Best Practices for Multi-Use Bioreactor Sterilization

APPLICATION BRIEF

GETINGE

Establishing an Effective and Reproducible Process

Abstract

Regardless of whether the bioreactor is being prepared for a same-use application or an entirely different one, all bioreactor components, as well as liquid media, must be made ready through an effective and reproducible process. In this brief different sterilization cycle types are tested for efficacy on an assembled bioreactor. Testing results demonstrate inefficient ambient air removal and subsequent inefficacy of sterilization in two common methods.

This brief describes how a multi-use bioreactor, headplate, tubing, vent filters, liquid media, and filled liquid addition bottle can be sterilized at once and in a single integrated process with confirmed sterilization efficacy. Successful sterilization of the complete assembly improves throughput, minimizes downtime, and returns the bioreactor to service with minimum labor.

Cycle Terminology

- **Purge (Conditioning)**
  - Upon cycle initiation, steam flows into the chamber to displace ambient air, which purges through the chamber to the drain. Both gravity and vacuum systems can manage this process, with vacuum being the most efficient.
  - Residual air trapped within the bioreactor tubing or other components will form a barrier against live steam and must be removed.
- **Pre-Vacuum**
  - Alternating vacuum and steam functions create pressure/vacuum pulses to thoroughly remove ambient air, which will be replaced at all levels by steam. This is important for tubing and other head plate components where air can become trapped.
- **Pressure Pulse(s)**
  - Steam pressure pulses following the pre-vacuum penetrate all areas and displace ambient air trapped in long silicone hoses. The use of pressure pulses to remove air ensures better penetration of steam and repeatable sterile results.
- **Heat Up**
  - During the ramp phase, all outlet valves are closed and both temperature and pressure increase to achieve setpoint.
- **Dwell (Sterilization Plateau/Exposure Phase)**
  - According to the programmed cycle, temperature and pressure dwell is maintained for an established amount of time, predicated on the standard operating procedure or other criteria.
- **Drying/Cool Dow**n
  - In the final phase of the sterilization procedure, the bioreactor and all components, including liquid media, are returned to ambient temperature and pressure.
    - A gravity cool-down sequence is the slowest and does not offer a complete drying function.
    - A vacuum-assisted cool-down sequence is useful where liquid media is sterilized but the pressure decrease rate must be measured to permit the liquid to cool by evaporation while boiling.

Introduction

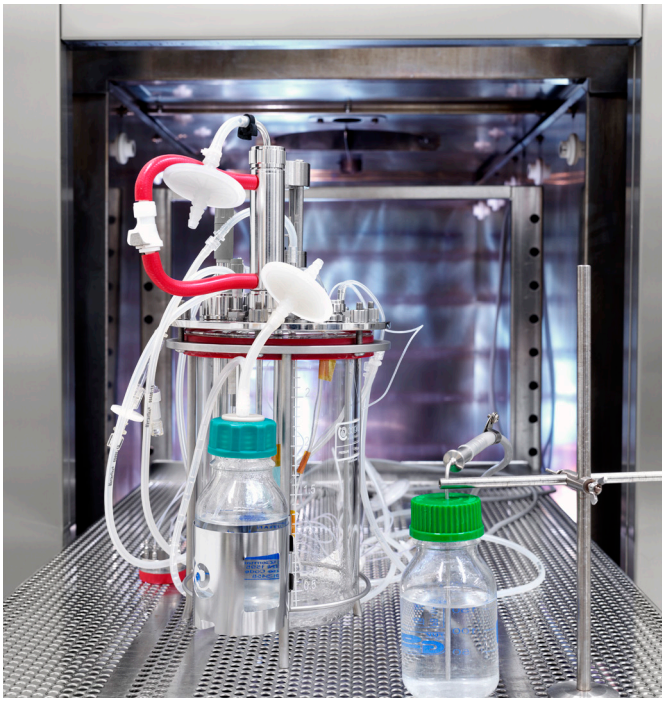
Bioreactors are commonly sterilized in simple laboratory sterilizers. In general, steam sterilizers typically only operate in two modes, gravity or vacuum:

- **Gravity** — The most simplistic sterilization uses injected steam to displace ambient air by gravity alone. Once air is removed through the chamber drains, the drain valve closes and the steam pressure rises to the programmed setpoint. When the cycle is completed the drain valve is opened to remove steam and equalize the pressure. Steam is replaced by ambient air through the vent, which helps dry and cool the load.

- **Liquid** — A gravity sterilization method that uses a slower exhaust rate to prevent and reduce the boil-over effect, which is caused when sterilized liquids are equalized too quickly. The drain valve is cycled while pressure is monitored to ensure slow repressurizing and prevent excess evaporation. Like the gravity cycle, this includes natural drying at end of cycle or Air-Over-Pressure.

- **Pre-Vacuum, Post-Vacuum** — Primarily used for hard or porous goods, this method removes ambient air mechanically within the load before steam is injected. Pre-vacuum sterilization removes more ambient air so steam can more effectively reach components. As temperature and pressure reduce, condensation evaporates into a vapor, which helps dry the load. A post-vacuum cycle removes this vapor, increasing condensation evaporation to dry the load and preventing recontamination once the bioreactor is removed from the sterilizer.

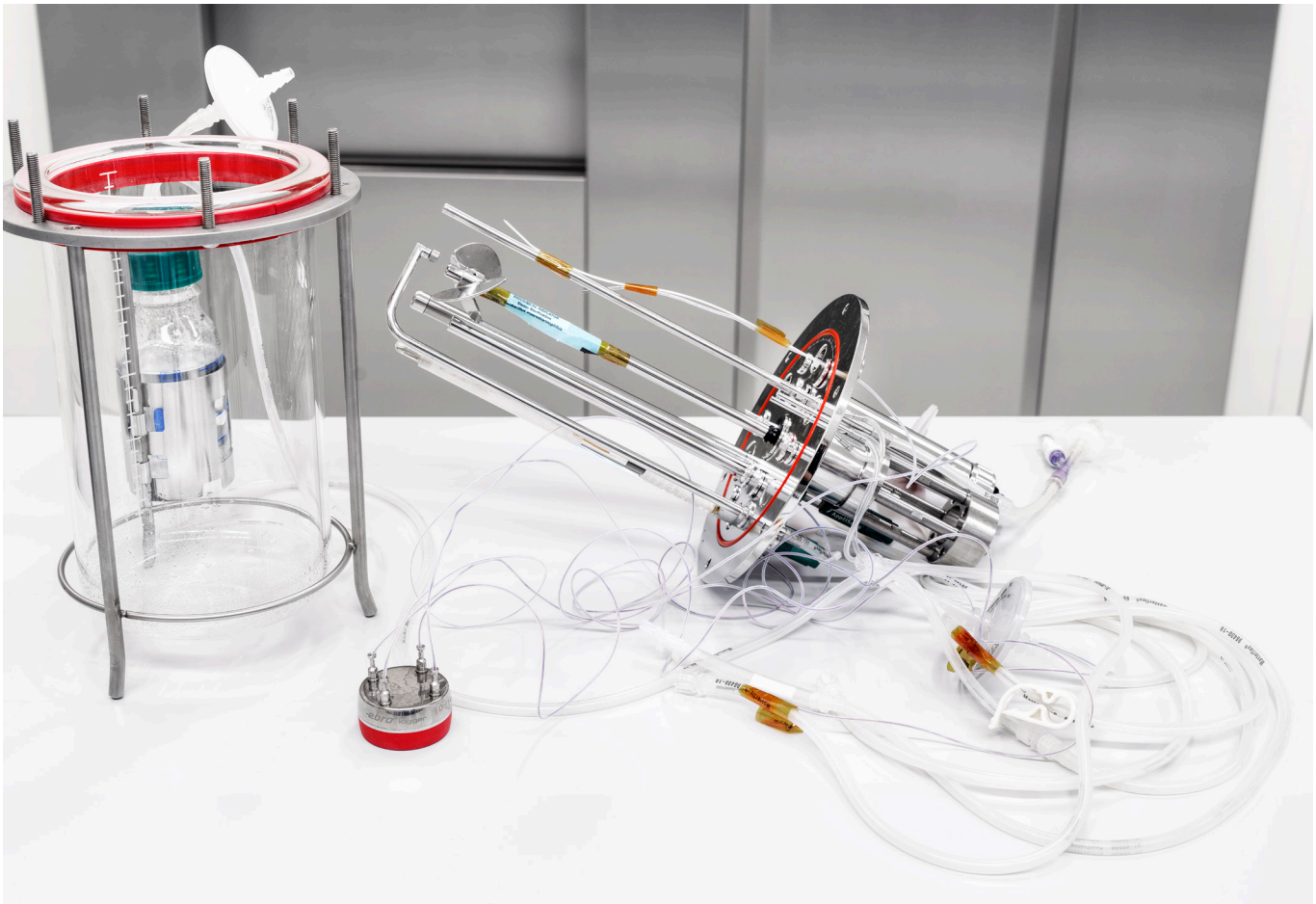
Regardless of operation mode, the sterilizer must meet the minimum temperature plateau of 250°F (121°C) with a minimum dwell time of 20 minutes to ensure the sterilization cycle's efficacy. To verify the requisite load temperature of 250°F (121°C) is met, the temperature should be measured in the coldest spot or the slowest area to heat. The combination of temperature and time is essential for eradicating thermoresistant spores and other organisms.



Comparison Testing

In a Sterilization Efficacy Evaluation completed by the Getinge Sterilization Test Lab, various bioreactor sterilization methods were studied. Testing was performed using temperature mapping and commercially available bioindicators. These tests were conducted through standard cycle selections, and verified using bioindicators to assure that all concealed areas were adequately exposed to steam. A 0.79 gal. (3L) bioreactor was used for all cycles in the study. A small amount , 0.3 oz. (10 ml) of water was added to the vessel before each test. The thermowell was also filled with water in each test to verify heating from the vessel, rather than from steam entering externally from the top. The temperature in the liquid addition bottle was verified by measurement in a reference bottle of same type and size.

Test Type	Method
Scenario 1: Standard Test Cycle	Steam injection to purge, followed by heat up to 250°F (121°C) for a minimum 20 minute dwell time
Scenario 2: Pre-Vacuum	A single pre-vacuum is added to increase air removal and improve steam penetration within vessel and tubing
Scenario 3: Pre-Vacuum and Pressure Pulses	A single pre-vacuum is followed by steam pressure pulses to remove any remaining air and provide maximum steam penetration in vessel and tubing



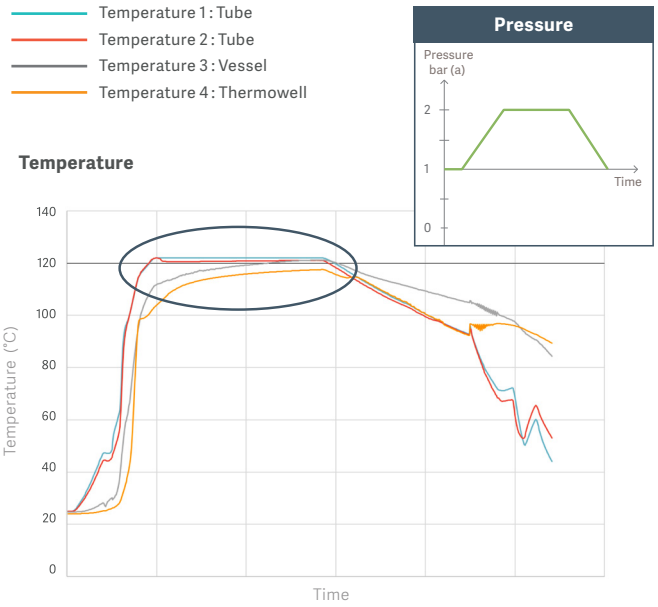
This figure illustrates a typical test set-up where the bioindicator is placed in the sterilizer adjacent to a Getinge/Applikon bioreactor properly disassembled, either partially (repeat process) or totally (new process). This test will confirm that all interior tubing, probe and other components are sterilized and ready for return to service.

Results

Scenario 1 — Liquid cycle, no vacuum (CC 890):

- ✖ Temperature sensors placed in tubes reach sterilization temperature.
- 🔍 Sensor placed in the vessel reaches and maintains sterilization temperature for the plateau's final four minutes.
- 🔍 Temperature sensor placed in the thermowell does not reach sterilization temperature (hitting a maximum temperature of only 243.5°F or 117.5°C).

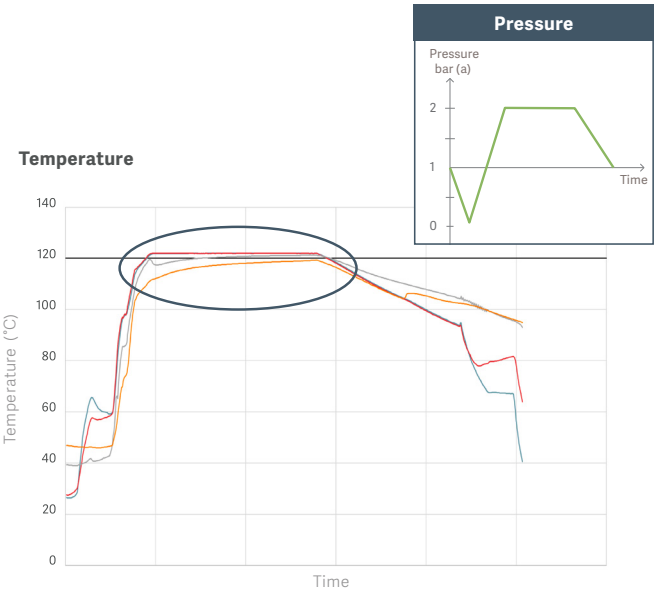
Conclusion: Minimum sterilization time at required temperature was not reached for two out of three bioreactor components. This result strongly indicates insufficient air removal.



Scenario 2 — Liquid cycle, one pre-vacuum (CC 895):

- ✖ Temperature sensors placed in tubes reach sterilization temperature.
- 🔍 Sensor placed in the vessel reaches and maintains sterilization temperature for 10 minutes of the plateau.
- 🔍 Temperature sensor placed in the thermowell does not reach sterilization temperature (hitting a maximum temperature of 246.7°F or 119.3°C).

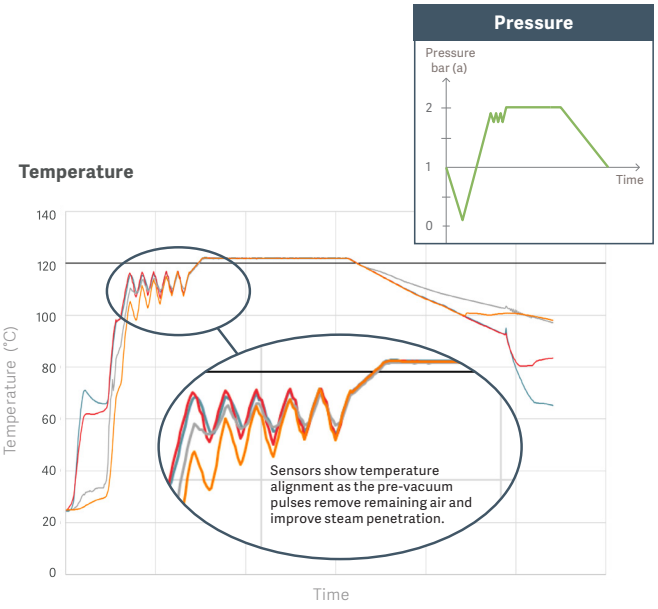
Conclusion: Minimum sterilization time at required temperature was not reached for two out of three bioreactor components. Though temperature profile was improved from Scenario 1, results still suggest remaining air in the vessel.



Scenario 3 — Liquid cycle, one pre-vacuum and pressure pulses (CC 894):

- ✖ Temperature sensors placed in tubes reach sterilization temperature.
- ✖ Sensor placed in the vessel reaches and maintains sterilization temperature for 20 minutes, entirety of plateau.
- ✖ Temperature sensor placed in the thermowell reaches sterilization temperature (250°F or 121°C) and maintains sterilization temperature for 20 minutes, entirety of plateau.

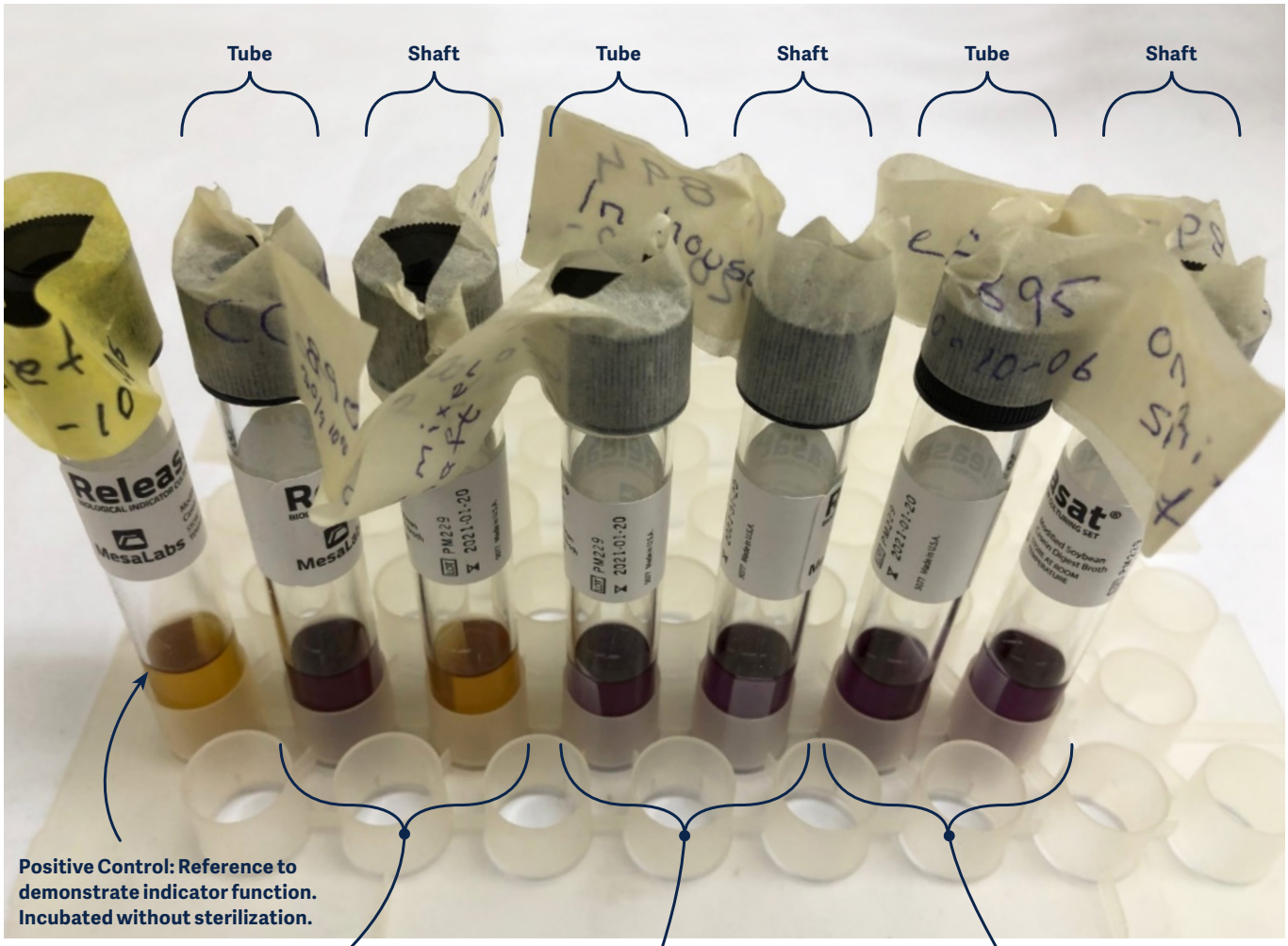
Conclusion: The necessary temperature and dwell time was reached for every bioreactor component. The temperature profile indicates good air removal in all positions.



Bioindicator Results

Following sterilization in each scenario, the bioindicator strips from each process were placed in culture medium, initially purple, alongside a positive control, which was incubated without sterilization. Samples are placed in an incubator at 131°F to 140°F (55°C to 60°C) for at least seven days.

If the color remained purple, it indicated that all spores and organisms were eradicated in the sterilization process. Color change to yellow indicated bacterial growth.



Liquid cycle, no vacuum **Scenario 1 (CC 890)**

Bioindicator Position	Bacterial Growth
Tube	Negative
Shaft	Positive

The positive control and the shaft-position bioindicator from Scenario 1 (CC 890) showed bacterial growth. This result confirms that Scenario 1 (CC 890) does not have sufficient air removal and steam penetration to ensure sterilization efficacy.

Liquid cycle, one pre-vacuum **Scenario 2 (CC 895)**

Bioindicator Position	Bacterial Growth
Tube	Negative
Shaft	Negative

While this scenario did not show bacterial growth, both the vessel sensor and thermowell sensor failed to meet the required temperature for the minimum dwell time indicating there is still risk of bacterial growth following future sterilization cycles.

Liquid cycle, one pre-vacuum and pressure pulses **Scenario 3 (CC 894)**

Bioindicator Position	Bacterial Growth
Tube	Negative
Shaft	Negative

Sterilization in this scenario is confirmed by the bioindicator results and verification that temperature and dwell time was reached for every bioreactor component.

Problems & Solutions

When ambient air remains in the vessel, thermowell, and/or long and narrow tubing used on the bioreactor, steam cannot penetrate all the way through to provide sterilization. This compromises sterilization efficacy and jeopardizes repeatability. Combining the pre-vacuum and pressure pulses solves this issue by forcing out additional ambient air as the pressurized steam travels into all areas of the bioreactor.

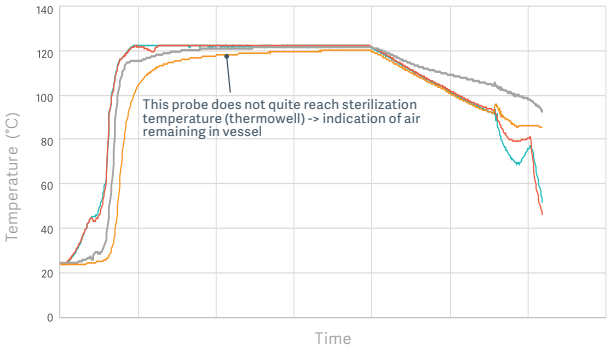
When hydrophobic vent filters are used, permeability must be good enough to allow steam penetration all the way through. The use of multiple vacuum pressure pulses allow for better steam penetration through vent filters and housings.

Another possible solution is to increase the overall plateau to ensure the minimum 20-minute dwell time is reached. In follow-up testing performed by the Getinge Sterilization Test Lab, the efficacy of an extended dwell time was evaluated. Testing compared the results of a 40-minute dwell time in an empty bioreactor (CC 899) with a 0.79 gal. (3L) vessel filled 0.63 gal. (2.4L) (CC 900) for the same dwell time.

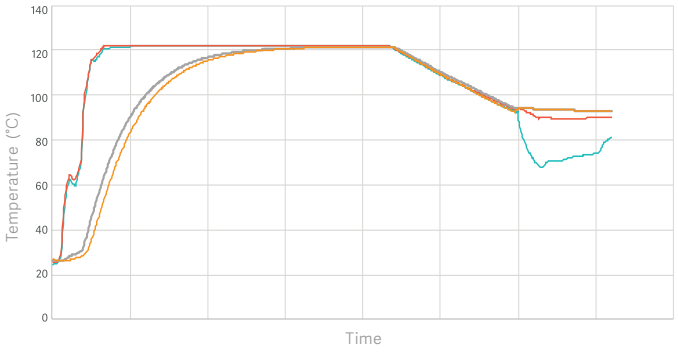
In test CC 899 — Empty Bioreactor, the temperature sensor placed in the thermowell still did not reach 250°F (121°C), even by the end of the 40-minute period. However, bioindicator test results indicated no bacterial growth verifying that extended plateau time improves sterilization results.

In test CC 900 — Full Bioreactor, the extended plateau time and liquid in the vessel allowed the minimum temperature to be reached throughout. Bioindicator results also verified sterilization in this scenario.

CC 899 — Empty Bioreactor



CC 900 — Full Bioreactor



Conclusion

The sterilization process is confirmed to be the best fit. This process combines pre-vacuum and pressure pulses to efficiently remove air and improve steam penetration into the entire bioreactor assembly. A standard steam sterilization cycle on an assembled bioreactor does not sufficiently remove ambient air. As a result, the long, narrow tubes and multiple deep vacuums of the bioreactor are not penetrated by steam for the entire plateau. While the wash cycle, conducted before sterilization, should have removed any bioburden and the overall temperature during sterilization should be sufficient to kill most spores/organisms, the bioreactor condition should still be verified before starting additional lab tests. This can be done by waiting several days post-sterilization to check for unexpected microbial growth.

Option 1 — Adequate Method

Separate the bioreactor into three separate loads based on type. This method requires the complete disassembly of the bioreactor, increases downtime and increases possible risks of integrity breach.

- Cycle 1: Hard Goods Cycle
- Cycle 2: Long/Narrow Tubes
- Cycle 3: Closed Vessel (Empty) or Closed Vessel (Liquid Filled)

Option 2 — Adequate Method

Pre-Vacuum and Steam Sterilization

While this method achieves confirmed sterilization, it is not recommended for use with liquid-filled bottles as it can cause the boil-over effect. The addition of the pre-vacuum cycle ensures more air is removed from the tubing yet does not effectively remove all ambient air.

- Cycle 1: Hard Goods & Long/Narrow Tubes
- Cycle 2: Closed Vessel (Empty) or Closed Vessel (Liquid Filled)

Option 3 – Leading Method

Getinge Applikon Bioreactor Sterilization Process

The sterilization process found to deliver the best results with the least number of steps combined pre-vacuum and pressure pulses. This cycle offered the simplest solution to assured sterilization for reproducibility and quality assurance.

This innovative approach accommodates three different types of loads, hard goods, tubing and liquid, in one cycle while removing all ambient air using a single pre-vacuum and multi-pulsed steam pressure. In loads with liquid media, the pre-vacuum removes ambient air while at room temperature, before steam is injected, so liquid media does not boil or evaporate. Once the pressure level is above the given liquid's saturation pressure, remaining air is removed through pressure pulses for complete steam penetration.

As demonstrated in Scenario 3 (CC 894), this combination of pre-vacuum and pressure pulses confirms sterilization efficacy for all bioreactor components in a single cycle and provides quality assurance.

Process parameter cycle considerations are included for sterilization of liquid media and ancillary components, which are unique to the bioreactor function. As vacuum cycles remove air from the media, a natural cool-down period permits the liquid to reach atmospheric equilibrium without boil-over before opening the sterilizer door. Additionally, because the liquid bottle remains connected to the bioreactor assembly, one breach of integrity risk is eliminated.

Summary and Best Practices

The Getinge Applikon Bioreactor Sterilization Process mitigates customer challenges in a single, combined cycle, allowing you to spend more time focusing on your work. However, if a process that combines pre-vacuum and pressure pulses before the sterilization phase is not available, extended plateau time or separate liquid bottle sterilization is recommended.

The following best practices improve throughput and overall confidence in bioreactor preparation.

- A properly cleaned (washed) bioreactor, headplate and components is the first step in the sequence.
- Once cleaned, staging the bioreactor, tubing and components in the sterilizer can be improved by eliminating areas where moisture may condense and pool in hard to reach areas.
- The ideal sterilization sequence requires a series of programmable vacuum and pressure pulses, which remove ambient air in advance of steam injection.

Proper sterilization begins with thorough cleaning using automated wash cycles that remove bioburden or other contaminants that adhere to the bioreactor vessel, impeller, ports, tubes, and other components essential to bioreactor function.

A companion White Paper[1] to this Application Brief details the development of a bioreactor and headplate, and how this design interfaces with a unique wash rack designed to thoroughly clean bioreactor components from the inside out using high-pressure water, detergents, additives, and programmed cycles that initiate cleaning at the touch of a button. These objectives include control over temperature, cycle time, mechanical impingement through and around the wash rack load, and chemical action required to break down difficult debris within the bioreactor assembly.

Reference

1. Getinge Bioreactor Preparation Solution Whitepaper

Getinge/Lancer Sales • 1150 Emma Oaks Trail, Ste 140, Lake Mary, FL 32746
Manufacturer • Ekebergsvägen 26 • Box 69 • SE-305 05 Getinge • Sweden

www.getinge.com