Course: MBIO 240

Laboratory Skills

Sterilization techniques
&
Microbiological culture media preparation

Aims

- To explore and understand various sterilization techniques commonly employed in microbiology.
- To learn the preparation of Nutrient Agar medium for the isolation and cultivation of bacteria.
- To learn the preparation of Potato Dextrose Agar (PDA) medium for the isolation and cultivation of fungi.

Sterilization:

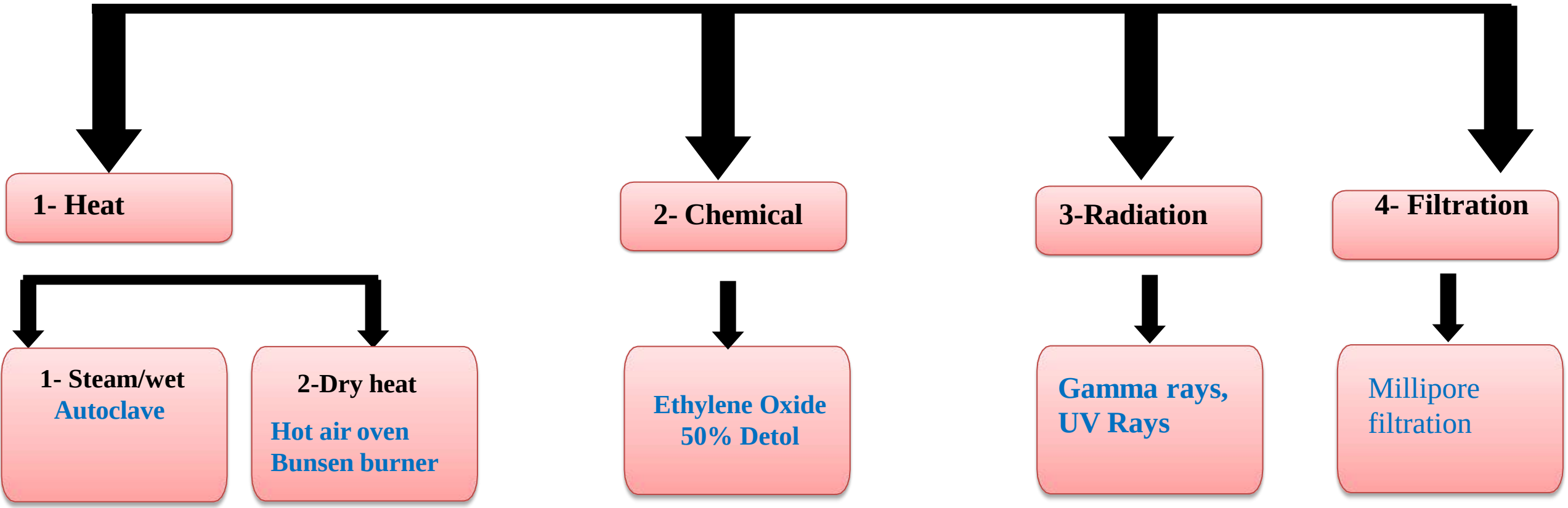
The process of eliminating all forms of microbial life, including bacteria, viruses, fungi, and their spores, from surfaces or objects.

Importance of Sterilization:

- Sterilization is essential in preventing infections, ensuring the safety of medical procedures, and maintaining the integrity of laboratory experiments.

- **In microbiology labs sterilization is a critical step in preparing culture media, and other materials that must be free from contamination.**

Sterilization methods



1. Heat Sterilization:

A. Moist Heat Sterilization (Autoclaving):

Autoclave

- The most useful approach is **autoclaving,** in which items are sterilized by exposure to steam at **121°C and 15 lbs** of pressure for **15-20 minutes** or longer, depending on the nature of the item.
- These conditions are adequate to **quickly and effectively kill all microbial forms even the hardiest spore formers**.
- **Moist heat is more effective than dry heat.**
- **Uses:** laboratory glassware, media, surgical instrument, aqueous solutions ..etc

PROCEDURE FOR AUTOCLAVING

1. Load the autoclave with the freshly prepared culture media.
1. Close and lock the autoclave door.
1. Set the autoclave time for 15 minutes or longer.
1. Make certain that the autoclave temperature is set to 121°C.
1. Start the autoclave by pushing the start button or twisting the knob to the start position.
1. When the period of sterilization is completed and the pressure in the chamber reads 0.
1. Carefully open the door and remove the containers, using heat-proof gloves.

<u>The following instructions must be considered when use the autoclave:</u>

- Always use **distilled water**.
- All bottles and containers must be heat-proof.
- Never fill flasks/tubes completely with the liquids.
- Never tightly cap the bottles and tubes.
- **Never** open the autoclave door until the **pressure falls to zero**.

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B. Dry Heat Sterilization:

Direct Flaming (Bunsen burner)

Another method of **dry sterilization** that involves exposing metallic instruments to a flame to kill microorganisms.

Uses:

- sterilization of inoculating loop and needled.
- mouth of test tubes, flasks are also sterilized by passing through the Bunsen burner flame for a few seconds

<u>To sterilize a glass spreader, and forceps follow these steps:</u>

1. Dip the glass spreader or the tip of the forceps in **70% ethanol**.
2. Ignite the residual ethanol by briefly inserting it into a Bunsen burner flame.
3. Allow the spreader to cool after the ethanol has burnt.

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Chemicals disinfectants:

- There are many chemicals that have a broad spectrum of antimicrobial activity and are fast acting.
- Examples: Formalin, phenols, ethanol are extensively used in microbiological labs for a variety of purposes:
- Such as swabbing a bench before and after use, for the sterilization of surfaces
- When using disinfectants, it is important to ensure that **they are used at the correct concentration** and that **they are left to work for the correct length of time.**

Microbial culture medium

- **A microbial culture medium is a mixture of substances that supports the growth of microorganisms by providing necessary nutrients, energy sources, growth-promoting factors, minerals, and buffer salts.**

Three Physical Forms Of Media Are Used

1. liquid, or broth media (No agar)

2. semisolid media

(0.3–0.5%)

3. solid media

(1.5–2.0% agar)

The major difference among these media is that solid and semisolid media contain a solidifying agent (usually agar), whereas a liquid medium does not.

LIQUID MEDIA (BROTH)	SEMISOLID MEDIA	SOLID MEDIA
• Do not contain an agar component. • Example: nutrient broth, tryptic soy broth, or brain- heart infusion broth. • All above can be used to propagate large numbers of microorganisms in fermentation studies and for various biochemical tests.	• Can also be used in fermentation studies. • In determining bacterial motility. • Promoting anaerobic growth.	• Such as nutrient agar or blood agar. • **Can be used:** (1) for the surface growth of microorganisms in order to observe colony appearance. (2) Pure culture isolations. (3) Storage of cultures. (4) Observe specific biochemical reactions.

Depending on the type and combination of nutrients, different categories of media can be made

CATEGORIES

1.1. Complex media are rich in nutrients, they contain water soluble extracts of plant or animal tissue (e.g., enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar, often glucose is added to serve as the main carbon and energy source.

2.The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called **complex**.

1.2. Defined media are media composed of pure ingredients in carefully measured concentrations dissolved in double distilled water i.e., the exact chemical composition of the medium is known. Typically, they contain a simple sugar as the carbon and energy source, an inorganic nitrogen source, various mineral salts and if necessary, growth factors (purified amino acids, vitamins, purines and pyrimidines).

1.3. Selective/differential media are media based on either of the two categories above supplemented with growth-promoting or growth-inhibiting additives. The additives may be species- or organism-selective (e.g., a specific substrate, or an inhibitor such as cycloheximide which inhibits all eucaryotic growth and is typically used to prevent fungal growth in mixed cultures).

Common Microbial culture medium

1.Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) is a common medium used for the growth of fungi and yeasts.
The medium contains essential nutrients, including carbohydrates from potato infusion and dextrose, and nitrogen from yeast extract. PDA is widely used in mycology research and in the food industry to detect and identify fungal contaminants.

2. Nutrient Agar Medium:

Nutrient Agar (NA) is a general-purpose medium used to grow a wide range of bacteria and fungi. It contains essential nutrients like peptides, sodium chloride, and agar, which support the growth of most microorganisms. NA is commonly used in microbiology labs for routine cultivation and isolation of bacteria and some fungi

Composition of Potato Dextrose Agar (PDA)

Ingredients	Gms/L
Potatoes, infusion from	200.0
Dextrose	20.0
Agar	15.0

Final pH (at 25°C) 5.6±0.2

Composition of Nutrient (NA)

Ingredients	Gram/liter
Peptone	5.0
Yeast extract	1.5
Beef extract	1.5
Sodium chloride	5.0
Agar	15.0

Final pH at 25°C: 7.4 ±0.2

Media preparation

Procedure:

- Follow the manufacturer's instructions to calculate and weigh the required amount of NA medium using an analytical balance and transfer it into a conical flask.
- Add the required amount of distilled water to the flask and stir the mixture to ensure the medium is fully dissolved.
- Adjust the pH of the medium to 7.0 by adding a dilute acid or base solution, as needed.
- Sterilize the medium by autoclaving at 121°C for 15 minutes.
- Store the prepared media in a refrigerator (4°C) for future use.

Calculation for Culture Media Preparation

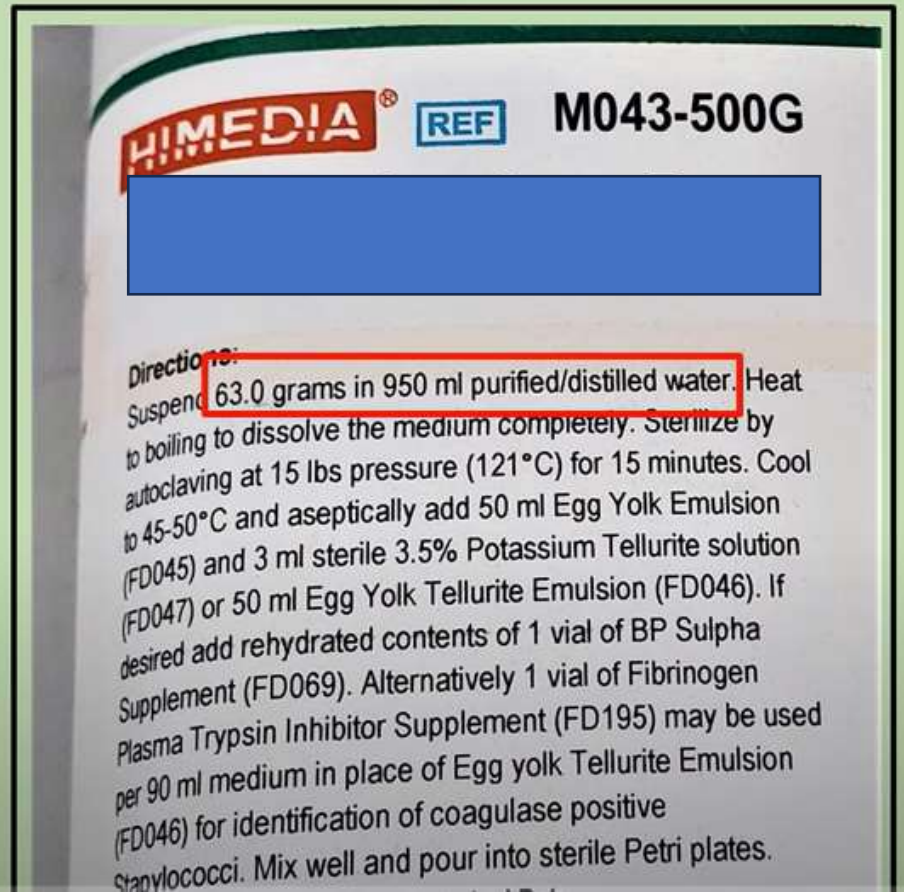
Calculation:

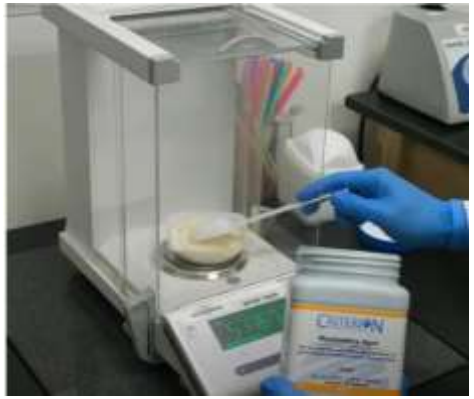
*As per the manufacturer instructions,
in 950 ml water, we need to dissolve 63 g Media

*So, in 250 ml water, we need to dissolve:

$$= \frac{63\ g \times 250\ ml}{950\ ml}$$

$$= 16.578\ g\ Media$$





Calculation and Weighing

Mixing with Water and dissolving

Sterilization
121°C for 15 minutes

Click to watch

Media Prep