# UPSTREAM PROCESSING - STERILIZATION IN BIOPROCESS TECHNOLOGY

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## Contents





## Summary

One of the most relevant parts of comprehensive upstream processing in bioprocess technology is sterilization. In the present article various methods for inlet gases, liquids, and substrates sterilization, sterilization of small and large industrial equipment and

validation of sterilization are discussed. Sterilization of inlet gases by absolute, ceramic, fibrous and stainless steel filters, filter cartridges and membrane filters as well as fibrous filter dimensioning are presented. Various methods of liquid sterilization by filtration as well as heat sterilization are discussed. Sterilization of small equipment including microbiocidal gases and chemical agents ionizing radiation and dry heat sterilization are discussed. Industrial methods of large equipment sterilization, including valves, piping and elimination of condensate and validation of sterilization are presented.

## 1. Introduction

Sterilization is understood as the elimination, by removal or killing, of all microorganisms and the inactivation of viruses present in or on a product. According to this definition sterility is an absolute concept.

Except in cases where obvious physical destruction of microorganisms is apparent, as for example, in flaming, the mechanism by which sterilizing agents induce death is by no means certain. In some instances, rupture of cell walls and evacuolation may be visible and may be the result of osmotic forces. However, in most examples of killing bacteria, the dead organisms remain as discrete entities, which differ from live ones in their reaction to certain stains, their inability to reproduce and in some case their loss of motility.

## 2. Sterilization of Gases

A suitable process is expected to achieve the desired degree of sterility without impairing the treated product. Of the above-mentioned processes, however, only filtration can fulfil this objective, and its application is restricted to the treatment of air and other gases and clear liquids.

The primary and generally acceptable method of sterilizing large quantities of inlet and outlet gases is by filtration. Over the years, many different types of filter materials have been tried, including porous ceramic filters, cotton fibers, steel wool, granular carbon, glass fibers, and a number of specially designed filter media or membranes. Of these, porous ceramic filters have generally been useful for small bioreactors due to size limitations. Granular carbon towers and steel wool are generally ineffective. Cotton fibers produce high-pressure drops and tend to support bacterial growth. At present, only glass fibers and special membrane-type filters are commonly used.

Filters capable of removing microorganisms from air can be divided into two broad categories: those in which the interstices of the filter are smaller than the particles to be removed and those in which they are larger.

### 2.1. Absolute Filters

The first group comprises the so-called absolute filters, which may be of ceramics, sintered glass or metal constructions. Their claim to be 100 percent efficient in removing microorganisms rests on the fact that there is no passage through them, which is large enough to permit a spore to pass. They have, in general, two main disadvantages

when sterilization of large volumes of air is required, namely, high cost and high-pressure drop.

### 2.2. Fibrous Filters

Fibrous filters, as a second group, do not offer an impenetrable barrier to microorganisms. They are commonly made from beds or pads of fibrous material such as paper, cotton wool or glass and mineral slag wool. The fiber diameters will usually be in the range of 0.5–15 μm and the gaps between fibers will in most cases be many times this figure. In spite of this, such filters can be effective enough in removing from air stream bacterial spores, which are of the order of 1 μm diameter or less.

It is clear that fibrous filters cannot, at least in theory, be absolute, but carefully designed fibrous filters may be effective enough, they are relatively cheap and of low pressure drop. This latter factor is of considerable importance in industrial systems, where high operating pressures may be very costly (Figure 1). It is also clear that the mechanism by which fibrous filters operate must be very different from the absolute filters.

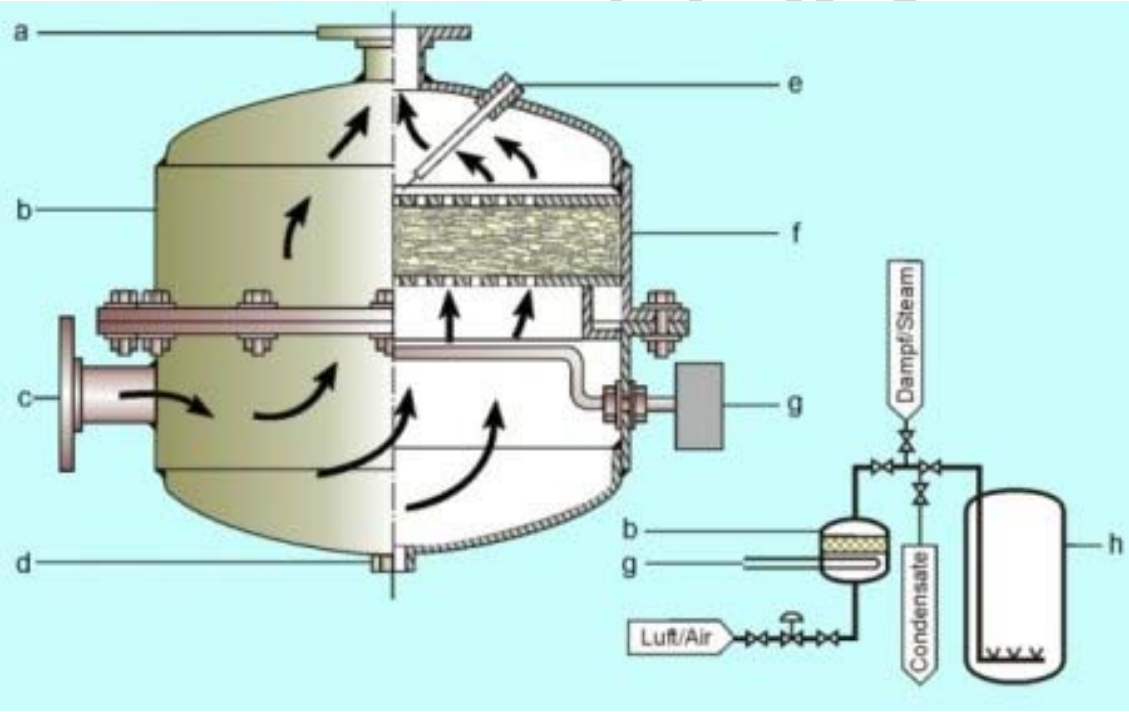


Figure 1: Industrial fibrous housing [adopted from Bioengineering AG, Switzerland]

In glass wool filters the particles would be trapped by a combination of physical effects. Particle filtration involves inertial effects, blocking effects, diffusion, gravity separation and electrostatic attraction. The last two mechanisms have a minimal effect on the removal of particles. The disadvantages of glass wool filters are shrinkage and solidification during steam sterilization. Glass fiber filter cartridges, which do not have these negative effects, have replaced glass wool filters.

It has been shown that electrostatic forces increase the efficiency of collection of particles, but it is difficult to quantify the effects of electrostatic forces. Filter efficiency might be relatively high at low airflow rates. By increasing the air-flow rate the filter efficiency would decrease to the point where interception predominates. As the airflow rate is increased to higher levels, the filter efficiency might increase due to implication.

As pointed out by Aiba and coworkers in 1973, it is of the greatest importance in fibrous depth filter design that channeling of air through or around the bed be prevented; if this is not done, only a fraction of the filter bed may be used and filtration efficiency will then be severely impaired. Similarly, movement of fibers in the bed must be prevented as this can result not only in channeling of air but also in dislodging of trapped organisms.

The use of bonded filter mats greatly reduces some of the problems associated with the use of loose wools. Some commercially available glass and slag-wool filter materials are bonded with resins and compressed into blocks or mats so that displacement of fibers is virtually eliminated. Resins have to be resistant to steam sterilization and fibers are usually absolutely hydrophobic. In any case extensive testing of bonded materials by repeated sterilization is desirable if steam is to be used.

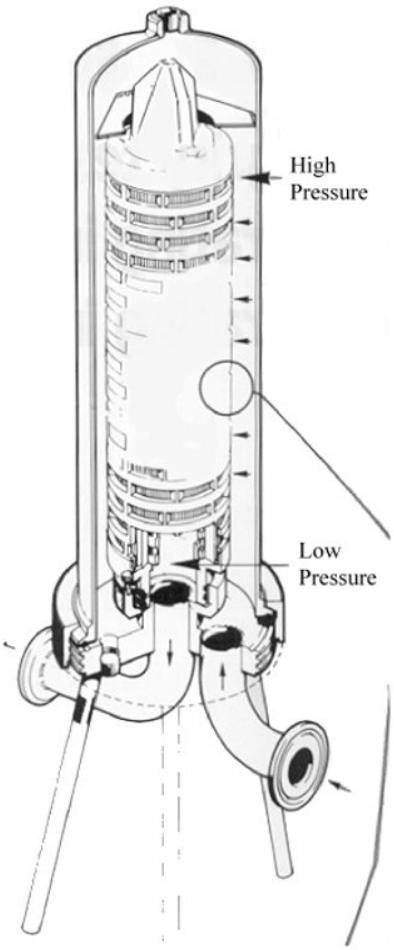


Figure 2: Cartridge housing for one or several filter cartridges [Dominic Hunter Ltd, UK]

The “fiber problem” associated with glass fibers, just as with asbestos fibers, has created a situation, which suggests the use of alternate materials. In the bioprocess field, on the other hand, the traditional glass-wool filter is being replaced more and more by hydrophobic membrane cartridge filters, which can be combined on the modular principle, depending on the air volume flow rate required, in a horizontal or vertical arrangement. Although difficulties appeared at first after repeated sterilization (leaks at the caps of the cartridge filters), they have now been largely eliminated by the use of suitable membrane materials (based on PTFE = polytetrafluoroethylene). Suitable cartridge housings for one or several filter cartridges are listed in Figure 2.

Numerous filterable additions that form clear solutions and are fed in continuously during the course of the bioprocess can be sterilized by microbial removal filtration. As a rule one uses suitable membrane filters (disk filters or cartridge filters, depending on the flow rate), which are stable to the material being filtered. Suitable nominal pore sizes for this purpose are not larger than 0.2 µm. In filtration processes that operate rapidly and continuously there is little risk of accidental microbial passage. This risk increases, however, with increasing contact time and particularly in intermittent processes. During the stationary phase, when the filtration process is interrupted, single microbial cells can grow into the filter labyrinth and then, when operation is resumed, be flung out by the pressure build-up.

### 2.3. Sterilizatiom of Gas Filters

Packed depth fibrous filters can be sterilized by steam or by dry heat. When steam sterilization is used, flowing steam is passed through the filter and it is necessary to ensure complete removal of condensate and air from the filter chamber and to steam long enough to ensure that very little condensation occurs in the filter.

A steam pressure of 1-2 atm for 30 minutes will usually suffice. Air must be purged from the filter effectively early in the process or the steam may not contact the entire filter adequately. Dry heat sterilization is an alternative to steam sterilization.

The filter is sterilized with air at high temperature. The normal method of accomplishing this is to install an electrically heated element close to the inlet side of the filter bed and to pass air through the heater and filter.

For sterilizing the filter bed, the air will be heated to 160–200°C and passed through for a period of about two hours. During this operation the filter is isolated from other equipment on the outlet side and the hot air is bled off after passing through the bed.

This method of dry heat sterilization obviates the possibility of the filter remaining damp at the end of sterilization operation—a risk in steam sterilization, which could lead to failure of the filter.

### 2.4. Sizing of the Fibrous Filter

To design a fibrous filter for sterilization of process air, one must first determine the required efficiency of the filter:

$$\eta_{eff} = N_0\text{-}N / N_0 = 1 - N / N_0 \quad (1)$$

Various equations have been given for the pressure drop across the filter, such as that of Wong and Johnstone:

$$\Delta P = 2\rho v_g^{\,2} \alpha L C / \pi D_f \quad (2)$$

That of Bader 1986:

$$\Delta P = 9.257 \cdot 10^{-6} v_g (1-\varepsilon)^m \mu L / D_f^{\,2} \quad (3)$$

Where the definition of linear air velocity, $v_g$, is expressed as:

$$v_g = V / S = V / \pi r^2 \quad (4)$$

From equations (2,3), the fibrous filter diameter can be calculated:

$$r = \sqrt[4]{(2\rho \alpha L C V) / \pi^3 \Delta P D_f}$$

or

$$r = \sqrt{9.257 \cdot 10^{-6} (1-\varepsilon)^m V\mu L / \pi \Delta P D_f^{\,2}} \quad (5)$$

Dept filters have many disadvantages in their use. There are a number of reasons discussed for the replacement of depth filters with specially designed air filtration cartridges.

### 2.5. Filter Cartridges

The term filter cartridge can be applied to any of a wide range of manufactured filters, which fit into specially designed filter housing. Such filters have existed for a long time but were generally not suitable for the sterilization of air.

During the past decade, a number of manufacturers have developed a specialized technology, which has enabled them to produce a filter medium membrane that is capable of removing micrometer and sub-micrometer-sized particles with a high degree of efficiency.

Some of these filter media have been capable of withstanding steam sterilization conditions with a relatively low pressure drop at high airflow rates.

In bioprocess technology, cartridges with glass fiber paper, ceramic and membrane filters are used (Figure 2).

A range of filters made from glass fiber paper, with a mean fiber size of 0.5 μm held in a reinforcing medium, are manufactured by Domnick Hunter Ltd., Sartorius and Pall.

### 2.6. Ceramic Filters

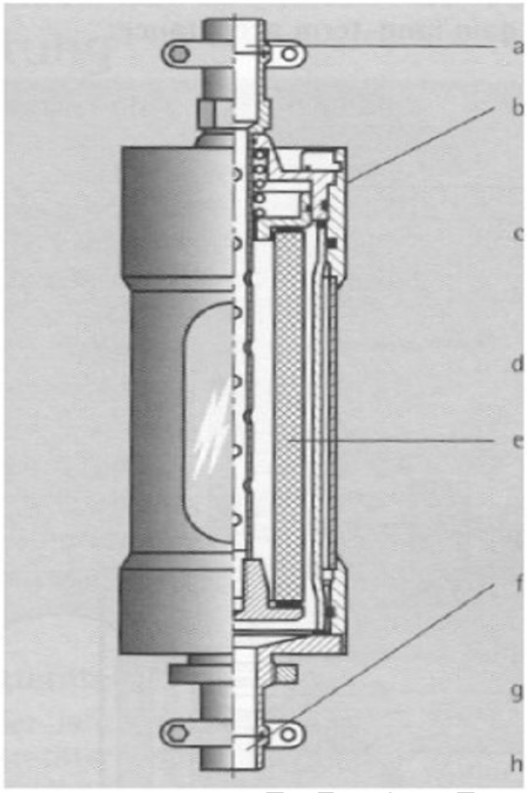


Figure 3: Ceramic filter for laboratory bioreactors [Bioengineering AG Switzerland]

An alternative to glass fiber filters are the ones constructed of ceramic (Figure 3). These suffer from the disadvantage of rather high-pressure drops and low flow rates in comparison to a similar size glass fiber filter.

These are, however, fairly inexpensive and withstand steam sterilization almost indefinitely. For laboratory and pilot plant application, with facilities to steam sterilize *in situ*, they deserve a better recognition than they have been given in the past.

### 2.7. Stainless Steel Filters

Although not usually used for sterilizing gases, because of the excessive pressure drops encountered, porous stainless steel filters are used for purification of steam. This is required for sealing lantern pump rings or condensed water as coolant for mechanical seals; all traces of particulate matter must be removed from the steam to prevent scoring of the fine surfaces. Chemical inertness is required to combat the highly corrosive effects of steam and stainless steel is excellent for this purpose.

The basic construction of the cartridge consists of an inner cylindrical core, which supports a sheet of filter material, which is contained within an outer protective cage. The top of the filter has an end cap, which also frequently supports a locating fin. The bottom of the filter generally has an end cap with a tube with one or more 0 rings that slip into the filter housing and seal the cartridge. Air always moves from the outside of the cartridge cylinder toward the center and exits through the bottom tube connector.

## 2.8. Membrane Filters

Membrane filters made from polyvinyl alcohol (PVA), cellulose esters, polysulfone or nylon plates, coated with heat resistant melamine resin, with an effective pore size of 20–30 µm, provide an excellent efficiency with a thickness of only 2–3 mm at linear air velocities of 4–5 $cms^{-1}$. These filters have an absolute filter effect owing to their membrane structure.

Absolute membranes generally consist of a solid sheet of a polymer through which small holes of defined size are cut, generally by a process of nuclear bombardment. On such filters all the pores in the membrane are of the same relative size and are small enough to prevent bacterial penetration.

Photomicrographs of polyvinyl alcohol plates show a fine fibrous structure, not a monolithic network of pores. It is interesting to note that in this kind of filter efficiency increases appreciably with increased linear gas velocity. Efficiency of these filters increases up to a critical value of 200 $cms^{-1}$, beyond, which its efficiency deteriorates sharply.

The drop in pressure of the air flowing through polyvinyl alcohol plates significantly affects the value of filter efficiency.

In the filtration of air it is advantageous that vegetative bacteria dry out very quickly in the steady flow of air and die, whereas the long-living forms (spores of bacilli) survive for a long period. Filters designed specifically for air filtration are best tested with a suitable microorganism.

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**Biographical Sketch**

**Marin Berovic** was born on 21 March 1951 in Novo mesto, Slovenia. He has B.Sc., M.Sc., and Ph.D degrees in Chemical and Biochemical Engineering, associated professor of Biochemical Engineering and Biotechnology on Faculty of Chemistry and Chemical Engineering, University of Ljubljana. He also has an M.A. from the Academy of Beautiful Arts, as professor of Art Technology, Dept. Restoration and Conservation of Art Monuments, University of Ljubljana. State representative in European Federation on Biotechnology (EFB) in Working Party on Bioreactor Performance and European Section on Bioprocess Sciences (ESBES), where he is a Head of Task Force Bioprocess Engineering Courses. He is a Member of the New York Academy of Sciences, International Organization on Biotechnology, Society of Chemical Industry of Great Britain, and American Chemical Society. Scientific Training: Technische Universitat Graz,Austria,Technical University Delft Leiden, The Netherlands, Institut fur Technische Chemie Technische Universitat Hannover, Germany, Technical University of Denmark, Lyngby, Denmark, University of Strathclyde, Glasgow, Great Britain. His scientific bibliography contains 235 various contributions from the field of biotechnology and bioreactor engineering. He obtained two National B. Kidric awards for research and innovations. His art bibliography includes 125 art exhibitions (personal 33 and 92 collective) in Slovenia, Austria, Italy, and Spain. He has obtained 10 awards in various art competitions.