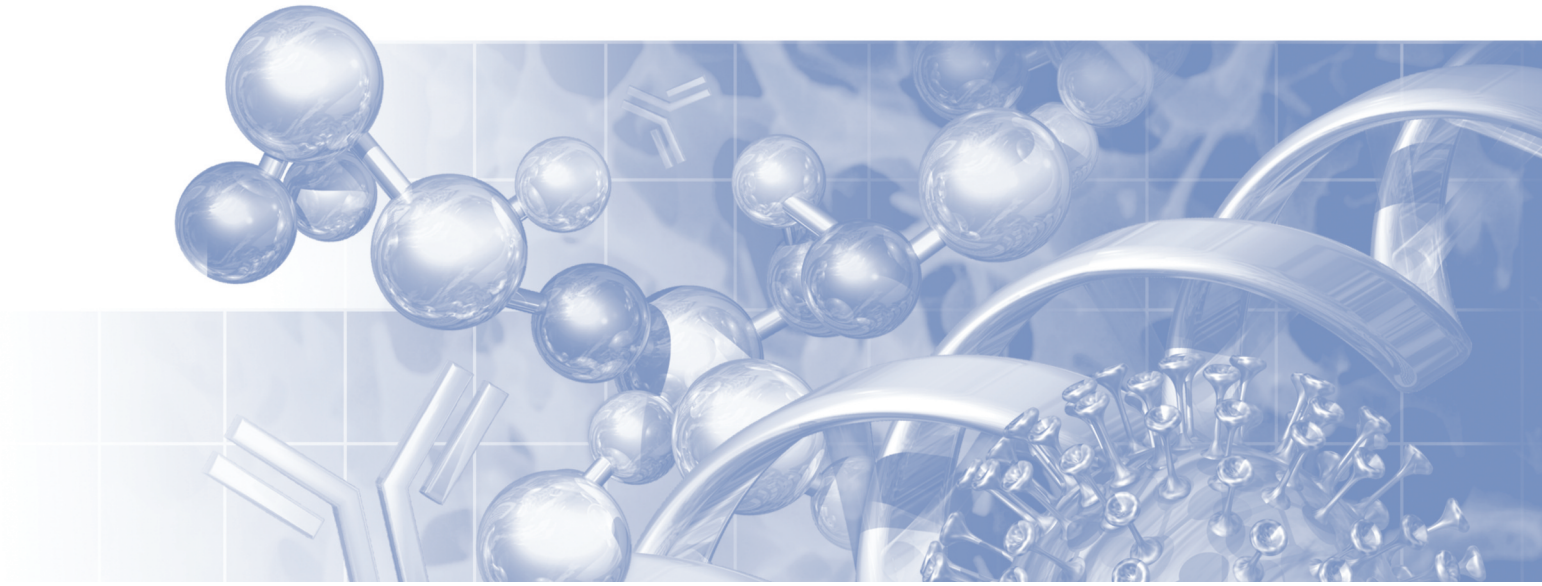
PALL Life Sciences

Application Note

USTR 2311a

Sterilizing Filtration of Enriched and Pure Gaseous Oxygen Employed in Cell Culture Applications



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Contents

1. Summary

Modern bioreactor aeration concepts increasingly using oxygen-enriched air or pure oxygen gas to improve cell culture productivity. As with air, these alternate oxygen source gas feeds must be filter-sterilized to prevent spoilage of bioreactors by contaminant organisms coming from the incoming and outgoing gas streams. Membrane filters are used to sterilize these gases, and must also be integrity testable to assure process security and meet GMP requirements. The Water Intrusion Test has become a preferred choice for integrity testing of sterilizing grade gas filters due to its avoidance of alcohol as a test liquid. Exposing filters to pure oxygen streams, however, presents specific engineering challenges, because many materials such as organic matter, plastics or even metals can potentially ignite when in contact with oxygen, particularly if also subjected to static electrical discharges, high temperatures, pneumatic shocks or mechanical impact. For that reason several countries have established mandatory requirements or restrictions on use of these materials with gaseous oxygen or oxygen-enriched gases. Apart from these conditions, installations for gaseous oxygen including those using filtration need a dedicated risk assessment prior to use.

Users are responsible for performing a risk assessment and obtaining expert advice in the design of any system that uses gaseous oxygen, as well as, for the selection of appropriate materials in contact with oxygen. Such an assessment will assist with the prevention of damage and injury to personnel. A sound method to facilitate an adequate risk assessment is to review safety related tests designed to evaluate the suitability of materials that come in contact with oxygen and are intended for use in oxygen service. For that reason, in support of use of Emflon® HTPFR filters in a system demanding sterilizing filtration of pure gaseous oxygen or oxygen enriched air, the construction materials of Emflon HTPFR filters have been subjected to a series of standard pressure shock tests[1] with 100% gaseous oxygen at 10 bar (145 psi) pressure at 60 °C (140 °F).

The safety related tests conducted by the BAM Federal *Institute for Materials Research and Testing* provide an important basis for a proper oxygen hazard analysis, which, as mentioned above, is necessary prior to introducing a material or component into oxygen service.

Together with aerosol bacterial and bacteriophage (virus) removal validation data and the liquid bacteria challenge test data correlated with an easy to use Water Intrusion Test, Emflon HTPFR filters can be considered qualified for sterilizing grade gaseous oxygen filtration in microbial and mammalian cell culture processes.

For the more demanding conditions of use and/or in cases where the risk assessment of polypropylene components exhibits increased safety consideration, all-fluoropolymer filters are also available from Pall. To help to identify additional risk factors in installations for gaseous oxygen, several important general safety guidelines about oxygen handling and filtration, such as preventing possible ignition mechanisms and single use mode of sterilizing gas filters, are also outlined in this paper.

In addition to the above safety aspects, oxygen or enriched oxygen gases can lead to accelerated oxidation or corrosion of component materials. This latter aspect is not the subject of this paper.

2. Where is Usage of Pure or Enriched Gaseous Oxygen an Issue?

Many valuable biopharmaceutical and biotechnological products, as well as chemical, food and beverage products, are produced by aerobic fermentation, which includes mammalian and microbial cell culture applications. Worldwide demand for fermentation products has been increasing steadily and this trend is expected to continue. To improve productivity, manufacturers are trying to run high strength broths with higher biomass for getting higher product yields. Because of this and the fact that the yield of a fermenter can be increased by eliminating oxygen starvation phases, improved aeration concepts have been established recently to satisfy the additional oxygen demands of the fermentation and cell culture industry.

2.1 Microbial and Yeast Fermentation

Bacteria and yeasts can grow and multiply very quickly leading to a very high oxygen demand particularly during the exponential growth phase. With ongoing growth and metabolite production towards the stationary phase, viscosity of the fermentation broth increases leading to an inhibition of oxygen transfer. Limited mass transfer of oxygen during the growth phase can inhibit biomass production, impacting the number of cells available to produce the desired product, whereas limited mass transfer during the stationary phase, due to higher viscosity, can also impact production of the primary metabolite pattern.

Air is often used as the sole oxygen source in fermentation with typical aeration rates in the range of 0.3 to 1.5 VVM (volume per volume per minute). However, air contains 21% oxygen, 78% nitrogen and some other gases in minor concentrations. In order to reach the cells, the oxygen from the air needs to dissolve in the broth and be dispersed within the fermenter in small enough bubbles so that the oxygen can be efficiently transferred to the cells. Typically, most of the oxygen available from air remains undissolved and vents from the fermenters to the atmosphere, making it difficult to obtain even the minimal dissolved oxygen level required to sustain organism growth, and maintain the desired production level. There are several fermenter designs that can be used for aerobic fermentations, including glass or stainless steel fermenters that have air spargers and impellers that serve to mix the broth as well as form dispersed air bubbles. Another type is the airlift fermenter that has a sparger that is designed to form air bubbles as well as mix the broth.

There are major differences between mechanically agitated fermenters and airlift fermenters. In airlift fermenters, air has the dual role of oxygenation and mixing of the entire fermenter. Gas bubble sizes are influenced by the sparger design and the bubble coalescence rate. The agitator in a mechanically agitated fermenter usually controls the mixing and the bubble formation and air has very little to do with the bulk mixing of the fermentation broth. It is necessary to supply very high air flow to an airlift fermenter to provide adequate mixing, suspension of solids and transfer of oxygen.

The simplest way to increase the oxygen supply to an air-based fermentation system is to increase the air flow. This can only reduce the oxygen starvation problem at moderate oxygen demand. At higher oxygen uptake rates, the air will start flooding the impellers in mechanically agitated fermenters. In an airlifted fermenter, excess air can fluidize the entire fermenter and can cause the broth to be blown out of the fermentation vessel. Installing large agitators and motors may improve the oxygen transfer rate, but this represents an increase in capital expenses as well as in operating cost due to increased energy requirements. In addition, excess heat may be generated and cooling may be required. Some cells may also be sensitive to heat, and increased agitation can damage the cells leading to lower viabilities and yields and a higher impurity level in the fermentation broth. Even if the increased capital and operating expenditure is not a concern, larger agitators and more powerful motors can provide only limited oxygen transfer rate improvements.

2.2 Growth Optimization of Mammalian Cell Cultures

Mammalian cells such as hybridomas or Chinese Hamster Ovary (CHO) cells are relatively big, complex structures and very susceptible to damage by shear forces. They grow to moderate concentrations of typically 1 to 5 x 10^6 cells per mL in comparison to the higher biomasses seen in microbial fermentations, which can be several orders of magnitude higher. Due to the lower cell growth rates and biomass, oxygen demand is lower for mammalian cell cultures (typically < 0.1 VVM). However, mammalian cells are fragile and can be damaged by fluid mechanical forces and shear generated by impellers or collapsing gas bubbles. High shear forces can result in a higher degree of cell lysis, and therefore, increased content of host cell protein (HCP) and other impurities in the supernatant. This may lead to higher associated costs of downstream processing and purification. In addition, the loss of cells as well as the higher concentration of impurities can have an impact on product yield.

Thus, the routine techniques of increasing aeration through high agitation rates and liberal sparging are often not possible in mammalian cell cultures. From a practical point of view, only a few methods are operationally feasible to aerate cells in bioreactors in order to increase cell density and product yield:

Head space or surface aeration alone is incapable of supplying enough oxygen to cell cultures of moderate density and size but is often used successively in conjunction with sparging. For larger sized fermentation vessels, the ratio of surface area to volume decreases, causing surface aeration alone is impractical for reactors larger than those of laboratory scale.

Direct sparging of air into the culture medium is proven to be an effective method for supplying air to stirred cell cultures in large fermenters. In many fermentation processes, a sintered sparger is used. For applications that involve a microcarrier, a sparger inside a rotating sieve cage can be used to protect the microcarrier. The sieve mesh allows the medium to pass and prevents the microcarriers with attached cells from contacting the gas bubbles. Rotation of the sieve prevents clogging. It must be noted, however, that cellular damage due to direct sparging of gas bubbles has been observed and it is necessary to limit the rate of sparging. Furthermore, heavy aeration and agitation of the medium can generate foam, especially if higher amounts of animal serum (or other protein-based components) are used in the culture medium. Foam formation is undesirable for a number of reasons: decrease in working liquid volume, accumulation of reactants or products in foam, outflow of reactants or products due to escape of foam, and marked limitation in aeration and agitation rate. If cells become entrapped in the foam layer or at the interfacial surfaces of the bubbles, they are likely to be damaged or killed. Therefore, it can be necessary to use a fermenter based on surface aeration without air sparging or to control foam by chemical or mechanical methods. In some applications, the addition of anti-foam is not desirable, since it is an impurity in the fermenter that ultimately needs to be removed from the final product.

Membrane tubing aeration systems have also been evaluated in stirred vessels as well as in other bioreactor designs. An appropriate length of gas-permeable tubing (such as silicone tubing) is installed in the bioreactor. Gas is passed through the tubing and gaseous oxygen diffuses through the silicone (which acts as a membrane) into the culture medium. This method eliminates the need to sparge gases directly and eliminates the problems inherent in direct sparging.

2.3 Using Oxygen Enrichment Techniques in Cell Culturing

Depending on the cell culture phase, different and well-adjusted oxygen transfer rates are applied for optimum growth and production conditions. For mammalian cell culture, it is recommended that the impeller is used solely for mixing and that a suitable and gentle aeration device is used to satisfy the oxygen demand of the cells. The addition of oxygen to the air stream (oxygen-enriched air) can provide significant increases in oxygen transfer. The air is enriched by adding oxygen directly to the air stream upstream of the sterilizing grade air filter that is used to sterilize the air introduced into the bioreactor via the sparger. This approach allows a higher oxygen transfer rate without an increased capital investment. Alternately, air and oxygen can be added directly to the fermenter. Since pure oxygen bubbles have an oxygen concentration approximately five times higher than that of air, and oxygen is dispersed in small bubbles, a very high oxygen transfer and dissolution rate can be achieved.

3. Filtration of Pure Oxygen or Oxygen-Enriched Air in Cell Culture

Keeping sterile or monoculture conditions in a bioreactor means that sterilizing filtration of incoming and outgoing gases is a strong requirement. For this reason, liquid challenge validated sterilizing grade filters with easy and gentle testability by a Water Intrusion Test (WIT) are the preferred choice to sterilize air prior to reaching the fermenter. Removal of microbial and viral contaminants from the air stream serves to protect the nutrient media and the cells in the bioreactor against spoiling organisms such as molds, yeasts, bacteria, viruses and bacteriophages. Fermentation vessels typically have sterilizing grade exhaust and vent filters, which act to protect the bioreactor from contamination and contain the platform culture. The vent filter also serves to protect the operator and the environment which is especially important for genetically modified microorganisms (GMOs). All of these filters for inlet air, exhaust and venting provide further protection from contamination by their ability to remove particulate contaminants. To secure a high process safety level, it is recommended that the sterilizing grade gas filters used for cell culture are tested with a correlated Water Intrusion Test (WIT) after both steaming and usage.

Supply of pure or blended gaseous oxygen/air mixtures into cell culture processes is carried out by mixing gaseous oxygen and air from the usual pressure line by means of certain mixing devices upstream of the bioreactor, and followed by a sterilizing gas filtration step. Additionally, further exhaust and vent filters on the fermenter may also be exposed to higher oxygen concentrations. It is well known [4,5], that metals and polymers are more flammable in oxygen-enriched environments than in air. Hence, handling and, in particular, filtration of oxygen is a safety challenge. The presence of higher concentrations of oxygen can lead to ignition of materials that may not be a problem in air.

Oxygen installations, including those for filtration, have to be operated safely and may need approval or testing by special institutes. One such institute, the BAM Federal Institute for Material Research and Testing in Berlin, Germany tests batches of materials for usage in gaseous oxygen. With a special test routine, the ignition sensitivity to gaseous oxygen pressure shocks of the construction materials of Emflon HTPFR filters has been evaluated in 2012.

Emflon HTPFR filters features a highly efficient double-layer inherently hydrophobic Pall-manufactured polytetrafluoroethylene (PTFE) membrane along with support and drainage layers made of polyphenyl-sulphide polymer and polypropylene hardware specially formulated with protective anti-oxidants.

In order to test the reactivity with gaseous oxygen of all material parts of Emflon HTPFR filters were exposed to pure oxygen for 14 and 28 days. They were successfully integrity tested with the Water Intrusion Test (WIT) pre and post oxygen exposure. Solid samples of all component parts from both unused and previously exposed to oxygen Emflon HTPFR filters, were tested for ignition sensitivity to oxygen pressure shocks.

3.1 BAM Reactivity Test Method

The materials are placed into a heatable steel tube. The sample tube is then connected by long pipe with a pneumatically operated quick opening valve to a high-pressure oxygen accumulator. A heater enables the sample tube to be set to a defined test temperature. After the tube and pipe are at starting pressure, usually atmosphere pressure, the quick opening valve is opened and preheated oxygen of 60 °C (140 °F) at pre-set pressure flows abruptly into the pipe and tube. In this way, the oxygen in the tube and pipe is almost adiabatically compressed and heated.

If there is a reaction of the sample with oxygen, indicated by a steep temperature rise in the tube, further tests with a new sample are performed at a lower pressure ratio. If, however, no reaction of the sample with oxygen can be detected after a waiting period of 30 seconds, the tube is de-pressurized and the test is repeated (up to four times) until a reaction takes place. This means each test series consists of a maximum of five single tests with the same material under the same conditions. If no reaction can be observed, even after the fifth single test of a test series, testing is continued with new samples at greater pressure ratios, until finally that pressure ratio is determined, at which no reaction can be observed within a test series of five single tests. If the repetition of that test series with a new sample shows the same result, the test can be finished or continued at a different test temperature.

The evaluation of component materials both unused and previously exposed to oxygen for 14 and 28 days respectively showed that Emflon HTPFR filter materials passed the reactivity tests with 100% gaseous oxygen at 10 bar pressure at 60 °C (140 °F).

Providing that the user performs an adequate risk assessment as described earlier, Emflon HTPFR filters may be considered for gaseous oxygen service in applications such as:

- Feed of oxygen enriched air or gaseous oxygen at maximum temperature up to 40 °C and a maximum pressure of 3 bar for a maximum service life of 28 days or
- Exhaust gas at maximum temperature up to 60 °C for a maximum service life of 28 days in microbial and mammalian cell culture

These conditions are slightly less severe than the conditions applied to filter material parts during the above described reactivity tests. Also, process temperatures, excess gas pressures, contact times and oxygen concentration in the feed and exhaust air employed for cell culture applications where sterilizing grade gaseous oxygen filtration must be applied are typically even less severe, thus allowing for an additional safety margin.

4. Consideration of Additional Safety Recommendations

While oxygen enrichment improves production yields and lowers overall processing costs, it can increase potential fire risks for sterilizing gas filtration operations. Reactivity testing conducted by the BAM Federal Institute for Materials Research and Testing for usage of the Emflon HTPFR filter in gaseous oxygen as described above demonstrated that the filter's construction materials offer a high resistance to ignition to gaseous impacts. Emflon HTPFR is therefore a good candidate for such demanding fermenter or bioreactor applications by enabling manufacturers to qualify it in their specific applications conditions and use oxygen enrichment more safely.

As covered in Section 1, it is the user's responsibility to ensure that both the system used to supply gaseous oxygen and the application, are designed and constructed according to a safety or risk assessment together with expert input and advice. It is essential that the equipment is operated in a well-ventilated area and that the risk of leakage at any point is considered in the assessment. The user needs to consider a means to independently monitor oxygen levels in the area close to the equipment. The monitoring system would be set-up to prevent the oxygen level from reaching 24% and enabling shut-down of the oxygen supply in the event of a rise in level.

Other considerations that need to be made in the risk assessment are as follows:

- Safe storage and positioning of oxygen supply vessels such as cylinders or tanks
- Safe design and installation of pipework and fittings for both the supply system and application
- System and component information to ensure that materials in contact with oxygen are compatible
- Material Safety Data Sheets (MSDS) for materials employed need to be checked to determine their combustibility. In particular components need to be free of oil and grease
- Worst case operating conditions for pressure, temperature and flow rates
- Any items which are subject to steam sterilization need to be reduced below 60 degrees centigrade before oxygen is introduced
- Ignition mechanisms, e.g. static electrical discharge, mechanical stress, particle impact

Static electrical discharge is a significant potential source of ignition. The risk of static discharge may increase with lower humidity or higher flow rate of the gases. It is therefore recommended that the housing is connected to earth with a grounding line.

Further important general recommendations for the operation of an application demanding filtration of pure gaseous oxygen or oxygen enriched gases are as follows:

- Single-use of sterilizing grade gas filters are recommended
- Usage of "clean" and organic trace-free filters

- Minimized linear velocities by generous sizing: A German accident prevention regulation no. UVV/10 recommends: “The linear velocity, counted as the maximum flow at the lowest operating pressure should be below 25 m/s at operating pressures of 1 to 40 barg…”
- Avoidance of pressure shocks and pulses
- Special instructions on filter installation and operation are required
- Oil-, aerosol- and particle-free pressurized air-line can be supported by usage of coalescers and stainless steel and particle filters

In addition, regular physical integrity tests of sterile gas filters are recommended to verify that the filter is capable of performing its stated function.

Concerning the usage of gaseous oxygen, users are referred to their own national safety requirements and guidelines. However, several general safety guidelines offer many useful safety recommendations for the installation and operation of gaseous oxygen systems. Some guidelines and papers are listed for reader’s convenience in Section 5.

5. References

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