Programme: Bachelor of Science (Firstyear)

Subject: Microbiology

Semester : II

Paper Code: MIG 102

Paper Title: Industrial and Food Microbiology

Unit 2 : Maintenance of sterility

Module Name : Sterilisation of Production media: batch and continuous.

Module Number : 5

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Notes

Sterilization of Culture Media:

The constituents of culture media, water and containers contribute

to the contamination by vegetative cells and spores.

The media must be free from contamination before use in fermentation.

Sterilization of the media is most commonly achieved by applying heat

and to a lesser extent by other means (physical methods, chemical

treatment, and radiation).

Heat sterilization:

- Heat is the most widely used sterilization technique.
- The quality and quantity of contamination (i.e., the type and load of microorganisms), composition of the media and its pH and size of

the suspended particles are the important factors that influence the success of heat sterilization.Vegetative cells are destroyed at lower temperature in a short time (around 60°C in 5-10 minutes). However, destruction of spores requires higher temperature and relatively longer time (around 120°C for 15-20 minutes). Spores of *Bacillus stearothermophilus* are the most heat resistant.

In fact, this organism is exploited for testing the sterility of fermentation equipment.

Sterilisation of media can be done by one of the following methods:

i) By boiling

ii) By passing live steam

iii) By subjecting the medium to steam under pressure (autoclaving).

Media sterilisation is a technique of making the medium sterile by the use of steam and is carried out in two ways:

1) Batch sterilisation.

2) Continuous sterilisation.

Other Physical methods:

The physical methods include

filtration,

centrifugation, and

adsorption (to ion-exchangers or activated carbon) are in use.

Among these, filtration is most widely used. Certain constituents (vitamins, blood components, antibiotics) of culture media are heat labile and therefore, are destroyed by heat sterilization. Such components of the medium are completely dissolved (absolutely essential or else they will be removed along with microorganisms) and then subjected to filter sterilization. Sometimes, a combination of filtration and heat sterilization are applied. For instance, the water used for media preparation is filtered while concentrated nutrient solution is subjected to heat sterilization.

The filtered water is then added for appropriate dilution of the media. The chemical methods (by using disinfectants) and radiation procedures (by using UV rays, y rays, X-rays) are not commonly used for media sterilization as they could destroy the nutrients present in the medium.

1) Batch sterilisation:

The culture media are subjected to sterilisation at 121°C in batch volumes, in the bioreactor.

Batch sterilisation can be done by injecting the steam into the medium (direct method) or by injecting the steam into interior coils (indirect method).

For the direct batch sterilization, the steam should be pure, and free from all chemical additives (that usually come from steam manufacturing process).

There are two disadvantages of batch sterilization:

1. Damage to culture media:

Alteration in nutrients, change in pH and discolouration of the culture media are common.

2. High energy consumption:

It takes a few hours (2-4 hrs.) for the entire contents of the bioreactor to attain the requisite temperature (i.e. 120°C). Another 20-60 minutes for the actual process of sterilisation, followed by cooling for 1-2 hours.

All this process involves wastage of energy, and therefore batch sterilization is quite costly.

2) Continuous sterilisation:

Continuous sterilisation is carried out at 140°C for a very short period of time ranging from 30 to 120 seconds. (This is in contrast to the batch fermentation done at 121°C for 20-60 minutes).

This is based on the principle that the time required for killing microorganisms is much shorter at higher temperature.

Continuous sterilisation is carried out by directly injecting the steam or by means of heat exchangers. In either case, the temperature is very quickly raised to 140°C, and maintained for 30- 120 seconds.

Continuous sterilisation is a high-temperature, short-exposure-time process, can reduce thermal damage to the medium significantly compared with batch sterilisation, while achieving high levels of cell destruction.

Other advantages include improved steam economy and more reliable scale-up.

The amount of steam needed for continuous sterilisation is 20 to 25% of that used in batch processes.

The time required is also significantly reduced because heating and cooling are virtually instantaneous.

- 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.
- 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.
- Another disadvantage is that certain compounds in the medium precipitate (e.g., calcium phosphate, calcium oxalate) due to very high temperature differences that occur in a very short time between sterilization and cooling. The starch-containing culture media becomes viscous in continuous sterilization and therefore is not used.