RAMA UNIVERSITY

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FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

MEDIA STERILIZATION

- Sterilization is defined as the complete destruction or elimination of all viable organisms (in or on an object being sterilized). There are no degrees of sterilization: an object is either sterile or not.

- Sterilization procedures involve the use of heat, radiation, chemicals or physical removal of cells. Media for industrial fermentations are usually sterilized. In some cases the economics of the fermentation makes it unrealistic to sterilize.

- The fermentations can proceed, however, these fermentations employ low pH and other contamination inhibitors (lactic acid) to hold in check the numbers of contaminating microorganisms. In other cases, sterilization is not required as the media components are poorly utilized by contaminating microorganisms.

- Fermentation media are sterilized by the use of: filtration, radiation, ultrasonic treatment, chemical treatment or heat (boiling or passing live steam through the medium, or by subjecting the medium to steam under pressure -autoclaving). Steam is used almost universally for the sterilization of fermentation media.

- The major exception is the use of filtration for the sterilization of animal cell.

Heat:

Heat is the most important and widely used method. For sterilization, the type of heat, time of application and temperature required to ensure destruction of all microorganisms must always be considered. Endospores of bacteria are the most thermo-resistant of all cells so their destruction usually guarantees sterility.

Incineration:

In this process, organisms are burned and physically destroyed. It is widely used for needles, inoculating wires, glassware, tubes etc.and objects that can not be destroyed in the incineration process.

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Boiling:

Boiling is done at >100 ℃ for 20-30 min. It kills everything except for some endospores. To kill endospores and therefore perfectly sterilize the solution, very long or intermittent boiling is required.

Autoclaving:

Autoclaving is the process of using steam under pressure in an autoclave or pressure cooker. It involves heating at 121 ℃ for 15-20 min under 15 psi pressure and can be used to sterilize almost anything. However heat labile substances will be denatured or destroyed. Sterilization of nutrient media is usually done using this process.

Dry Heat (Hot Air Oven):

The process involves heating at 160 ℃for 2 hours or at 170 ℃for 1 hour. It is used for glassware, metal and objects that will not melt.

Sterilization in industry-scale fermenters (or bioreactors)is more complex. Steam is used to sterilize fermentation media. The medium can be sterilized in situ within the bioreactor. However, if the medium is sterilized in a separate vessel, the bioreactor needs to be sterilized before the sterile medium is added to it.

Bioreactors are sterilized by passing steam through spargers. Spargers are devices that distribute gas bubbles (usually sterile air or steam) in a liquid phase. They have particular design criteria, e.g., providing small sized bubbles (the sparger breaks the incoming air into small bubbles).Various designs can be used such as porous materials made of glass or metal. However, the most commonly used type of sparger used in modern bioreactors is the sparge ring. A sparge ring consists of a hollow tube in which small holes have been drilled and is easier to clean than porous materials and is also less likely to block during fermentation. During sparging, steam pressure is held at 15 psi in the vessel for 20 min.

Batch sterilization

•Batch sterilization is the reduction of contaminant organisms through the heating of a vessel.

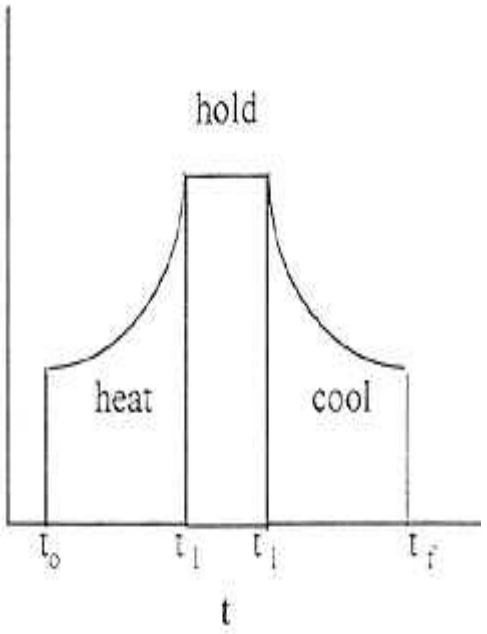
•The entire volume of media is sterilized at once through the use of thermal or radiation techniques. When running a thermal batch sterilization, a system goes through 3 steps: heating, holding, and cooling.

•Heating requires the addition of energy throughout the entire medium volume. This can be done by adding heat through a jacket on the vessel.

•The temperature is increased until it reaches the sterilization temperature where it is held for a set period of time.

•During this phase, most of the unwanted microorganisms are destroyed. Finally, the system is cooled to bring the sterile media back to the desired temperature.

•For radiation sterilization, the process is similar to above, although it uses radiation intensity instead of heat.



$$\nabla_{total} = \nabla_{heat} + \nabla_{hold} + \nabla_{cool}$$

$$\nabla_{heat} = \ln \frac{N_o V_o}{N_1 V_1} = \int_{t_0}^{t_1} k dt$$

$$\nabla_{hold} = \ln \frac{N_1 V_1}{N_2 V_2} = k(t_2 - t_1) \quad \text{constant T}$$

$$\nabla_{cool} = \ln \frac{N_2 V_2}{N_f V_f} = \int_{t_2}^{t_f} k dt$$

t = time
N_o = initial spore concentration
N_f = final spore concentration
V_o = initial batch volume
V_f = final batch volume
k = death rate constant

By the Arrhenius equation, the death kinetics are $k = Ae^{-E/RT}$.

In order to sterilize a batch, calculate the total area underneath the curve. Therefore, model death using first order kinetics and integrate as seen above. This will yield a temperature and the corresponding duration of time needed to sterilize the media.

Advantages:

- Most widely used technique
- Simple operation
- No additional materials are added to the media itself

Disadvantages:

- More expensive heat requirements than continuous sterilization
- Best results occur in well-mixed closed vessels

Continuous sterilization

➢Continuous sterilization is the rapid transfer of heat to medium through steam condensate without the use of a heat exchanger. Once the media is in a holding loop, steam is injected to the system via a nozzle.

➢The medium stays in this loop for a predetermined holding time until the entire medium is sterile. This is more efficient than batch sterilization because instead of expending energy to heat, hold, and cool the entire system, small portions of the inlet streams are heated at a time.

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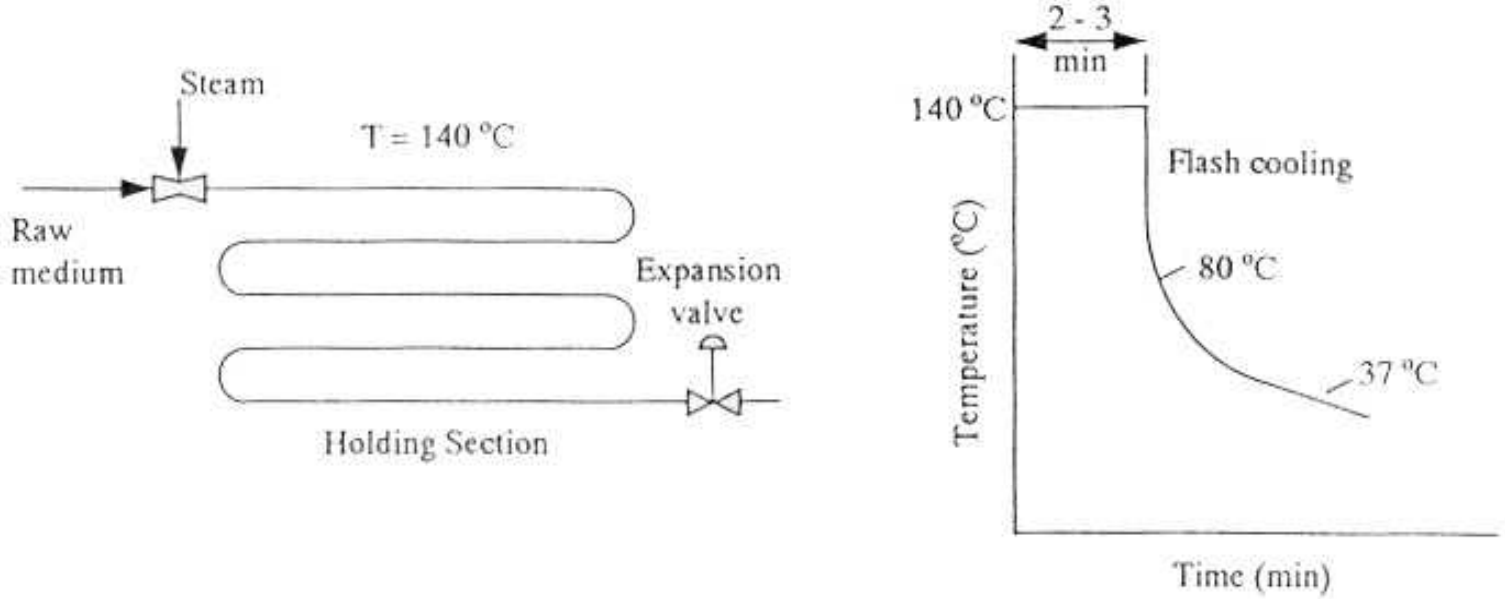
➢By looping sterile media tubes (which are at higher temperatures) past inlet tubes, the difference in temperature is used to help heat the unsterile medium. So instead of having a cold-water stream cool the sterile media, the lower temperature unsterile media stream absorbs heat from the warm stream, cooling the sterile media.

➢Finally, the sterile media is flash cooled through an expansion valve to adjust the temperature to meet process parameters.



Advantages:

Uniform steam requirements throughout the duration of the sterilization

Simplified process control

Shorter sterilization time means less thermal degradation of medium

Disadvantages:

High demand for steam in a shorter period of time than batch

Concentration of media becomes dilute due to steam condensation

Since steam is actually dispersed in media, steam must be clean to avoid contamination