A Parker domnick hunter White Paper

Controlling bioburden within biopharmaceutical manufacturing processes

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Introduction

The manufacture of biopharmaceuticals requires multiple operations in order to deliver a product of appropriate quality and potency to the patient. Operations such as fermentations and chromatography are technically complex requiring considerable understanding and control.

Throughout the process it is necessary to ensure either sterility or the control of bioburden within process streams. Conditions that are commonly encountered within bioprocesses are conducive to the growth of microorganisms within process solutions. These solutions are often moderate with respect to temperature, pressure, acidity and osmolarity and in addition can contain carbon sources required for microbial growth.

The growth of microbes within process streams can be deleterious to product quality and potency for many reasons, These include;

- Loss of productivity and reduced product quality in contaminated bioreactors.
- Increased costs due to replacing contaminated fermentations.
- Increased costs due to replacement of contaminated consumables such as chromatography resins or ultrafiltration membranes.
- Microbial degradation of biopharmaceutical product.
- Release of impurities, immunogenic or toxic molecules such as endotoxin into the process stream from the contaminating organism
- Changes in process stream characteristics leading to process measurements being ‘out of specification’
- Costs of quality investigations into high bioburden and endotoxin test results.
- Delays or prevention of the batch being released.
- Infection of patients receiving the biopharmaceutical with the contaminating organism.

Minimizing bioburden in biopharmaceutical manufacturing processes

Preventing contaminations within manufacturing process requires consideration to be given to many facets of facility design and operation. The operating philosophy of the facility can change the approach to contamination control. For example, a facility which is being operated “24/7” and in which equipment is adequately sized to maximize throughput can rapidly purify product being harvested from the bioreactor and minimize the risk of microbial contamination by reducing to a minimum the time product is being held in intermediate pool tanks. In cases where facilities are not being used so intensively a manufacturer might utilize sterile filtration in order to protect intermediate product pools from bioburden.

The risk of contaminations can be minimized by the quarantining and testing of all new production organism cultures, the adherence to aseptic procedures in the laboratory and Good Manufacturing Practice in manufacturing. In addition appropriate precautions should be taken with raw materials used in manufacturing. These can be supplied in a pre-sterilized form, sterilized within the manufacturing facility or alternatively can be screened for contamination prior to being used.

Bioburden controlling filtration is used throughout biomanufacturing plants in order to minimise the risk of serious microbial contaminations. Table 1 provides a list of applications within upstream and downstream processes in which filtration can be used to minimize microbial growth (adapted from Ref 1).

Location	Application	Category of filtration
Upstream processing	Growth media sterilization	Liquid filtration
	Bioreactor additions (pH control, nutrient feeds, antifoam)	Liquid filtration
	Bioreactor sparge gas inlet sterilization	Gas filtration
	Bioreactor exhaust gas sterilization	Gas filtration
	Post-harvest product hold tank	Liquid filtration
Downstream processing	Buffer filtration	Liquid filtration
	Buffer tanks venting	Gas filtration
	Product stream bioburdrn control	Liquid filtration
	Intermediate product hold tank venting	Gas filtration
	Final filtration	Liquid filtration

Table 1. Normal flow filtration applications within the biopharmaceutical industry

Modern sterilizing-liquid filter membranes are typically manufactured from polyethersulphone (PES) or polyvinyl difluoride (PVDF). Sterilizing-gas filters can be made from a range of hydrophobic materials including polytetrafluoroethylene (PTFE).

Bioburden control in upstream processing operations

Ensuring bioreactors contain only the genetically-modified, biopharmaceutical-producing organism is critical to bioreactor productivity, facility throughput and product quality. Heat sterilization of growth media is possible for prokaryotic fermentations that do not require heat-labile media components. For mammalian cell cultures, which typically require more complex medias, media sterilization by filtration is often required (Ref 2). This typically requires a traditional sterilizing-grade filter capable of retaining 10^7 colony forming units of *Brevundimonas diminuta* per cm^2 of effective filtration surface area under process conditions (Ref 3), however, concerns from biomanufacturers about contaminations from a group of microorganisms called 'Mycoplasma' that are small in size and have a flexible morphology have lead to the use of Mycoplasma retentive filters capable of the retention of Mycoplasma species such as *Acholeplasma laidlawii* under process conditions.

A sterile boundary must be in place around the bioreactor during operations to prevent the introduction of undesirable microbes. This should cover all inlets and outlets that could be sources of contaminations. During the course of fermentations, liquid additions are often made in order to replace consumed nutrients, control pH or foaming. These additions can be filtered into the bioreactor using sterilizing or Mycoplasma retentative filters. Gases sparged into the bioreactor to provide oxygen, agitation or pH control must also be filtered through a suitable sterilizing-grade gas filter. To complete the sterile boundary filters on the gas outlet serve both to prevent contamination through this line but also to contain potentially hazardous genetically modified organisms within the process.

Once the fermentation has completed, product is harvested from the bioreactor. Harvesting procedures vary depending on whether the product is intracellularly or extracellularly expressed. Typical operations might include centrifugation, depth filtration, homogenization or tangential flow microfiltration. In each case the goal of the bioreactor harvest is to ensure clarified, soluble product is prepared for purification during downstream processing operations. Often the clarified

soluble pool is stored while downstream operations are scheduled. At this stage it is possible, particularly with extracellularly expressed products, that the pool contains nutrient-rich components derived from the bioreactor which creates an environment that is conducive to the growth of contaminating microbes. Some biopharmaceutical manufacturers will chill this pool to within 2 to 8^0C which not only limits bioburden growth but can also stabilize products and limit the actions of proteolytic enzymes derived from the host organism that can degrade the product. Filter sterilization is an option to eliminate bioburden prior to downstream processing, however, some manufacturers prefer to use a bioburden reducing filtration step that does not sterilize but can significantly reduce bioburden while maintaining high flow rates and short processing times. This latter option is particularly useful when the storage time prior to downstream processing operations is short.

Bioburden control in downstream processing operations

Typical operations used in the purification of biopharmaceuticals include chromatography, tangential flow ultrafiltration, virus filtration and final product filtration. While sterile filtration is often a requirement in upstream processing in order to protect the bioreactor from contamination, manufacturers can be more flexible in options around minimizing bioburden in downstream processing until the final filter sterilization of the formulated product.

Chromatography and ultrafiltration steps are frequently performed as non-sterile operations and, at least until the end of the process, the control of bioburden downstream of the bioreactor is arguably less reliant on sterile filtration. Buffer solutions used in chromatography and ultrafiltration steps are often filtered through a membrane to remove solids particulates. The filtration of buffers may sterilize or at least reduce bioburden in addition to removing particulates as part of an overall bioburden control strategy (Ref 4). Similarly membrane filters can be used to protect chromatography columns, ultrafiltration membranes and virus filters from particulate matter without needing to sterilize the process stream. As with the harvest pool, intermediately purified product pools may be chilled to 2 to 8^0C, sterile filtered or bioburden control filtered to minimize the effect of microbes on the process stream quality and the approach will be determined by the operating philosophy and the speed with which batches pass though the facility (Ref 5).

Where maintaining the sterility of the contents of stainless steel tanks is important vent filters

should be fitted to allow the sterile transfer of gases into the tank which may contain intermediate product pools, buffers or growth media.

The final filtration of the biologic drug substance is critical to ensuring patient safety and must be sterilizing. A comprehensive process validation should be performed that ensures the filter will perform as required under the process conditions experienced. While leachable studies are important for consumables used throughout the process particular attention must be made to the final filter as leachable from here have a direct route into the vialled drug product.

Controlling bioburden by effective design of process equipment

The equipment used in bioprocesses should be validated, easy to clean, be built of non-reactive materials of construction, be inert or have no effect on the product and must be designed so as to prevent product contamination. The American Society of Mechanical Engineers (ASME) developed ASME BPE Standards for equipment used in biotechnology, fermentation and cell culture. The use of equipment designed to ASME BPE standards should minimize the risk of microbial growth occurring and persisting within the process.

The adoption of single-use processing technology has now been widely adopted within the biopharmaceutical facilities to a greater or lesser extent. Single-use assemblies can be supplied having been gamma-irradiated by the vendour. The Bio-Process Systems Alliance (BPSA) produces technical guidelines which provide basic information and recommendations on the use of single-use technologies for the biopharmaceutical industry. Generally, a minimum gamma-irrdation doss of 25 kGy is desired for microbial control or sterilization and will achieve a sterility assurance level of 10^{-6} at low bioburden levels (Ref 6).

Conclusions

Controlling bioburden with manufacturing is of critical importance to manufacturers of biopharmaceuticals. Preventing contaminations and reducing their impact can be achieved by a variety of means. Bioburden controlling and sterilizing filtration is a key method for reducing the risk of contaminations in upstream and downstream processes. The appropriate design of stainless steel equipment and sterilization of single-use systems are further ways in which

unwanted and damaging microbial growth can be minimized or even prevented.

References

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