Filtration in Preparation of Cell Culture Media and Buffers

Cell culture media is used in all biopharmaceutical operations. The media carries cells to fermenters or bioreactors and facilitates their growth, whether mammalian, yeast, insect, or bacterial cells. Media can be made up of numerous ingredients that are either mixed on-site or purchased pre-mixed in disposable containers.

Process buffers are used in diafiltration processes, as diluents for solutions entering chromatography columns, and as solvents or carriers for proteins. As with culture media, the buffers are often made up of multiple ingredients and can be mixed on-site or pre-mixed off-site and delivered in disposable containers.

Filtration of culture media and buffers is designed to enhance the operation of fermenters, bioreactors and downstream processes.

This application guide shows the functions of filters in the preparation of both media and buffers, whether on-site or at a separate facility. The filtration media/membrane and devices most often used to perform these functions are included in the discussion. There is a brief overview of the Critical Process Filtration technologies that may be applied in media and buffer preparation systems at the end of this guide.

Preparing Cell Culture Media

Figure 1 below is a schematic of a generic cell culture media preparation system. Once mixed, culture media is usually added to the fermentation tank or bioreactor on a batch basis. However, some operations employ continuous processing. In those cases, the cell culture media preparation system will feed the fermenter or bioreactor during the entire production run.

As components are delivered to the mix tank, filters remove particulates and/or bacteria that may be in them. Because the media, by its nature, promotes the growth of bacteria, assuring that unwanted bacteria are removed also assures the safe and effective operation of the fermenter or bioreactor.

If a mix tank is used, vent or process gas filters are used to maintain the quality of the solution in the tank. These filters protect the tank contents from particles and bacteria in surrounding air or process gas that may be used as a blanket over the solution in the tank.

While the schematic shows one mix process, there may be multiple media prepared and fed to the fermenter or bioreactor. There may be multiple mix tank and feed systems in place, or some media may be purchased from an outside source and delivered in rigid, reusable containers or flexible, disposable packages. In either case, the filtration needed to promote good system operation remains the same.

The locations of normal flow filters are noted in Figure 1 and their various functions are discussed in more detail later in this guide.

Contact Critical Process Filtration, Inc. if you have questions regarding any information in this guide, or if you have an application not covered in this guide, by calling (603) 880-4420, or access our website at www.criticalprocess.com.
Buffer Preparation

Figure 2 is a schematic of a generic buffer mix system delivering the buffer to a chromatography column. The buffer can also be delivered to a number of other downstream processes. The buffer preparation process is similar to cell culture media preparation. Individual components, usually water, salts, and pH adjusting chemicals, are individually filtered and mixed in a tank. The finished solution is then delivered to the appropriate process. Buffer preparation is most frequently a batch process, but some operations utilize continuous processing. Those systems must provide a steady stream of filtered buffer for the entire production run.

As the components are delivered to the mix tank, filters remove particulates or bacteria that may be in them. Downstream purification processes that use the buffers are sensitive to bacteria and particle contamination. To allow those processes to operate as intended, the finished buffer solution is filtered to remove those contaminants.

If a mix tank is used, the solution is protected from particle and bacterial contamination by a vent or process gas filter, depending on whether the tank is vented to atmosphere or a process gas blanket is used over the solution in the tank.

Filter Applications

Both Figure 1 and Figure 2 show “typical” filter applications. Additional filters may be required if either the solution components or the mixed solution contain materials such as gels or colloids. These deformable particles can cause premature filter plugging.

Filters are ideal for preventing the fouling of downstream processes such as chromatography and diafiltration, but the efficient operation of the filters themselves is required to make sure those processes run smoothly. Contact the Critical Process Filtration Technical Services Team for assistance in finding the right filter if you are faced with an unusual contaminant in your process.

Particle Removal Filters

The figures only show one location for particle removal filters (housings marked 1). These are at the point of entry for solution components. However, depending on the particle load in the components, and the nature of the mixing processes, removing particles may be required in multiple locations.

Particle filtration, as the name implies, is designed to capture particles larger than those the downstream filters will be asked to remove. They are called upon most often to remove inorganic particles such as undissolved powders and similar contaminants. Some large organisms, such as molds or yeasts, can also be captured by these filters. Most operators use depth filters for this step with pleated depth filters used most often. Pleated filtration products, with large filtration media surface areas, can remove several times more particle contaminants than standard melt-blown or nano-spun depth filters.

Critical Process Filtration offers both standard depth filters in polypropylene and nylon materials, and pleated depth filters using polypropylene or fiberglass flat sheet depth media. The pleated filters are available in “pharmaceutical grade” versions with appropriate documentation and quality certification.

Figure 2 – Buffer Preparation

Figure 3 – Pleated polypropylene and pleated fiberglass depth filters are available in all standard length and end cap configurations.
Bioburden Reduction Filters

As the term implies, filters that do not remove bacteria (bioburden) to levels required for “sterilizing” are designated as “bioburden reduction” or “bioburden control” filters. The filter micron rating may be 0.22 μm, 0.45 μm or even 0.65 μm. As with sterilizing grade filters, the pore size rating is a secondary consideration. Documentation of filter capability to remove the target organisms and validation of results in the application are of primary importance. Again, the goal of bioburden reduction is to remove most, but not all, organisms and promote system efficiency.

The filters marked “2” in the figures above are based on microporous membranes. Critical Process Filtration’s “Biopharmaceutical Grade” filters are made using asymmetric polyethersulfone (PES); high capacity PES; high capacity polyvinylidene fluoride (PVDF); and nylon 6,6 membranes. Available in both cartridge and disposable capsule formats, these filters can be validated for performance to assure that the system will consistently deliver media or buffer that meets the requirements of your processes. They also protect any downstream, sterilizing filters from overloading and premature fouling. Of course, all of the filters described in this guide are designed to work together and keep your fermenters, bioreactors and downstream processes running smoothly.

Sterilizing Filters

Typical sterilizing grade filters ( housings marked “3” in the figures) are rated to remove particles and bacteria that are 0.22 or 0.1 micron in size. In the past, the standard has been 0.22 micron, but a few organisms, such as Acholeplasma laidlawii, have been found to pass through these filters, so some users have moved to using a finer, 0.1 micron filter to remove them.

The filters are shown in only one location for media preparation and two locations in buffer preparation. Some operators may use additional locations, but these are the critical ones in both systems. The media must be free from bacteria that would interfere with the cell culture process, so a bacteria filter will be the last step as the media is introduced to the fermenter or bioreactor. In buffer preparation, bacteria are removed as the solution is introduced to the mix tank, filtering a smaller stream to remove bacteria with fewer filters. The final buffer solution is then filtered just before its use in a column or other process, as assurance against potential contamination.

Critical Process Filtration has “Pharmaceutical Grade” sterilizing cartridge and disposable capsule filters made using asymmetric PES and nylon 6,6 membrane. They are available with both 0.1 μm and 0.22 μm pore size ratings. Using these filters will assure that the components of your media or buffer and the final solutions are free from bacteria that could have a severe impact on your cell culture or downstream purification processes.

Tank Vent Filters

Filters marked “4” in Figures 1 and 2 are used to protect the media or buffer solutions stored in tanks from bacterial and particulate contamination. As a tank is filled, the air inside must be allowed to escape. However, as the tank is emptied, air (or a process gas such as nitrogen) must be allowed to enter the tank to replace lost liquid volume. The filter removes particle and bacterial content from the air or gas to assure the quality of the solution.

It is critical that the vent filter membrane remain dry so that air or gas can pass freely through the filter. To assure that dryness, filters are made using hydrophobic membranes, usually PTFE. Critical Process Filtration’s PTM sterilizing grade PTFE membrane cartridges or capsule filters are most often used.

If a tank is kept at an elevated temperature, then the PTM/HT high temperature PTFE membrane cartridges are used.

NOTE: Vent filters play a critical role in the design of tank systems. Stainless steel tanks are susceptible to collapse if subjected to vacuum conditions. It is critical to properly size the filter to allow the air flow required at the maximum rate of tank outflow with a pressure drop well below the vacuum rating of the tank. Improper sizing can result in permanent tank damage. Tanks should also have rupture discs, vacuum switches or other features to protect against accidental pressure or vacuum excursions that might cause tank failure.

If a large, flexible, collapsible bag is used instead of a tank, the bag will expand and collapse without the need to allow air into the container. Vent filters are not required for flexible bag containers.

References

United States Pharmacopeia 41, United States Pharmacopoeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD (2017)
All Critical Process Filtration filter media discussed in this guide are available in cartridge and capsule configurations. Cartridges are available in 5", 10", 20", 30", and 40" nominal lengths. Capsules are available in 2", 5", 10", 20", and 30" nominal lengths. All flat sheet depth media and membranes are available in disc filter format in 13mm, 25mm, 47mm, 90mm, and 142mm diameters.

Filter Membrane and Media Options for Cell Culture Media and Buffer Preparation

<table>
<thead>
<tr>
<th>Process Area</th>
<th>Filter Application</th>
<th>Filter Function</th>
<th>Critical Process Media*</th>
<th>Typical Pore Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Control</td>
<td>Particle Removal</td>
<td>Reduce particulate load to protect performance of downstream filters and processes</td>
<td>MB, NS, PD, GF</td>
<td>1 to 5μm</td>
</tr>
<tr>
<td>Bioburden Control and Sterilizing</td>
<td>Bioburden Reduction</td>
<td>Remove most, but not all, bacteria from the fluid stream</td>
<td>CWPS, PVWL, NM, PS</td>
<td>0.22 to 0.45μm</td>
</tr>
<tr>
<td></td>
<td>Bacteria Removal (Sterilizing)</td>
<td>Remove all bacteria from the fluid stream</td>
<td>PS, NM</td>
<td>0.10 to 0.22μm</td>
</tr>
<tr>
<td></td>
<td>Tank Vent Filtration</td>
<td>Prevent bacteria and particulates from entering tanks when liquids are drawn from the tanks. Protect solution quality</td>
<td>TM, TM/HT</td>
<td>0.22μm</td>
</tr>
</tbody>
</table>

*Media Codes
MB - Melt-Blown Polypropylene Media
PD - Pleated Polypropylene Depth Media
PS = Polyethersulfone Membrane
TM – PTFE Membrane
NS - Nano-Spun Polypropylene Depth Media
CWPS = High Capacity PES Membrane
PVWB = High Capacity Hydrophobic PVDF Membrane
NM = Nylon 6,6 Membrane
PVWL = High Capacity Hydrophilic PVDF Membrane
GF - Pleated Fiberglass Depth Media

Housings

Critical Process Filtration can provide housings for all cartridge filters. Sanitary housings are available for 1, 3, 6, 8, 12, and 21 cartridges from 10" to 40" long.

Visit our website at www.criticalprocess.com or contact Critical Process Filtration for more information, application assistance and access to datasheets for all of our products.