STERILIZATION - PHYSICAL METHOD

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Sterilization

- Sterilization describes a process that destroys or eliminates all forms of microbial life by physical or chemical methods.

- Sterilization is distinct from disinfection, sanitization, and pasteurization, in that those methods reduce rather than eliminate all forms of life and biological agents present.

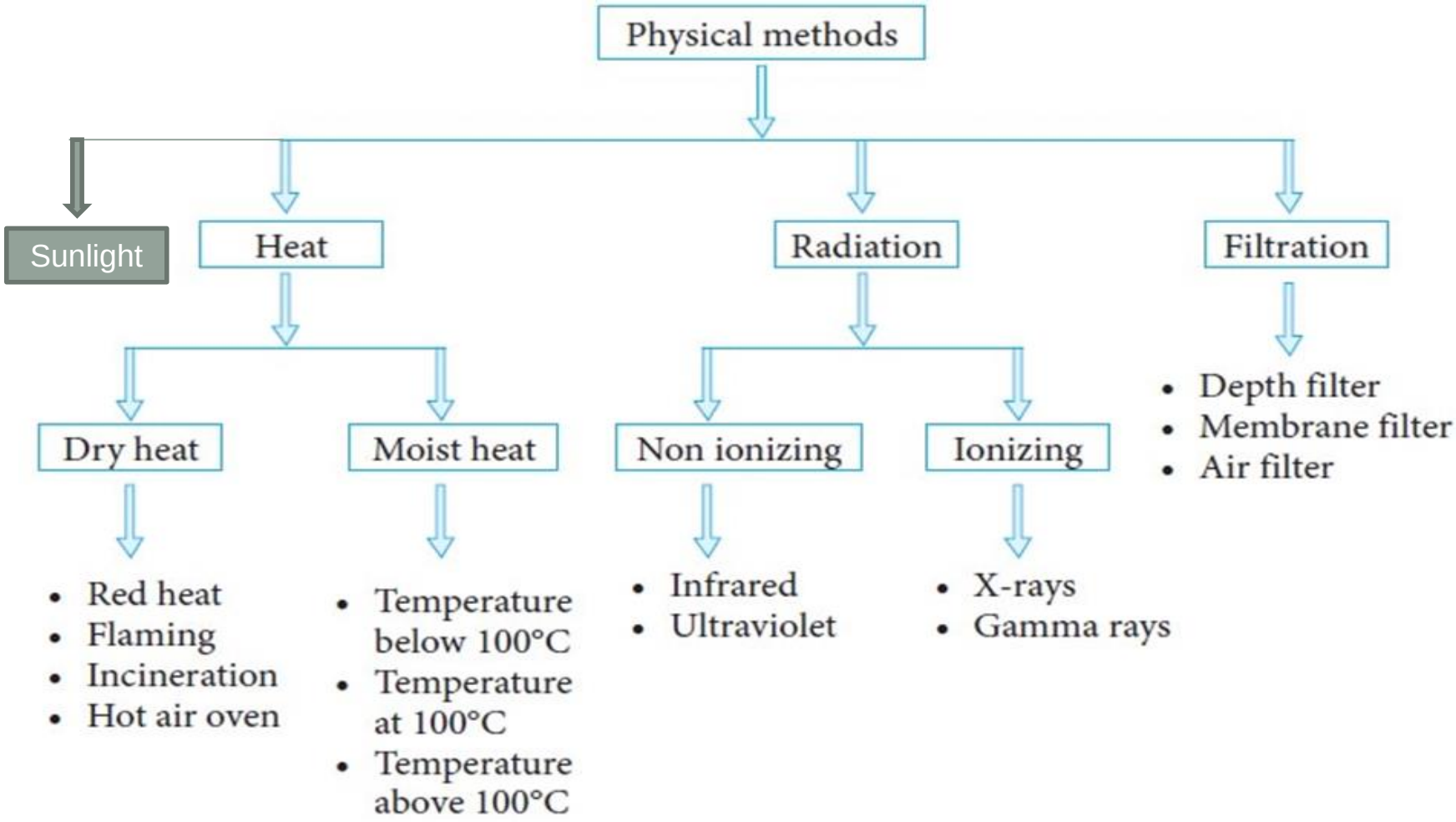
- After sterilization, an object is referred to as being sterile or aseptic.

Methods of sterilization:

Physical methods

Chemical methods

Physical Methods of sterilization



1. Sun light

- Sunlight plays an important role in the spontaneous sterilization that occurs under normal conditions.
- It possess appreciable germicidal activity.
- The action is mainly due to UV rays.

2. Heat:

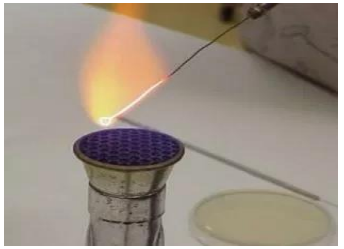
- The application of heat is simple, cheap and effective method of killing microbes.
- The time required for sterilization by heat is inversely proportional to the temperature of exposure.
- Generally, heat is of two types, they are dry heat and moist heat.

A. Dry heat:

- Dry heat kills microbes by oxidation of essential cell constituents.
- It is less effective than moist heat.

i. Red heat:

Sterilization is done by holding materials in a bunsen burner flame until they become red hot. It is used to sterilize inoculation, straight wires, wire loops, tips of forceps and spatulas.



ii. Flaming:

This is a method of passing article over a flame, but not heating it to redness. It is used to sterilize mouth of test tubes & flasks, used scalpels, glass slides & cover slips.

iii. Inceneration:

- Incineration is the process of sterilization along with a significant reduction in the volume of the wastes.
- It is usually conducted during the final disposal of the hospital or other residues.
- The scraps are heated till they become ash which is then disposed of later.
- This process is conducted in a device called incinerator.

iv. Hot air oven:

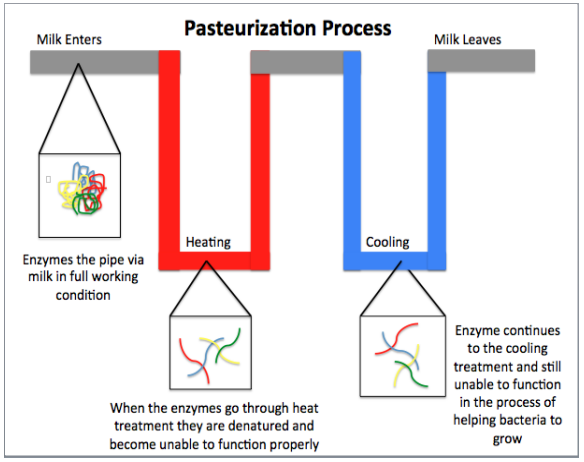
- It sterilizes the objects that cannot be sterilized by moist heat.
- It uses the principle of conduction in which the heat is first absorbed by the outer surface and is then passed into the inner layer.
- A hot air oven consists of an insulated chamber that contains a fan, thermocouples, temperature sensor, shelves and door locking controls.
- The commonly-used temperatures and time that hot air ovens need to sterilize materials are 170°C for 30 minutes, 160°C for 60 minutes, and 150°C for 150 minutes.
- These ovens have applications in the sterilization of glassware, Petri plates, and even powder samples.

B. Moist heat:

- Moist heat sterilization is one of the most effective methods of sterilization where the steam under pressure acts as a bactericidal agent.
- In this method, the microorganisms are killed by coagulating their proteins, and this method is much more effective than dry heat sterilization where microbes are killed through oxidation.
- It is used for heat sensitive materials and materials through which steam is permeable.
- Moist heat can be used in sterilization at different temperatures:
  - **Temperature below 100°C**
  - **Temperature at 100°C**
  - **Temperature above 100°C**

i. Temperature below 100°C

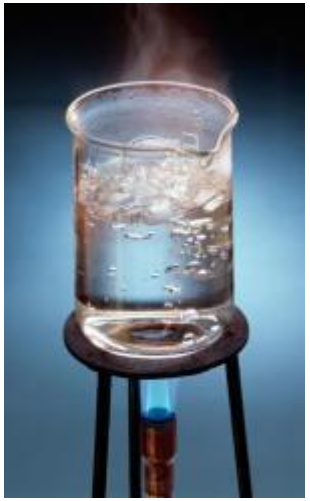
- The sterilization technique employed at a temperature below 100°C involves pasteurization.
- In this process, all non-spore forming microbes are killed in milk by subjecting the milk to a temperature of 63°C for 30 minutes (the holder method) or 73°C for 20 seconds (the flash method).
- In pasteurization, however, not all the pathogenic organisms are killed. The principle of pasteurization is the logarithmic reduction in the number of viable microbes so that they can no longer cause diseases.
- All mesophilic non-sporing bacteria can be killed by exposure to a moist heat at 60C for half an hour with the exception of some organisms which require different temperature-time cycles.
- The milk is not heated above its boiling point as the milk might curdle, and its nutritional value might be destroyed.
- Besides milk, other fluids and equipment like vaccines of non-sporing bacteria are also pasteurized at 60°C for 1 hour



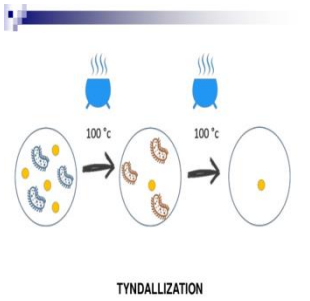
ii. Temperature at 100°C

a. Boiling:

- Boiling at 100°C is a moist heat sterilization technique that doesn't ensure complete sterility, but is enough for the removal of pathogenic vegetative microbes and some spores.
- In this case, the items to be sterilized are immersed in boiling distilled water for 30-40 minutes.
- **Boiling** is a very simple method of water **disinfection**. Heating water to a high temperature, 100°C, kills most of the pathogenic organisms, particularly viruses and bacteria causing waterborne diseases. In order for **boiling** to be most effective, the water must **boil** for at least 20 minutes.



- b. Tyndalization:
- Tyndallization is a method that is used for sterilization of media with sugar and gelatin at 100°C for 30 minutes on three successive days so as to preserve sugar which might be decomposed at a higher temperature.



iii. Temperature above 100°C

- Moist heat sterilization above 100°C involves sterilization by steam under pressure.
- When pressure is increased in a closed vessel the temperature increases proportionately i.e. for about 15 pounds of pressure per square inch (Psi) the rise to 121°C.
- This pressure and temperature is kept constant for 20 minutes during autoclaving.
- It is sufficient to kill all the vegetative forms and spores of the organisms.

3. Radiation:

- It is the process of exposing surfaces and objects to different kinds of radiation for sterilization.
- Mainly electromagnetic radiation is used for sterilization.
- The major target for these radiations is considered to be microbial DNA, where damage occurs as a result of ionization and free radical production (gamma-rays and electrons) or excitation (UV light).
- i. Non ionization:
- Ultraviolet radiation includes light rays from 150-3900 Å, of which 2600 Å has the highest bactericidal effect.
- Non-ionizing waves have a very little penetration power, so microorganisms only on the surface are killed.
- It is, however, applied in the sterilization of air, for the surface sterilization of aseptic work areas, and the treatment of manufacturing-grade water.

ii. Ionizing radiation:

- X-ray and gamma rays are the commonly used ionizing radiation for sterilization.
- These are high energy radiation which causes ionization of various substances along with water.
- The ionization results in the formation of a large number of toxic O_2 metabolites like hydroxyl radical, superoxide ion, and H_2O_2 through ionization of water.
- These metabolites are highly oxidizing agents and kill microorganisms by oxidizing various cellular components.
- It is generally exposed to items in the dried state which include surgical instruments, plastic syringes, and dry pharmaceutical products.

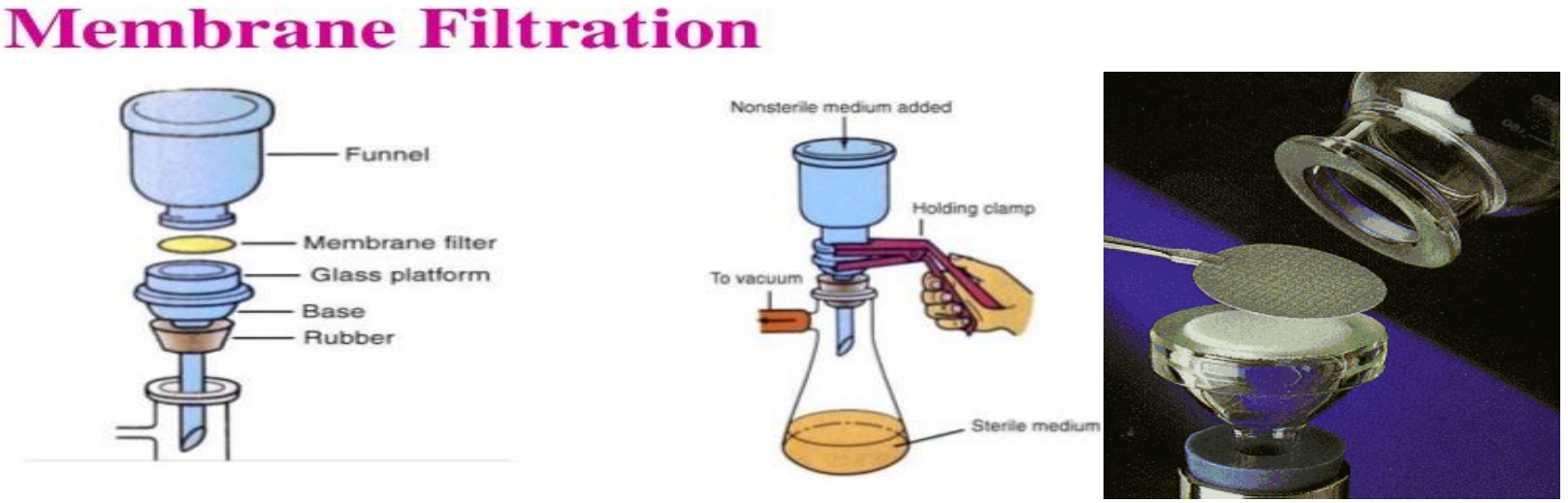
4. Filtration:

- Filtration sterilization used for heat sensitive materials to sterilize
- This process does not destroy but removes the microorganisms.
- It allows for the exclusion of organisms based upon size.

A. Depth filter:

- Depth filters are the oldest type and consist of overlapping layers of fibrous sheets of paper, asbestos or glass fibers.
- Retention of particles and microorganisms mainly in the depth of the filter matrix by mechanical retention and adsorption.

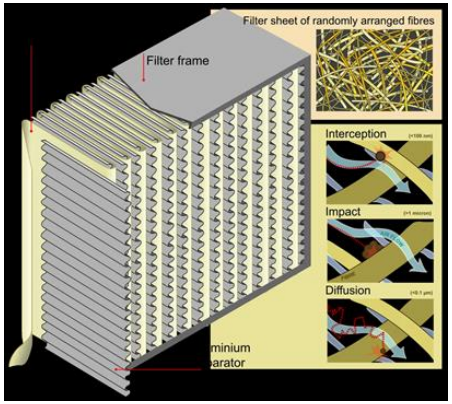
B. Membrane filters:



- They are made up of cellulose derivative (acetate or nitrate). They are very fine and are fixed in some suitable holders.
- Nominal pore size is 0.22 ± 0.02 mm or less is required.
- They are suitable for sterilizing aqueous and oily solutions but not for organic solvents such as alcohol, chloroform etc.
- Membrane filters are generally blocked by dirt particles and organisms, pre-filtration reduces the risk of membrane filter.

C. Air filter:

HEPA filter



- HEPA is an acronym for “High Efficiency Particulate Air”.
- It is constructed of borosilicate microfibers in the form of pleated sheet.
- Sheet is pleated to increase the over all filtration surface area.
- The pleats are separated by serrated aluminum baffles, which direct airflow through the filter.
- This type of air filter can remove 99.97% of dust, pollen, mold, bacteria and any airborne particles with a size of 0.3 micrometers.

Thank you