Sterilization and Aseptic Technique in Fermentation

Sterilization :
Is the process of Killing or removing all forms of microbial life (including endospores) in a material or an object.

Disinfection:
Reducing the number of pathogenic microorganisms to the point where they no longer cause diseases. Usually involves the removal of vegetative or non-endospore forming pathogens. May use physical or chemical methods.

In the fermentation industry it is mandatory to keep uncontaminated process from the preliminary culture to the production fermenter. But the risk of contamination is always present during inoculum development. Therefore, every effort must be made to detect as well as prevent contamination

Avoidance of contamination can be achieved by:

- Use pure inoculum to start fermentation.
- Sterilize the media.
- Sterilize fermenter vessel.
- Sterilize all materials to be added to the fermentation during the process.
- Maintaining aseptic conditions during the fermentation.

The most common contaminants of different industrial processes are considerably different. Some examples are given below:

1. ***In canning industry***, *Clostridium botulinum* is the chief concern. This obligate anaerobe can grow in sealed cans, and produce heat resistant spores and a deadly toxin. However, it is not a problem for catsup (too acidic), jam and jellies (too high sugar concentration) and milk (stored at low temperature).

2. Organisms like *lactobacillus* are a problem in ***production of wine***.

3. ***In antibiotic industry***, potential contaminants are many, e.g., molds, yeast, and many bacteria, including *Bacillus*.

4. The most dreaded contaminants of fermentation industry are ***phages***. The only effective protection against phages is to develop resistant strains.

Sterilization Procedures - The various methods for achieving sterility include:

(I) Physical Methods.

- Heat :
It is the most commonly used and the least expensive sterilizing agent, may be applied dry or moist. Moist heat can be employed in industry to kill microorganisms during boiling, tyndalization, and autoclaving.

-Filtration:
Used in industry and in the laboratory to free fluids and gases of dust and other particles and microorganisms (depth filter or a screen filter).

-Radiation:
The radiations used for sterilizing ultra violet light (In industry it is used for sterilizing the air in fermentation halls and other such large open spaces) , x-rays and gamma rays. In general, vegetative cells are much more susceptible than bacterial spores.

(II) Chemical Methods: These can be divided into two groups:

- Chemosterilants: which kill both vegetative cells as well as spores of bacteria, fungi, viruses, and protozoa.

- Disinfectants: which may not kill spores, or even some vegetative cells, but at least kill unwanted (pathogenic or spoilage) organisms.

Aspects of Sterilization in industry:

***I- Fermentor Sterilization*:**

The fermentor itself, unless sterilized, is a source of contamination. Steam is the most practical for fermentor sterilization. Sterilized either in empty condition, or when filled with nutrient solution. Reactor is heated up with the saturated vapor (between 121 to 141^{o} C) through heat transfer system (double

jacket or other internal exchangers). Ensure that all parts of reactor contact with medium are maintained at desired temperature ,for sufficient length of time. This applies particularly to ports, ducts, seals, etc., which often present danger of contamination.

II - Air Sterilization:

Air employed as carrier of O2 in aerobic processes, and any other gases which may be fed into reactor must be sterile. Air sterilization is generally carried out by filtration. If spent air from fermenter contains potentially pathological microorganisms, air must also be sterilized. Sterilization by filtration is employed to remove all particles and M.O (bacteria, spores, yeast, mould, algae) from mass flows. All filters themselves must be sterilized before they can be used to sterilize the air. Filters are also used to sterilize the effluent gases from fermentors, especially in case of pathogenic microorganisms. Sterile air filters are operated for around one year in industrial practice, and are required to withstand several sterilization runs (e.g. ~ 50 runs/y when used for penicillin fermentation).

III- Medium Sterilization:

Growth media are required for industrial fermentation, since any microbe requires: water, oxygen, an energy source, a carbon source, a nitrogen source and micronutrients for growth. Some fermentations must be supplemented with specific ***precursors***, enhances production of a secondary metabolite.
Inducers are often necessary in fermentations of genetically modified microorganisms (GMMs), generally all hydrolytic enzymes are inducible enzymes.
Inhibitor are those compounds which either stop a pathway at a specific point or redirect the metabolism toward a specific bioproduct.
Sunflower oil, olive oil to prevent foaming.

The most common means of achieving sterilization is moist heat. All forms of vegetative microbes are killed by applying heat (60^0C /10-15min.).The spores are heat resistant & majority of are destroyed only if exposed to (100^0C/10min.).In few cases, like spore of *Bacillus stearothermophilus* temperature over 120^0C is needed for their destruction. Complete sterility of culture media is achieved by subjecting the medium to121^0C/ 15min. But sterilization of oils will require a few hours, and concentrated media (10-20% solid) must be agitated for effective sterilization.

Batch Sterilization:

Media are normally sterilized in batches, in separate vessels or in fermenter .Medium is heated by means of saturated vapour at 121°C, either indirectly, through the double wall or heat exchanger inside reactor, or directly, by means of direct injection into medium. Steam-injection is faster and easier, but involves an increase in medium volume of 10-15%, due to formation of condensate. In practice, sterilization times range from 30 to 60 min at 121°C.

Particular disadvantages of batch sterilization are :
(1) High level of power consumption and
(2) High damage caused to media due to decomposition of thermobile components, which is caused primarily by long thermal sterilization periods at relatively high temperature.
Although batch sterilization process is less successful in avoiding destruction of nutrients than continuous one, objective in designing batch process is still to achieve required probability of obtaining sterility with minimum loss of nutritive quality. Highest temperature, which appears to be feasible for batch sterilization is121°C so procedure should be designed such that exposure of medium to this temp. is kept to min. This is achieved by taking into account contribution made to sterilization by heating and cooling periods of batch treatment.

Continuous sterilization

Continuous medium sterilization is particularly suitable for fermentation involving continuous feed. Two basic categories of continuous sterilization**:**

(1) injection sterilizers:

Steam is fed directly into raw medium, so that temperature rises to desired level almost immediately. Duration of sterilization is regulated by length of piping in holding stage of unit and flow rate. Sterilized medium is channeled through an expansion valve into vacuum chamber, where it is immediately cooled down.

(2) plate exchanger sterilizers:

Medium flows along steam-heated panels and is quickly brought to desired temp. (approx. 20 sec.). After retention in pipe channel, medium is cooled down to fermentation temp. by cooled panels.
Continuous sterilization is generally carried out at 141°C, whereby sterilization times of (2-3 min.) are sufficient.
An advantage over batch processes is low level of power consumption.
A negative effect of continuous sterilization is changes in viscosity (e.g. strength) which are caused by high temp. level. Short duration of sterilization

involved in continuous process is generally insufficient for sterilizing media containing solid matter. Batch process is recommended for sterilizing such media.

Sterile Filtration:

Filtration process does not destroy but removes the microorganisms.

It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material.In filtration processes that operated rapidly and continuously there is little risk of accidental germ passage through membrane (pore width of 0.2 *M).*This risk increases, however, with increasing contact time and particularly in intermittent processes.

Component parts of a fermentor

Formulation of media to be used in culturing the organism during development of inoculum and in the production fermentor

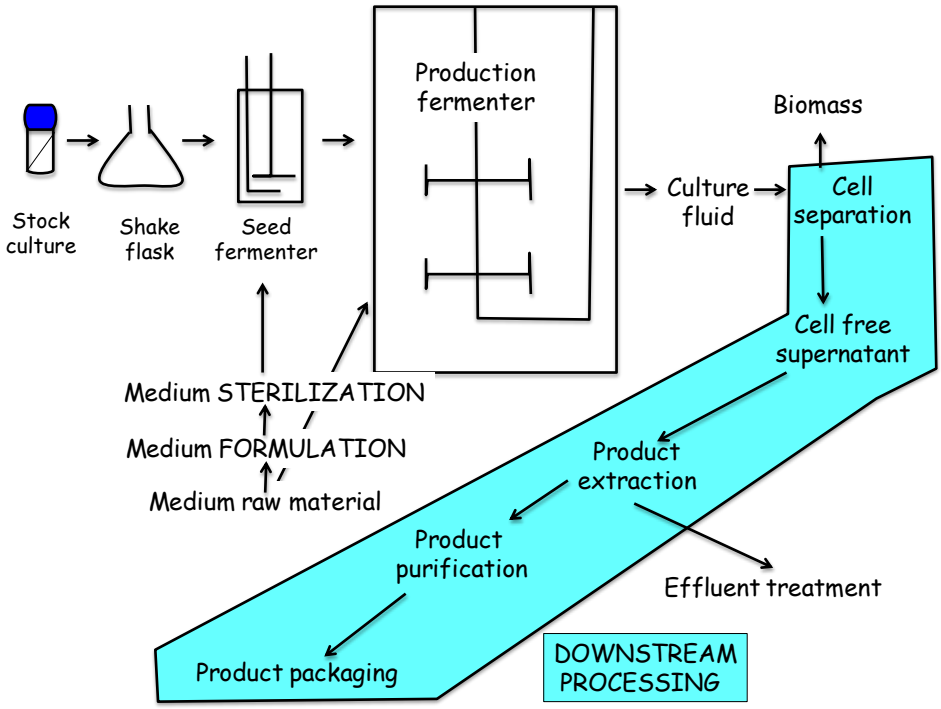
Sterilization of the medium, fermenter and ancillary equipment

Production of an active, pure culture in sufficient quantity to inoculate the production vessel

The growth of the organism in the production fermentor under optimum conditions for product formation

The extraction of the product and its purification

Disposal of effluents produced by the process



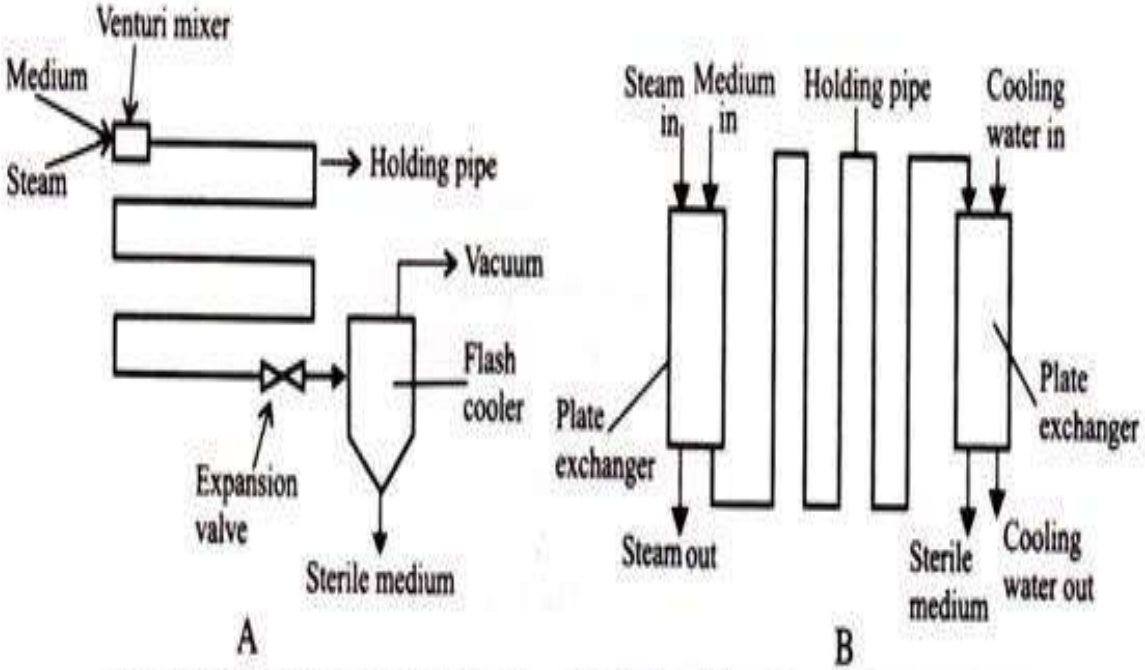


Fig. 4.10 Continuous sterilization : A – with flash cooling; B – with heat exchanger