Tutorial session 2: Sterilization methods

I. Introduction

In microbiology, it is essential to work under sterile conditions for two main reasons:

- To avoid contaminating our samples with other microorganisms.
- To prevent our samples from contaminating us, especially when working with pathogenic microorganisms.

Thus, the goal is to protect both the sample and ourselves from potential contamination.

II. Definition

Sterilization is a process that involves destroying (eliminating) all microorganisms (bacteria, viruses, fungi, parasites) present on an object, surface, or in a product.
A product is considered biologically sterile when it no longer contains any revivable microorganisms (non-living microorganisms or those capable of reviving from a dormant state).
Microorganisms exist in two states:

- **Vegetative state**: This is the active form in which cells reproduce and multiply.
- **Sporulating state**: This is a resistant, dormant form that develops under unfavourable nutritional conditions.

Therefore, the goal of sterilization is to eliminate both forms of microorganisms.

III. Methods of sterilization

Sterilization methods can be classified based on their mechanism of action:

- **Destruction** :
  - **Physical methods** :
    - Heat (temperature)
    - Radiation (energy from radiation)
  - **Chemical methods** :
    - Disinfectants (e.g., alcohol, bleach)
- **Elimination** by filtration (based on size)

III.1. Heat sterilization

III.1.1. Principle of operation: Heat sterilization involves heating air, leading to the destruction of microorganisms through hydrolysis and protein denaturation.

III.1.2. Sensitivity of microorganisms to heat

A. Factors related to microorganisms:

- Microbial species: Heat sensitivity varies by species.
- State of microorganisms:
  - Vegetative bacteria: Destroyed at temperatures of 52–60°C in an aqueous environment for 5–60 minutes.
  - Spores: More resistant than vegetative forms and require higher temperatures for destruction.
- Number of microorganisms.

B. Factors related to the environment:

- Nature of the environment and presence of active agents: Influences the choice of heat treatment. For example, sodium bicarbonate and sodium salicylate have bactericidal effects when heated.
- pH of the environment.

C. Factors Related to destruction methods:

- Temperature
- Time

III.1.3. Types of heat sterilization

Heat sterilization methods are the most commonly used. They are categorized into **dry heat** and **moist heat** processes.

- **Dry heat sterilization**

It destroys microorganisms by causing agitation of water molecules and the destruction/denaturation of biological molecules (e.g., DNA, RNA, proteins).

This technique is used for sterilizing materials that are relatively resistant to high temperatures or unsuitable for steam sterilization, such as oxidizable materials, empty glassware, porcelain and metal instruments.It is not suitable for rubber, liquid culture media, or plastics.

In practice, various standard combinations of temperature and time can be applied.

Table 1: Examples of Temperature/Time Combinations used for sterilization in a Pasteur oven

Temperature (°C)	121	140	160	170
Time to Sterilize (Min)	600	180	120	60

It is produced in the laboratory by:

1. **Pasteur oven** :

This is an electric hot air oven with a thermostat, using dry heat. It is used exclusively for sterilizing pre-cleaned and dried glassware or metal instruments (e.g., dissection tools) that can withstand very high temperatures.

2. **Flaming**:

Passing the surface of non-flammable small equipment through a flame (Bunsen burner) ensures perfect sterilization. This method is used to sterilize platinum wires, test tubes, and Pasteur pipettes.

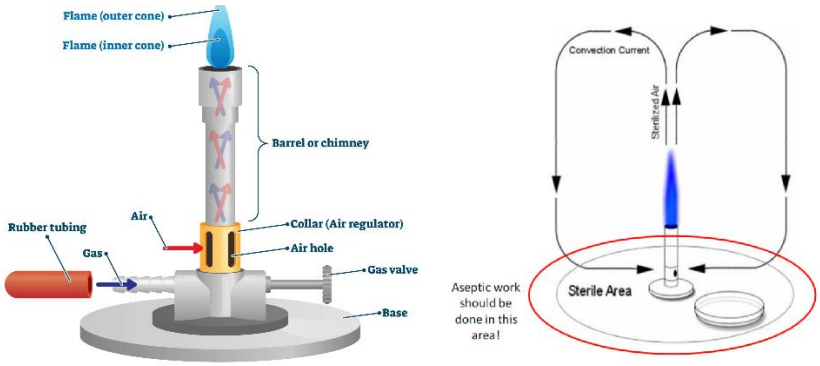

Figure 1 : The Bunsen burner

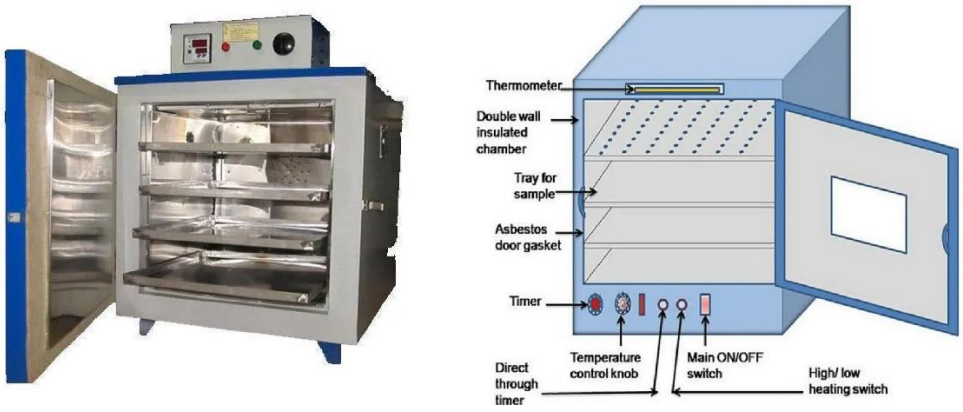

Figure 2 : The Pasteur oven

- **Sterilization by moist (wet) heat**

Moist heat sterilization involves three main methods:

a. Autoclave sterilization

Heating occurs under pressurized steam at a temperature and duration that vary depending on the medium and the volume of the containers. This process kills all vegetative cells and endospores. In practice, various standard combinations of temperature, time, and pressure can be applied.

For example, culture media, other solutions, and materials sensitive to dry heat are typically sterilized using a standard cycle of 15 to 20 minutes at 121°C in a steam-saturated atmosphere. This ensures the complete destruction of all life forms, including both vegetative and sporulated microorganisms.

Figure 3: Autoclave design diagram and parts

b. Pasteurization

Pasteurization is a heat treatment process applied to liquids. Its purpose is to destroy all non-sporulating pathogenic microorganisms and significantly reduce the vegetative flora present in a product. The temperatures used in pasteurization typically range between **75°C and 80°C**.

c. Steaming (tyndallization)

It is a process discovered by John Tyndall in 19th century for sterilizing substances to kill the spores of bacteria. The process of exposure of materials to steam at 100°C for 20 min for three consecutive days is known as tyndallization. First exposure kills all the vegetative forms and in the intervals between heating, the remaining spores germinate into vegetative forms which are killed on subsequent heating. Tyndallization is also called **fractional sterilization** or **intermittent boiling.**

III.2. Sterilization by filtration

It is a technique that involves passing a liquid or air through a filter with pore sizes ranging from 0.2 µm to 0.45 µm. Microorganisms are too large to pass through the pores and are therefore retained by the filter.

This method is particularly useful for heat-sensitive products, such as certain amino acids, vitamins, growth hormones, nucleic acids, and many antibiotics. Unlike other sterilization methods, filtration does not destroy microorganisms but instead physically retains them on the filter.

Examples of Filtration Applications:

1. Wastewater and drinking water treatment: Filtration is used to remove microorganisms from wastewater and to purify drinking water, ensuring it is safe for consumption.

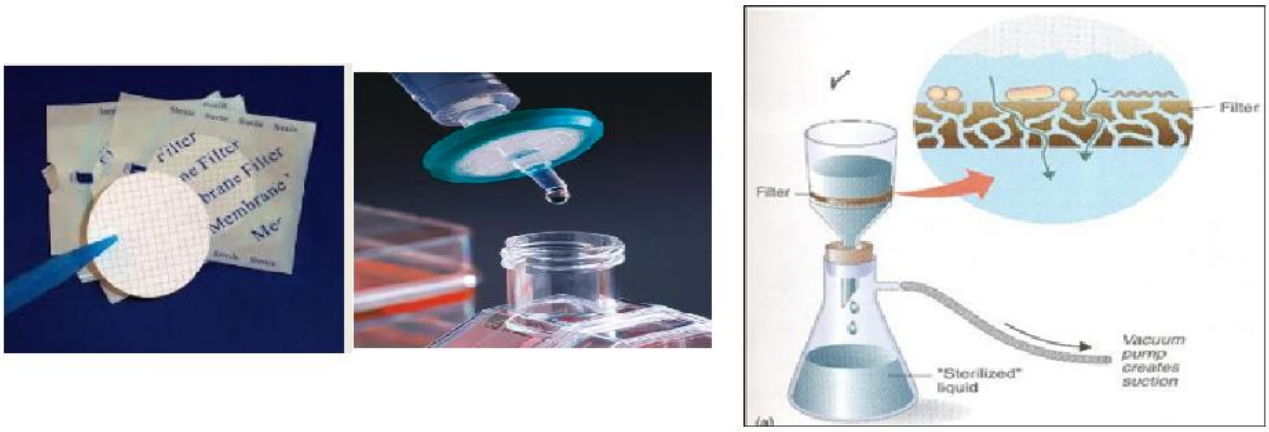

Figure 4: Liquide filtration using membrane filtration

2. In microbiology Laboratories, working under sterile conditions is essential. Air in the work area is filtered to maintain sterility. This is achieved using laminar flow hoods or microbiological safety cabinets (MSCs), which are equipped with filters that prevent the passage of microorganisms.

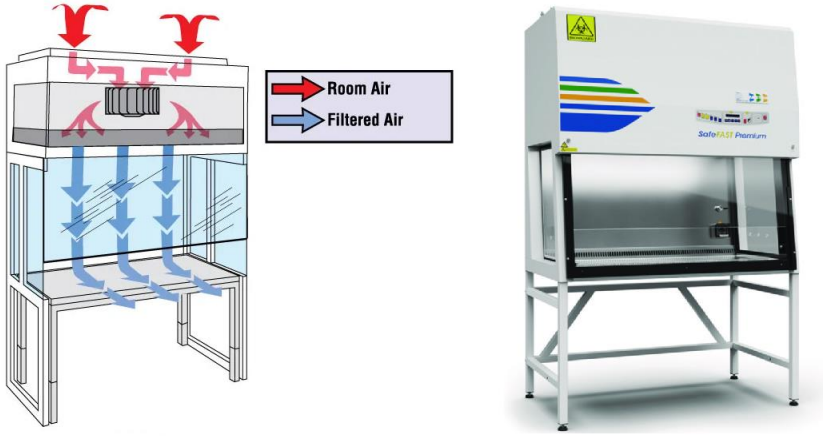

Figure 5. Air filtration using microbiological safety cabinets

III.3. Sterilization by radiation

Sterilization using ultraviolet (UV) radiation is commonly employed in laboratories for decontaminating air and work surfaces, particularly those under protective hoods. Germicidal ultraviolet radiation is a sterilization method that uses UV light at specific wavelengths to alter the DNA or RNA of microorganisms, thereby preventing their reproduction and rendering them harmless.

- Applications of UV sterilization:

- Used in virology, cell cultures, and the preparation of pharmaceutical products and culture media.
- Other forms of radiation, such as X-rays, are used for the industrial sterilization of plastic Petri dishes and the preservation of certain food products.

Figure 6: Example of a UV sterilization device

III.4. Chemical Methods of Sterilization

Chemical methods of sterilization are used in microbiology for biological specimens and plastic equipment. In this method, several chemicals work as bactericidal agents. They can be of two types: gaseous or liquid.

- **Gaseous sterilization**: The gaseous chemical agents used for sterilization include ethylene oxide, formaldehyde, nitrogen dioxide and ozone.
- **Liquid sterilization**: This method is less effective than gaseous sterilization and is used to remove low levels of contamination. Common liquid chemical agents that are used for sterilization include hydrogen peroxide, glutaraldehyde and hypochlorite solution.

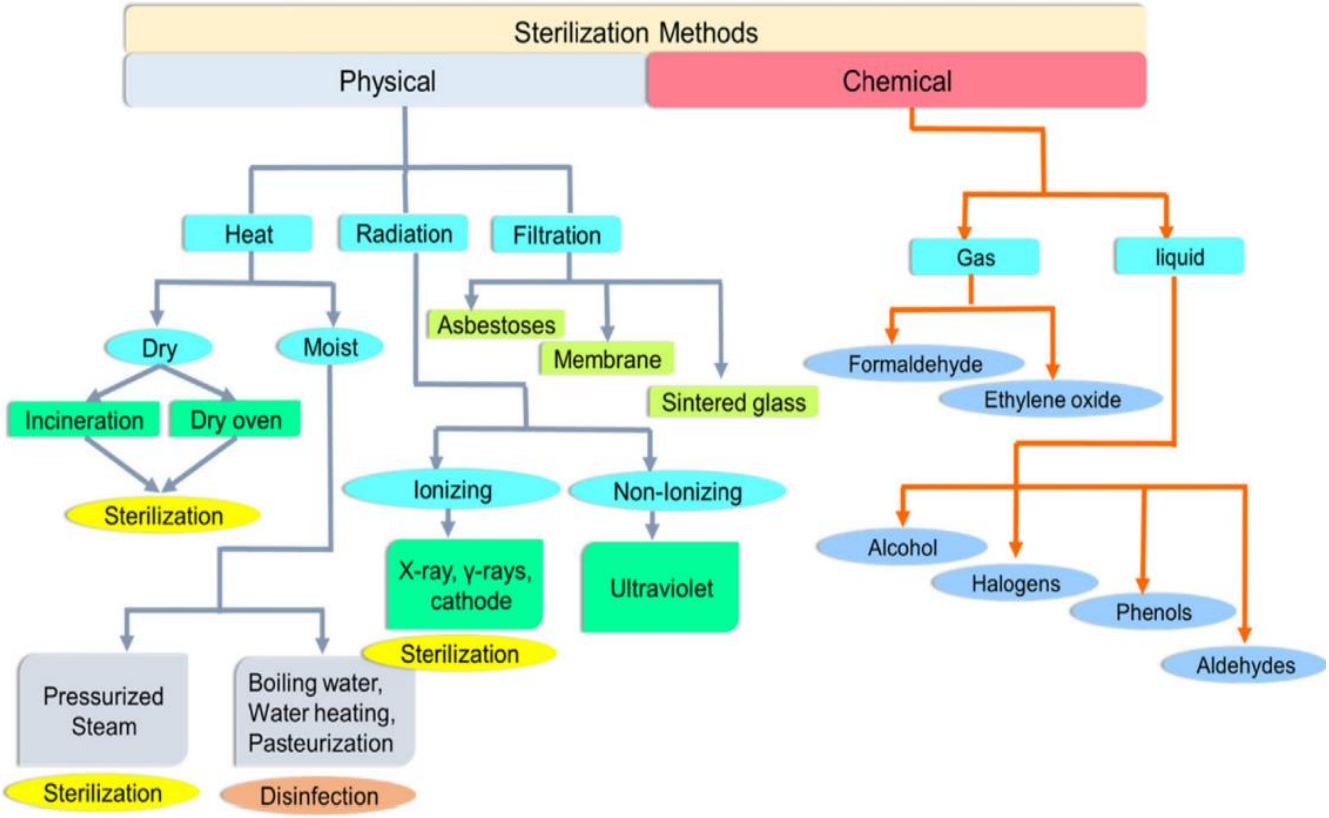

Figure 7: Methods of sterilization.