

## COURSE- M.SC. BOTANY PART -II PAPER- XVI

### TOPIC- METHODS OF STERILIZATION

#### (BIOTECHNOLOGY & BIOINFORMATICS)

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Sterilization is the complete removal of microorganisms from an object or surfaces.

Sterilization is obtained when microorganisms are subjected to antimicrobial agents for sufficient time and at optimum conditions.

Sterilization is a process of eradicating live microorganisms from substances. It is done to preserve things for a long time and kill germs. If something is not sterilized, it may cause infection to those who use it. Therefore, it should not be taken for granted. There are several methods of sterilization, including:

#### A] Physical methods of sterilization:

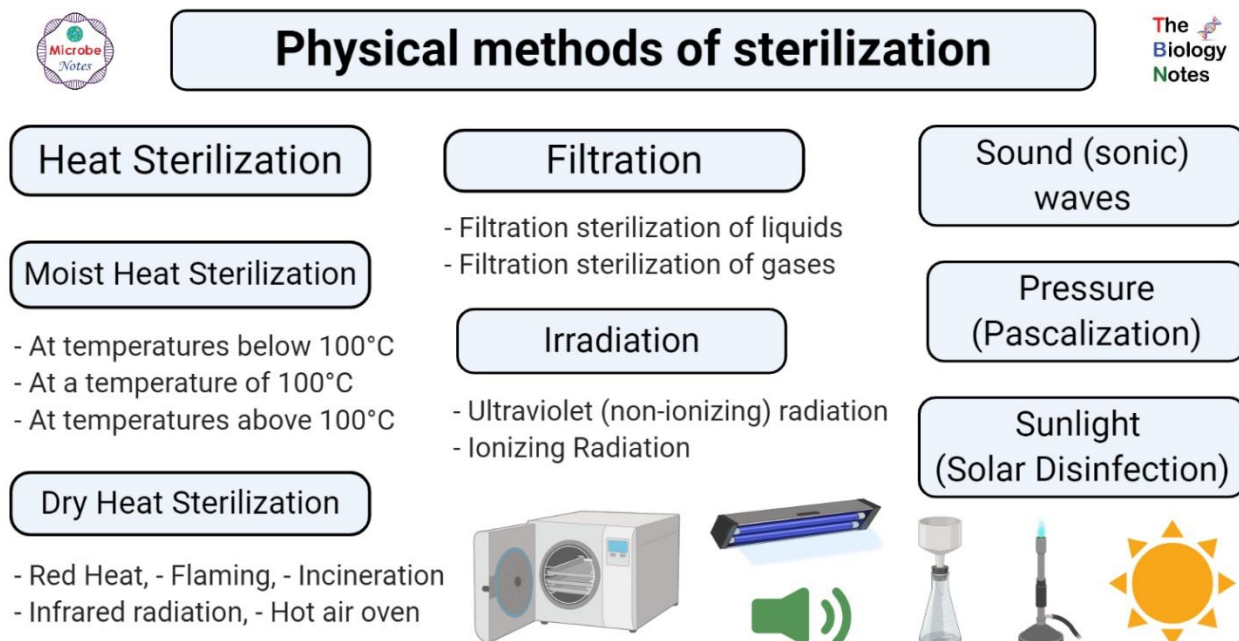


Figure: Physical methods of sterilization.

Some physical methods associated with sterilization are explained below:

## 1) Heat Sterilization:

Heat sterilization is the most effective and widely used method of sterilization, where the bactericidal activity results through the destruction of enzymes and other essential cell constituents.

This method of sterilization is applicable to thermostable products. Still, it can be applied to both moisture-sensitive and moisture-resistant products, for which dry (160–180°C) and moist (121–134°C) heat sterilization procedures are respectively used.

### i.) Moist Heat Sterilization:

An **autoclave** is a device that works on the principle of moist heat sterilization through the generation of steam under pressure.

Moist heat sterilization is one of the most effective methods of sterilization where the steam under pressure (at Temp. in the range 121–134°C) acts as a bactericidal agent. High pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.

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.

In the pharmaceutical and medical sectors, it is used in the sterilization of dressings, sheets, surgical and diagnostic equipment, containers, and aqueous injections, ophthalmic preparations, and irrigation fluids, in addition to the processing of soiled and contaminated items.

Moist heat can be used in sterilization at different temperatures:

#### **At temperatures below 100°C**

The sterilization technique employed at a temperature below 100°C involves **pasteurization**.

In this process, all mesophilic 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).

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 in special water baths. Similarly, serum and body fluids with congealable proteins are also sterilized at 56°C for 1 hour in water baths.

### **At a temperature of 100°C**

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.

**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.

Moist heat at 100°C is applicable for contaminated dishes, beddings, pipettes, and other instruments that are not soiled or contaminated as well as for objects that are temperature sensitive.

### **At temperatures above 100°C**

Moist heat sterilization above 100°C involves sterilization by steam under pressure.

This principle is employed in an **autoclave** where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg. As a result, the steam under pressure has a higher penetrating power. When this steam comes in contact on the surface, it kills the microbes by giving off latent heat.

**Autoclaves** are used for the sterilization of contaminated instruments along with different culture media as it ensures complete sterility.

## **ii.) Dry heat sterilization**

Dry sterilization is the process of removing microorganisms by applying moisture-free heat which is appropriate for moisture-sensitive substances.

Dry moisture-less heat destroys microorganisms by causing denaturation of proteins and also lyses the proteins in many organisms, causes oxidative free radical damage, causes drying of cells, and can even burn them to ashes, as in incineration

Dry heat sterilization is used for the sterilization of materials which are difficult to sterilize by moist heat sterilization for several reasons.

Substances like oil, powder, and related products cannot be sterilized by moist heat because moisture cannot penetrate into deeper parts of oily materials, and powders are destroyed by moisture.

Similarly, laboratory equipment like Petri dishes and pipettes are challenging to sterilize by moist heat due to the penetration problem.

Thus, in dry heat sterilization usually higher temperatures in the range 160–180°C are employed and also require exposure times of up to 2 hours depending upon the temperature employed.

This principle is used in instruments like **hot air oven** and **incineration**, which generates very hot moisture-free air.

The primary industrial application of dry heat sterilization is in the sterilization of glass bottles which are to be filled aseptically.

For the purposes of **depyrogenation** (destruction of bacterial endotoxins, the products of **Gram-negative bacteria also called pyrogens**, which cause fever when injected into the body) of glass, temperatures of approximately 250°C are used.

There are different types of dry heat sterilization which are explained below:

#### a) Red Heat

Red heat sterilization is the process of instant sterilization by holding the instruments in a Bunsen flame till they become red hot.

This method is based on dry heat sterilization is commonly used for sterilization of instruments like **incubation loops, wires, and points of forceps**.

#### b) Flaming

Flaming is a type of dry sterilization that involves exposure of metallic objects to flame for some time where the flame burns microbes and other dust presents in the instrument.

In the case of flaming, the instrument is dipped in alcohol or spirit before burning it in a gas flame.

#### c) Incineration

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**.

#### **d) Infrared radiation**

Infrared radiation (IR) is a method of thermal sterilization in which the radiation is absorbed and then converted into heat energy.

During this process, the instruments will be exposed to the radiation, which will result in a temperature of about 180°C for about 17 minutes.

IR is applicable for mass sterilization of packaged items like syringes and catheters.

#### **e) Hot air oven**

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.

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.

## **2) Filtration:**

The process of filtration is unique among sterilization techniques in that it **removes, rather than destroys, microorganisms**.

Further, it is capable of preventing the passage of both viable and nonviable particles and can thus be used for both the clarification and **sterilization of liquids and gases**.

The primary mechanisms involved in filtration are **sieving, adsorption, and trapping** within the matrix of the filter material.

Filtration uses membranous filters that have tiny pores that let the liquid pass through but prevent bigger particles such as bacteria from passing through the filter.

Therefore, the smaller the pore, the more likely the filter is to stop more things from going through it. Certain types of filter (**membrane filters**) also have an essential role in sterility testing, where they can be employed to trap and concentrate contaminating organisms from solutions under test.

The principal application of sterilizing-grade filters is the treatment of heat-sensitive injections and ophthalmic solutions, biological products, air, and other gases for supply to aseptic areas.

They may also be required in industrial applications where they become part of venting systems on fermenters, centrifuges, autoclaves, and freeze dryers.

### **Filtration sterilization of liquids:**

**Membrane filters**, in the form of discs, can be assembled into pressure-operated filter holders for syringe mounting and in-line use or vacuum filtration tower devices for filtration of liquid.

Membrane filters are often used in combination with a coarse-grade fiberglass depth prefilter to improve their dirt-handling capacity.

### **Filtration sterilization of gases:**

Filters employed for this generally consist of pleated sheets of glass microfibres separated and supported by corrugated sheets of Kraft paper or aluminum which are employed in ducts, wall or ceiling panels, or laminar air flow cabinets.

These **high-efficiency particulate air (HEPA) filters** can remove up to 99.997% of particles  $>0.3\mu\text{m}$  in diameter and thus are acting as depth filters.

In practice, their microorganism removal efficiency is rather better as the majority of bacteria are found associated with dust particles.

Other applications of filters include sterilization of venting or displacement air in tissue and microbiological culture (carbon filters and hydrophobic membrane filters); decontamination of air in mechanical ventilators (glass fiber filters); treatment of exhausting air from microbiological safety cabinets (HEPA filters); and the clarification and sterilization of medical gases (glass wool depth filters and hydrophobic membrane filters).

## **3) Irradiation:**

Irradiation 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).

### **Ultraviolet (non-ionizing) radiation**

Ultraviolet radiation includes light rays from  $150\text{-}3900\text{ \AA}$ , of which  $2600\text{ \AA}$  has the highest bactericidal effect. Upon exposure, these waves are absorbed by many materials, particularly nucleic acids.

The waves, as a result, cause the formation of pyrimidine dimers which bring error in DNA replication and cause the death of microbes by mutation.

It is, however, applied in the sterilization of air, for the surface sterilization of aseptic work areas, and the treatment of manufacturing-grade water.

### **Ionizing Radiation**

X-ray and gamma rays are the commonly used ionizing radiation for sterilization.

The ionization results in the formation of a large number of toxic O<sub>2</sub> metabolites like hydroxyl radical, superoxide ion, and H<sub>2</sub>O<sub>2</sub> through ionization of water.

These metabolites are highly oxidizing agents and kill microorganisms by oxidizing various cellular components.

Radiation sterilization is generally exposed to items in the dried state which include surgical instruments, sutures, prostheses, unit-dose ointments, plastic syringes, and dry pharmaceutical products.

### **4) Sound (sonic) Waves Vibration:**

Sonic waves can be used as bactericidal agents which employ ultrasound (usually from 20–40 kHz) to vibrate a fluid.

As the sound waves propagate through a detergent solution, these ultrasonic waves produce alternating tensile and compressive forces that oscillate, and these oscillating forces cause millions of microscopically-sized cavities to form in the detergent solution.

Once they reach a maximum size, these cavities violently collapse, causing submicroscopic voids to form which induce the formation of high-energy hydraulic shock waves.

These shock waves, Temp. as high as 10,000°F and hydrodynamic pressures as low as 10,000 PSI, physically loosen and remove microorganisms and other adhering debris from even the most inaccessible surfaces of a contaminated instrument.

These sonic waves are found to be effective against bacteria spores depending on the chemical composition of these spores. This method is routinely used by healthcare facilities to clean surgical and dental instruments before the terminal sterilization.

## 5) Pressure (Pascalization):

**Pascalization or High-Pressure Processing (HPP)** is a method employed for preservation and sterilization of food, in which products are processed under very high pressure (hundreds of megapascal), leading to the death of and inactivation of enzymes in the food.

HPP treatments may be applied at room temperature, and with the exception of some vegetables, shape, color, and nutrients of most foods are not affected.

Hydrostatic pressures are nonthermal, and covalent bonds are not broken, so that flavor is unaffected. At 400-600 MPa, proteins are easily denatured, and cell morphology is altered, and ribosomes are destroyed.

Changes occur in the lipid-protein complex of cell membranes, and increased membrane fluidity is also observed, which causes leakage of nucleic acids.

Pascalization is especially useful on acidic foods, such as yogurts and fruits, because spores which are pressure-tolerant don't have the ability to live in environments with low pH. The treatment works equally well for both solid and liquid products.

## 6) Sunlight (Solar Disinfection):

Solar disinfection is a process used for the removal of microorganisms with the help of sunlight. This process is commonly used to purify or disinfect drinking water.

Solar disinfection is based on the inactivation of pathogenic organisms as a result of the UV-A (wavelength 320–400 nm) part of the sunlight, which reacts with oxygen dissolved in the water and releases highly reactive forms of oxygen (oxygen free radicals and hydrogen peroxides).

These metabolites damage pathogens, while it also interferes with metabolism and destroys bacterial cell structures, and simultaneously the full band of solar energy (from infrared to UV) heats the surface.

However, the efficacy of solar disinfection is significantly low as it requires a long period of exposure. But this process is economical and an environment-friendly option.

## B] Chemical Sterilization:

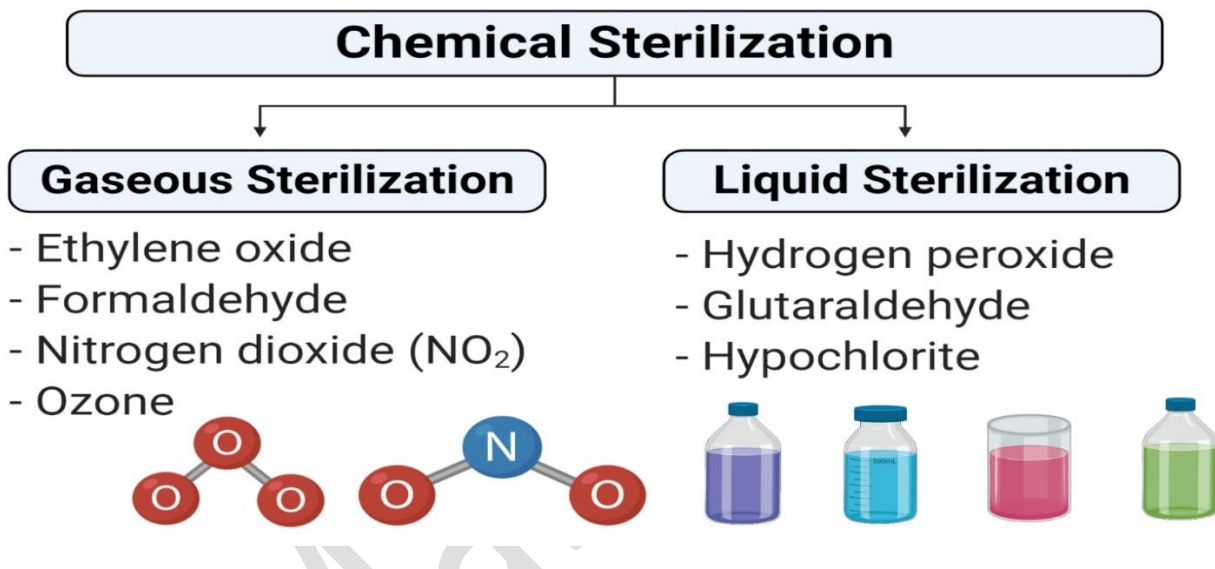
Chemical Sterilization is the process of removal of microorganisms by the use of chemical bactericidal agents.



Even if physical methods of sterilization are more appropriate for effective sterilization, it is not always appropriate to use for heat-sensitive materials like plastics, fiber optics, and biological specimens.

Under such conditions, chemical either in liquid or gaseous state can be used for sterilization. However, it is crucial to ensure that the materials undergoing sterilization are compatible with the chemical being used.

Besides, it is important to adopt safety rules in the workplace safety during the use of chemical agents. The chemical method of sterilization can be categorized as **liquid and gaseous sterilization**.



### 1) Gaseous sterilization:

Gaseous sterilization involves the process of exposing equipment or devices to different gases in a closed heated or pressurized chamber.

Gaseous sterilization is a more effective technique as gases can pass through a tiny orifice and provide more effective results.

Besides, gases are commonly used along with heat treatment which also facilitates the functioning of the gases. The mechanism of action is different for different types of gases.

Some of the common gases used for gaseous sterilization are explained below:

**Ethylene oxide (EO)** gas is a common gas used for chemical treatment applied to sterilize, pasteurize, or disinfect different types of equipment and surfaces because of its wide range of compatibility with different materials.

Ethylene oxide kills all known microorganisms, such as bacteria (including spores).

**Formaldehyde** is another important highly reactive gas which is used for sterilization.

It possesses broad-spectrum biocidal activity and has found application in the sterilization of reusable surgical instruments, specific medical, diagnostic and electrical equipment, and the surface sterilization of powders.

**Nitrogen dioxide** is a rapid and effective sterilant that can be used for the removal of common bacteria, fungi, and even spores.

**Ozone** is a highly reactive industrial gas that is commonly used to sterilize air and water and as a disinfectant for surfaces.

Ozone is a potent oxidizing property that is capable of destroying a wide range of organisms including prions, without the use of hazardous chemicals as ozone is usually generated from medical-grade oxygen.

## 2) Liquid sterilization:

Liquid sterilization is the process of sterilization which involves the submerging of equipment in the liquid sterilant to kill all viable microorganisms and their spores.

Although liquid sterilization is not as effective as gaseous sterilization, it is appropriate in conditions where a low level of contamination is present.

Different liquid chemicals used for liquid sterilization includes the following:

**Hydrogen peroxide** is a liquid chemical sterilizing agent which is a strong oxidant and can destroy a wide range of microorganisms.

It is useful in the sterilization of heat or temperature-sensitive equipment like endoscopes. In medical applications, a higher concentration (35-90%) is used.

**Glutaraldehyde** is an accepted liquid sterilizing agent which requires comparatively long immersion time. For the removal of all spores, it requires as long as 22 hours of immersion time.

**Hypochlorite solution**, which is also called liquid bleach, is another liquid chemical that can be used as a disinfectant.

Appropriate concentrations of hypochlorite can be used for the disinfection of workstations and even surfaces to clean blood spills and other liquids.

We have reached the end of the Sterilization Methods Series where we explored the different ways to kill or remove deadly bacteria, viruses, and harmful pathogens.

## How do we select the appropriate sterilization method when there are so many choices available? Our decision will depend on many different parameters, including:

The purpose of sterilization. Sterilization in the lab is different than in hospitals, which is also different than pharmaceutical sterilization. Each industry and application have their own unique requirements.

- The material that needs to be sterilized. Is the material heat sensitive? Is it sensitive to moisture, gas or radiation?
- The nature of the microorganisms that need to be destroyed or removed.
- Additional considerations are: time, safety and budget.

Sterilization Method	Advantages	Disadvantages	Common Uses
Steam	Cost effective Short cycle times	High temperature High moisture levels	Dental, Medical, Sterile Processing, Laboratory, Pharmaceutical, small clinics and more
Flaming	Fast Cost Effective	Applied on the instrument directly Applicable on metallic devices only	Small metal or glass objects in microbiology labs
Incineration	Load volume decreases by 90%	Total destruction of load High construction and installation costs	Waste processing
Dry Heat	Relatively low cost Ideal for moisture sensitive Items	Long cycle duration	Small clinics, metals, ceramics
Formaldehyde	After sterilization, most loads are available for immediate use	Long cycle duration	Endoscopes
EtO	High efficiency Large sterilizing volume	MutagenicCarcinogenic Excessively Long cycle	PVC, Rubber, Endoscopes
Filtration	Used for sterilizing gases and liquids	Does not differentiate between viable and non viable particles	Heat sensitive injections, solutions and air (venting systems)
Radiation	High penetration power	Expensive Can produce undesirable changes in irradiated products	Industrial sterilization of heat sensitive products Biomedical devices
Ozone	Uses medical grade oxygen	Long cycle time	Medical
Plasma	Does not leave chemical residues	Cannot sterilize: liquids, powders, and strong absorbers	Endoscopes Soft contact lenses Surgical Dental

This infographic provides a visual overview of each method.