasma is the common name of the prokaryotic (bacteria) class of microorganisms Mollicutes. They are deformable, due to the lack of a cell wall, and are small in dimension (0.05-0.4µm), enabling them to penetrate 0.2µm and even some 0.1µm rated 'sterilising-grade' membranes (see Figure 1).

MYCOPLASMA CONTAMINATION

Mycoplasma may be introduced to cell culture media through both animal-derived nutrient components, such as serum and serum albumin (4), and animal-free components, such as soy peptones (5), as well as from operator contamination. Once in culture media, mycoplasma can reach high concentrations without causing cytopathic effects or detectable changes in visual appearance (such as colour), turbidity or pH of the solution, but may alter culture properties that could affect cell metabolism and growth (6).

The limits for some rapid mycoplasma detection methods range from 10, to 10⁵cfu/mL (7-11). Therefore, even when detection by culture is one cfu/sample, failure to detect mycoplasma in a bioreactor or culture media feed might not indicate complete absence of mycoplasma. Also, the success of a detection method relies heavily on the sampling rate. For example, failure to detect a contamination in one sample per

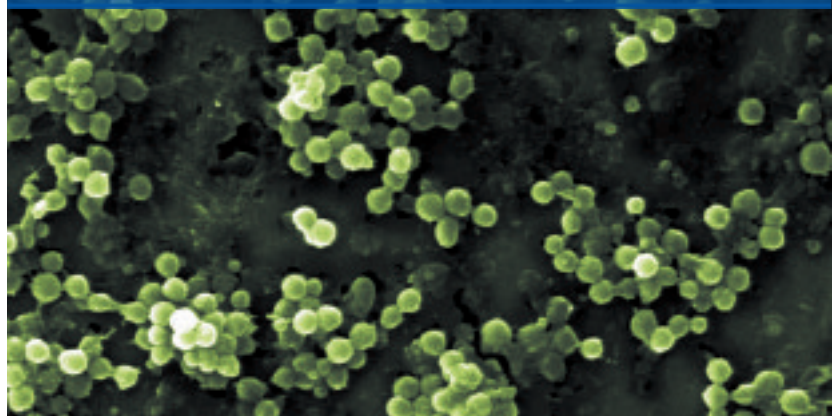
10,000L batch is not representative, and does not guarantee the absence of mycoplasma; thus a higher sampling rate (which in turn leads to higher expense) must be implemented. Even when detected, the inactivation of mycoplasma is laborious, costly, and at times ineffective. Several elimination methods have been suggested, which include the use of antibiotics, but these methods do not ensure certainty of complete mycoplasma elimination (11-13).

Several approaches have been taken to reduce the risk of mycoplasma contamination, such as screening raw sources for presence of mycoplasma (or using pre-sterilised raw materials where possible), and then preventing their introduction into bioreactors through the use of heat or sterile barriers. For example, moist heat can be effectively used for sterilising some cell culture media, although the stability of the media components at elevated temperatures must be ensured, as heat-labile components of media for mammalian cell culture (such as glucose and protein growth factors) are susceptible to damage. For heat-sensitive media and other cell culture media, sterilisation through direct-flow filtration with 0.2µm or 0.1µm sterilising-grade membrane filters is widely practiced (13). In a typical process, media is pumped or pushed under pneumatic pressure from the media mixing tank through a sterilising grade filter or a filter train directly into the bioreactor. Prior to use, the filters are sterilised either by steam-in-place (SIP) operation or autoclaving, or are supplied pre-sterilised using gamma irradiation. In some applications, high temperature short time (HTST) – a pasteurisation process that applies an elevated temperature for a short period of time – is used in-line with the sterilising filter(s) to reduce the risk of viral contamination (14).

FILTERS WITH HIGH RETENTION EFFICIENCY

In the biopharmaceutical industry, the most widely practiced approach to reduce the contamination risk for processing a large batch of cell culture media is to use 0.1µm rated filters with a high level of mycoplasma retention (validated for retention of

Figure 1: SEM photomicrograph showing the mycoplasma *Acholeplasma laidlawii* (white spheres). Cells are rendered monodisperse prior to filter challenges using sonication.



Acholeplasma laidlawii (ATCC 23206)). A higher retention efficiency (expressed as log reduction value, LRV) provides assurance of lower risk of contamination (see Figure 2). As an example, a 0.1µm filter with 10 LRV (penetration risk of 1/10₁₀) provides 1,000 times higher sterility assurance for mycoplasma than a filter with 7 LRV (penetration risk of 1/10₇). To illustrate this further, Figure 2b shows the risk of mycoplasma contamination for three 0.1µm rated filters subjected to different mycoplasma challenge levels (representing different levels of mycoplasma contamination in culture media prior to filtration). Clearly, filters with lower mycoplasma retention ratings (<=LRV 7) do not provide the same level of risk mitigation as higher retention filters.

Along with high mycoplasma LRV, overall process economics and ease of use are two other important factors that govern the selection of a filter for cell culture media filtration. Depending on the nature and composition of media components, cell culture media may vary in their filter-fouling properties, and membrane filters can vary in their fouling resistance or filter throughput, which is defined as fluid volume processed per unit membrane area (L/m², for example). 0.1µm rated mycoplasma-retentive membrane filters can demonstrate media throughputs ranging from several hundred to several thousand L/m². Typically, culture media with high concentrations of glucose, unfiltered peptones, unfiltered serum, and antifoam are high-fouling in nature (15). Bench scale filterability studies allow for initial screening of 0.1µm rated filters for cell culture media filtration, whereas a scale-up study is

required to determine the appropriate filter sizing for processing a batch at manufacturing scale (16,17). It is important to note that two filters providing equal throughputs per unit area (L/m²) at bench scale using discs, may exhibit significantly different filter performance in a 10” pleated cartridge format depending on the cartridge membrane pleating characteristics. As an example, implementation of the laid over pleat design and narrow core allows for incorporation up to twice the membrane area per 10” cartridge element compared to a similar size cartridge with a traditional ‘fan’ or ‘star’ pleating geometry (see Figure 3). The effective filter surface area (EFA) is maximised through the use of select high lateral flow pleat support and drainage layers. This effect is illustrated in Figures 4a and 4b, where filter A and filter F have similar membrane throughput in units of L/m² at bench scale using disc. But fewer cartridge elements are required to process a batch of media with filter A because of the larger membrane area in a 10” filter element resulting from differences in pleating geometry.

PROCESSING COST ANALYSIS

Analysis of process economics for the design and selection of a filter train for media filtration should ideally incorporate the costs of filters, hardware, buffers, water for injection (WFI), labour and filter disposal costs. To illustrate such an analysis, we compared the processing costs of filtering a 10,000L batch of two media; Tryptic Soy Broth (TSB), and Dulbecco’s Modified Eagle Medium (DMEM) solution supplemented with proteose peptone (PP3) in

two hours filtration time for three different 0.1µm filters (16) (see Figure 2). Filter A had an *A laidlawii* mycoplasma LRV of >10 and a narrow-core laid over membrane pleating design (see Figure 3a). Filters F and G had *A laidlawii* LRVs of six and seven respectively, and both of them incorporated a traditional ‘fan pleat’ membrane pleating geometry in a cartridge format (see Figure 3b). For calculation purposes, the filterability data (throughput values) were taken from bench scale testing with flat discs (16). The analysis considered various operational steps involved in a sterile filtration process, which include filter installation, pre-flushing of filters, SIP, pre/post use integrity testing to ensure integrity of filter pre/post filtration, media filtration, clean-in-place (CIP), and finally filter disposal. The cost of media filtration per batch was then calculated, accounting for the various costs to process a 10,000L batch of media solution in two hours.

First, the filters’ throughput values (see Figure 4a and Figure 5a, page 72) and membrane areas per 10” cartridge element were used to determine the total number of cartridges required to process a 10,000L batch (Figure 4b and Figure 5b) in two hours. Next, appropriate filter housing was selected and the costs of filters, filter housing and flushing volumes were taken from an internal database. The costs of the labour, WFI and filter

Figure 2: A summary of (a) 10” cartridge flow permeabilities and manufacturers’ claimed mycoplasma LRV’s for various 0.1µm rated filters and (b) mycoplasma contamination risk for processing 1,000L batch through 1m² filter area at different contaminant loading. A contamination risk of unity corresponds to a contaminated batch of media, even after filtering it through a 0.1µm rated filter.

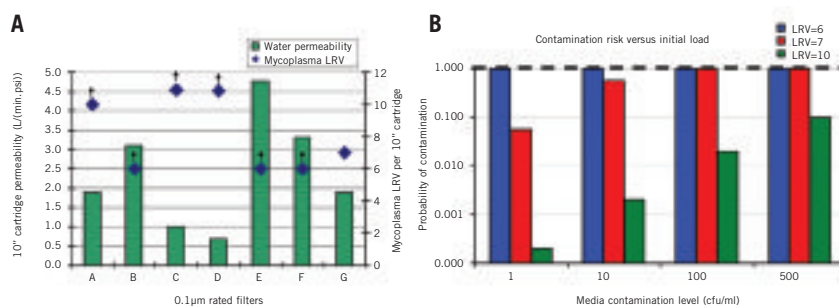
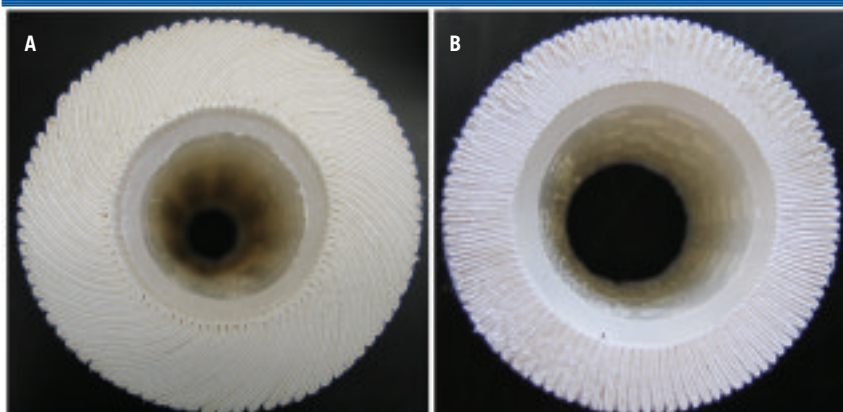


Figure 3: Different membrane pleating structures: (a) narrow-core laid over pleat design and (b) traditional fan pleat design with standard diameter core. The greater membrane packing density in narrow-core laid over pleat cartridge design enables more membrane area per 10” filter element.



disposal cost were representative of those reported for the medium to large scale biopharmaceutical industry (18,19). The costs of WFI and filter disposal were proportional to the number of filter cartridges and the estimated labour cost for a person dedicated to the process for the duration of the filtration operation. The cost of filter housings (considered as capital investment) was distributed uniformly over a five-year period. The analysis does not include the costs common to all the membranes, irrespective of their performance, such as the cost of pumps and surrounding hardware. The relative mycoplasma contamination risk and the cost for filtering a 10,000L batch of media in two hours for three 0.1µm filters is shown in Figures 4c and 4d for TSB and in Figures 5c and 5d for DMEM supplemented with PP3. Figures 4c and 5c indicate that at a mycoplasma challenge level of 10cfu/ml (detection limit for rapid mycoplasma detection methods), neither filter F nor filter G provide sufficient protection against mycoplasma contamination risk.

ANALYSIS RESULTS

The data suggest that the cost of filters (which accounts for membrane throughput, membrane area in a cartridge element, as well as the cost of a cartridge element) constitutes most of the process cost. For similar throughput values, a cartridge utilising the laid over pleat technology, with a larger membrane area per cartridge element, will in turn require fewer cartridge elements to process similar batch volume (see Figures 4b and 5b). Along with the obvious advantages of reduced space requirement for filter inventory, a smaller floor area for filter housing footprint and a much easier operation process, the use of fewer cartridges helps to reduce the processing costs further by reducing capital cost (smaller filter housing), reducing costs associated with smaller quantities of WFI for SIP and CIP operations, and reducing filter disposal costs.

In summary, to meet with the current trends in biopharmaceutical industries when qualifying filters for cell culture media sterilisation, it is essential to consider important factors such as mycoplasma contamination risk, overall process economics, and operational complexity in a filtration process. A filter with higher mycoplasma retention efficiency (LRV), complemented with an advanced cartridge pleating design that provides more membrane surface area per cartridge element, is important. Specifically, the reduced number of filter cartridges reduces filter inventory cost, the size of filter housing, and the filter disposal cost, and thus significantly reduces operational complexity. Moreover, such factors become of even greater importance over the life span of a biopharmaceutical drug product. It is important to highlight that, in some cases, a 0.2µm pre-filter is used in line with 0.1µm filter for media filtration. Selection of 0.1µm filters that incorporate high-capacity 0.2µm membrane prefilter layers and are targeted for high filter capacity applications could, in some cases, eliminate the need for a separate 0.2µm pre-filter cartridge assembly and thus bring additional economy for the culture media filtration system.

CONCLUSION

For cell culture media filtration, the end-user must consider:

- Mycoplasma contamination risks
- Process economics, which accounts for the total cost for processing a batch of culture media

- The operational simplicity, primarily resulting from reduced number of filter cartridge elements required to process a batch of cell culture media

A filter validated to provide higher mycoplasma LRV will provide a higher safety assurance and minimise the risk of contamination. Filters with higher area and throughput per 10” element mean a reduced number of cartridges elements, reduced cost of raw materials (CIP/SIP/initial flushing volumes and so

Figure 4: (a) Throughput for 0.1µm filters; (b) number of cartridge elements required to process 10,000L batch of TSB; (c) probability of contaminated media filtrate at challenge level of 10cfu/mL, and (d) various costs for processing 10,000L solution of TSB in two hours.

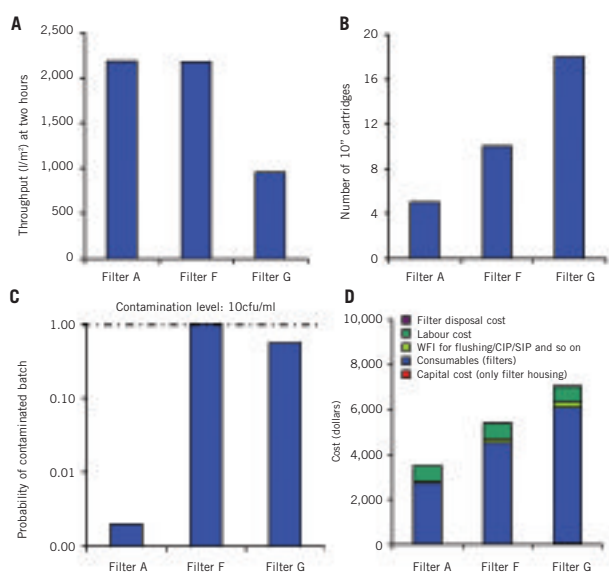
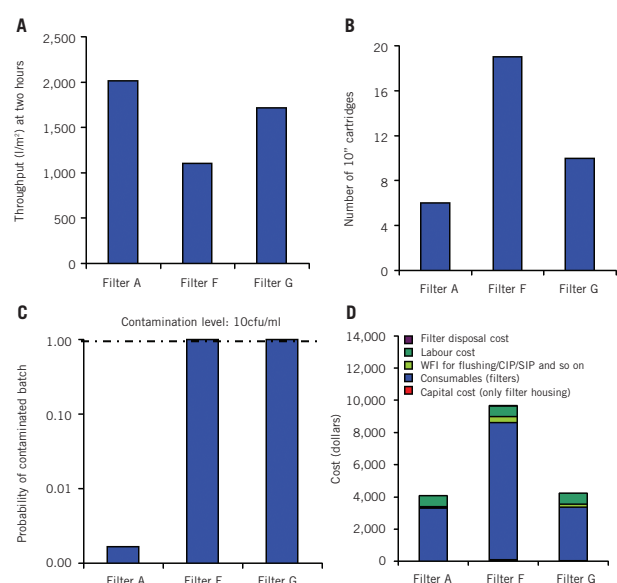


Figure 5: (a) Throughput for 0.1µm filters, (b) number of cartridge elements required to process 10,000L batch of DMEM supplemented with PP3, (c) probability of contaminated media filtrate at challenge level of 10cfu/mL, and (d) various costs for processing 10,000L solution of DMEM supplemented with PP3 in two hours.



on), smaller filter housings and minimise post-use filter disposal cost. Moreover, fewer cartridges require a reduced inventory, which means a smaller floor area because of the smaller filter housing footprint, so it is more operationally efficient from an end-user perspective. All these factors should be carefully considered in filter selection along with cell growth studies that are process specific.

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