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STERILIZATION TECHNIQUES IN FERMENTATION PROCESSES

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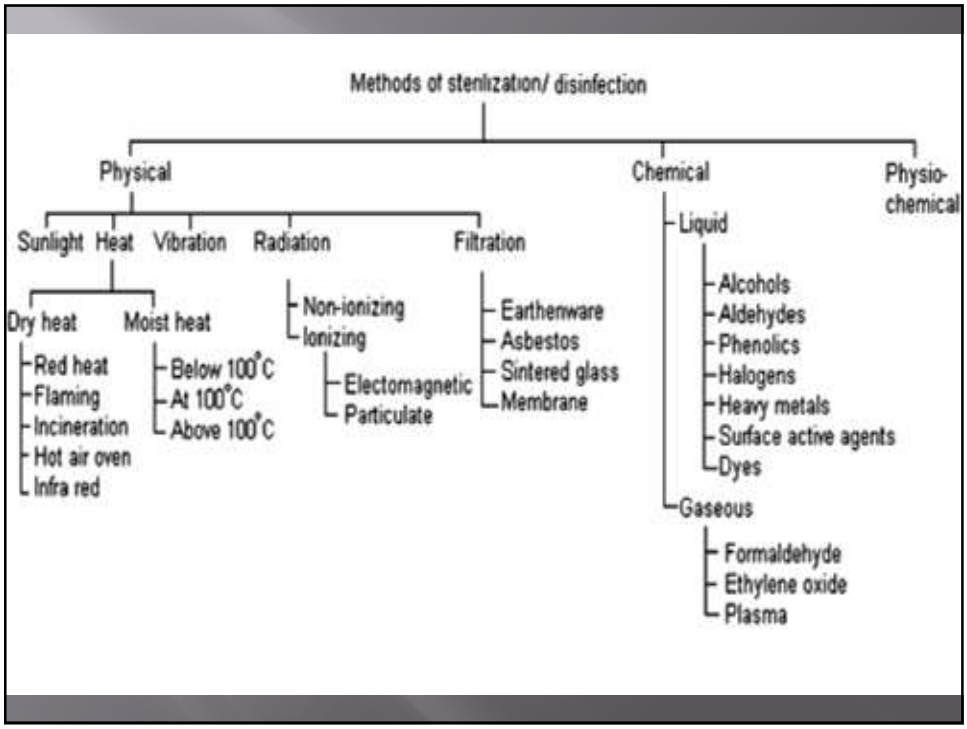
Learning Outcome

- Learner will be able to define sterilization.
- Learner will be able to classify different methods of sterilization.
- Learner will be able to understand each method in detail.

Sterilization

· Sterilization refers to the killing or elimination of all microorganisms, including highly resistant bacterial spores.
· Sterilization is an absolute term, i.e. the objects must be sterile which means the absence of all microorganisms in that object.
· Sterilization can be referred to any process that removes all forms of life, including transmissible agents (such as bacteria, fungi, viruses, spore forms, etc.) present on a surface, in a fluid, drugs or biological culture media.
· Sterilization can be achieved by applying heat, chemicals, irradiation, high pressure, and filtration or combinations.
There are three methods of sterilization:
1.Physical Sterilization (by heat, filtration or radiation)
2.Chemical Sterilization
3. Physiochemical Sterilization



Physical Sterilization

A. Heat- The most effective and a rapid method of sterilization and disinfection is Heat. Excessive heat acts by thickening or clotting of cell proteins. Less heat interferes metabolic reactions. The two most common methods for sterilization used in the laboratory are:(a) sterilization by hot air in a hot air oven, (b) sterilization by autoclaving.

Types of Heat:

i. Sterilization by Moist Heat
ii. Sterilization by Dry Heat
iii.Sterilization by Hot Air

B. Filtration- Sterilization by filtration is done mainly for thermolabile solutions. These may be sterilized by passage through sterile bacteria-retaining filters, for e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. Filters containing Asbestos should not be used. Normally, the membranes of not greater than 0.22 µm having, nominal pore size is used.

C. Radiation-

Types of Radiation:

i. ionizing and
ii. non-ionizing.

Sterilization by Moist Heat

Moist heat sterilization describes sterilization techniques that utilize saturated steam.Moist heat can denature and coagulate the protein. It can cause breakage of DNA strands and loss of functional integrity of cell membrane.

Methods include:

• **Boiling Water:** Boiling at 100°C is done in a water bath for 30 minutes. By this method Syringes, rubber goods and surgical instruments can be sterilized. The method is effective against all bacteria and some spores.

• **Steaming under pressure:** Steam is more effective than dry heat at the same temperature as: (a) Bacteria are more susceptible to steam, (b) Steam has great penetrating power, and (c) Steam has more sterilizing power as more heat is given up during condensation.eg Autoclaving

• **Fractional Sterilization** works at 100°C under normal atmospheric pressure i.e. without extra pressure. It is suitable for sterilizing the objects which may be damaged at higher than 100°C temperature. Eg.Tyndallization

Sterilization by Dry Heat

- Dry heat sterilization is done at 160°C is applied by holding the temperature for one hour, which is required to kill the most resistant spores. The articles remain dry.
- **Mechanisms include**

1) Protein denaturation,
2) Oxidative damage,
3) Toxic effect of elevated electrolyte (in absence of water).

Methods include:

1. **Red Heat:** Wire loops that are used in microbiology laboratory are sterilized by heating to 'red' in bunsen burner or spirit lamp flame. Temperature is above 100°C so it leads to sterilization.
2. **Flaming:** The object is passed through flame without allowing it to become red hot, e.g. scalpel. Temperature is not high to cause sterilization.
3. **Sterilization by Hot Air:** Hot Air Oven (Sterilizer) It is the most common method used for sterilization. The substances such as Glasswares, swab sticks, all-glass syringes, powder and oily substances are sterilized in a hot air oven. For sterilization, a temperature of 160°C is maintained (holding) for one hour. Spores are killed or removed at this temperature which leads to sterilization.

Filtration

- Sterilization by filtration is done mainly for thermolabile solutions.
- These may be sterilized by passage through sterile bacteria-retaining filters, for e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these.
- Normally, the membranes of not greater than 0.22 μm having, nominal pore size is used.

Different types of filters

1.Earthenware filters
2. Asbestos filters
3. Sintered glass filters
4. Membrane filters

Radiation

1. **Non-ionizing rays:** Rays having the wavelength longer than the visible light are non-ionizing. A high-pressure mercury vapor lamp is used to generate UV rays. UV rays induce the formation of thymine-thymine dimers and eventually inhibit the replication of DNA. UV radiation induces mutations in cells of bacteria, viruses, yeast, etc. When exposed to the effective UV radiation is inactivated within seconds.
2. **Ionizing rays:**

- Ionizing rays are high-energy rays which have good penetrative power.
- It is termed as "cold sterilization", as the radiation does not generate heat.
- There are two types of ionizing rays; particulate and electromagnetic rays.
- Electron beams are particulate in nature while gamma rays are electromagnetic in nature.

References and Further Readings

- Sterilization Techniques used in Fermentation Processes. August 2018 DOI:10.1002/9781119460381.ch3; In book: Principles and Applications of Fermentation Technology. Shivani Sharma ,Arindam Kuila,Vinay Sharma
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