BIOPROCESSING DESIGN CONSIDERATIONS FOR STERILITY CONTROL

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Using strict sterilization criteria during sensitive fermentation operations can minimize, or even prevent, loss of time or materials due to contamination or process upsets. Maintaining a sterile environment is crucial to successful fermentation operations and should be made a priority in design of any sensitive fermentation operations.

Establishing boundaries for a sterile environment can be achieved inherently through process design (commonly done for feed streams into the sterile system) or with adaptive control schemes (usually for the pieces of equipment being sterilized). A typical configuration of the different mechanisms to control the sterile boundary can be found in Figure 1.

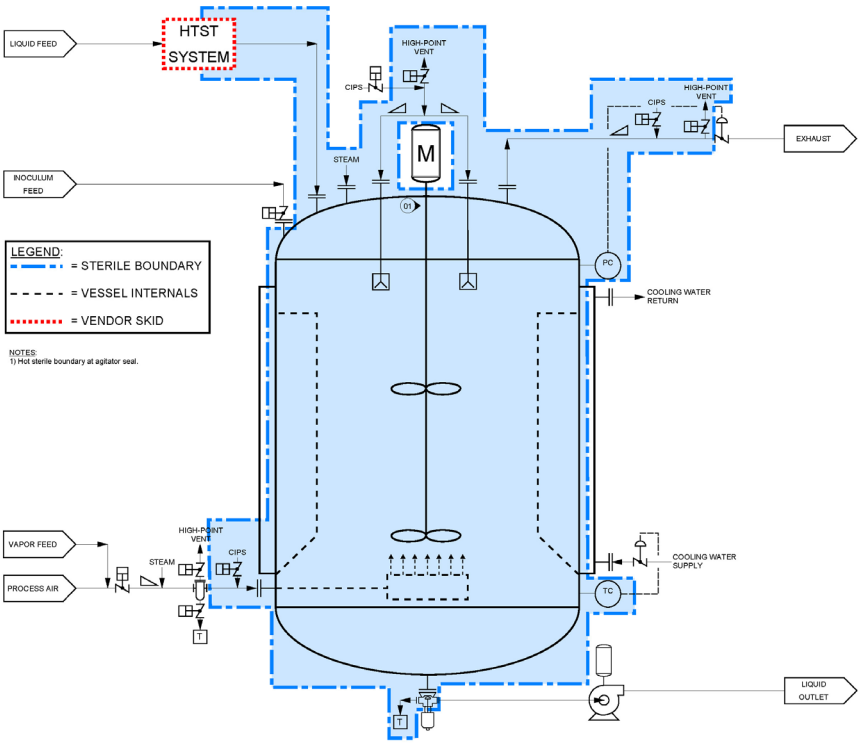

Figure 1: Sterile Boundary Control Points based on ASME BPE-2019

Generally, sterilization of a fermentor can be done in one of two ways:

In situ or full sterilization:

- All feed streams are collected inside the fermentor and steam is introduced while it's full. Steam is introduced either directly into the fermentor or indirectly with a jacket.
- This is convenient for smaller scale systems (<5,000 gallons), due to the capital cost savings from reduced equipment complexity.

Empty sterilization:

- Steam is introduced into an empty fermentor to sterilize, then the separately sterilized feed streams are introduced.
- This is more practical for larger scale systems, since it's quicker and allows for heat integration to reduce utility consumption.

STERILE CONTROL OF FEED STREAMS

Sterilization methodology for feed streams is handled differently for vapor streams than for liquid or solids-laden broth/slurry streams. Non-sterile vapor streams (usually air for aerobic processes, or anhydrous ammonia for pH control) are sterile filtered before being introduced to the sterile fermentor. The sterile filter is steam sterilized along with the fermentor during the empty sterilization. Often, coalescing filters are used upstream of the sterile air filter to remove large particles and excess entrained water.

Clean liquid streams can be sterile filtered if they can be passed through an appropriately selected micron filter. However, most liquids and slurry/broth streams are instead sent to a High-Temperature, Short Time (HTST) sterilization skid. The HTST introduces the heating medium to the feed either directly or indirectly and routes the heated streams through a holding tube. The holding tube maintains a minimum temperature of the flowing feed stream for the time required for sterilization, after which the liquid stream is cooled down to normal process temperatures and introduced to the sterile system. Considerable temperature changes and heat exchanges in an HTST make it an attractive opportunity for recovering 75-80% of heat.

STERILE CONTROL OF EQUIPMENT

Prior to receiving sterile feed streams, the system must be cleaned and sterilized. In a batch operation, this is usually done by performing a Clean-In-Place (CIP) procedure at the end of the previous batch, followed by a Sterilization/Steam-In-Place (SIP) procedure at beginning of the current batch. The following points are considered when designing around the SIP procedure, and are described in more detail in the ASME BPE Standard [1]:

- The sterile boundary for a fermentor includes any vessel internals; inlet/outlet piping up to, and including, isolation valving; and spargers/spray devices.
  - Double mechanical seals are commonly used on agitators to prevent contamination, with the sterile boundary stopping at the inboard side of the primary seal. A sterile barrier fluid separates the primary and secondary seals and provides a flushing mechanism to improve seal life. Although steam can be used as a sterile barrier fluid, food-grade lubricants are also acceptable and improve the seal lifetime when compared to steam.
  - If using an external heat exchanger, the pump, heat exchanger, and associated piping are all within the sterile boundary.
- Unless explicitly necessary, all equipment is to be empty and have no liquid hold-up prior to SIP.
  - Steam sterilization commonly requires condensation of steam on equipment surfaces, which is why purging the air inside the fermentor with steam is critically important.
- SIP uses a single steam supply point at the vessel and flows out of the vessel at several low-point drains (condensate traps) or high-point vents (unlike what is done for CIP, where multiple supply points flow into the vessel with a common return line).
- All inlet/outlet ports of the system are closed, with only a pressure-controlled high-point vent, steam inlet line, and condensate trap lines still opened..
  - Any air or other process vapor in the head space of the system will be displaced by steam and exhausted out through the vent at a controlled rate, ensuring the design pressure of the system is not exceeded.
  - The design of these high-point vents will prevent downstream condensation and route all upstream condensation back into the system.
- All flow and backpressure control devices are located outside of the sterile boundary and do not need to be sterilized.
- All instruments inside the sterile boundary must be designed for SIP.
- The system has been sterilized after a minimum temperature is achieved for a predetermined amount of time on the time scale of minutes. Appropriately placed temperature sensors can be used to determine required sterilization.

Bioprocessing designs can make use of these sterility control methodologies to achieve a sterile boundary. The burden for maintaining a sterile boundary can be divided into separate components (i.e. feed streams and pieces of equipment) and subsequently addressed in controllable sections. By robustly enforcing sterilization criteria for the individual components of a sterile system, time and material losses due to contamination can be minimized or prevented.

REFERENCES

[1] American Society of Mechanical Engineers (ASME), "Bioprocessing Equipment," ASME BPE-2019, pp. 66-85, 2019.