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Preparation of Culture Media

Culture Media or Growth Media

Culture Media may be defined as substrates which are employed for cultivation of microorganisms.

Objectives:

- To multiply the microorganisms under controlled laboratory conditions.
- To study the characteristics or properties of microorganisms.
- To determine the cause of infectious disease by letting the agent multiply in a predetermined medium.

Properties or requirements of a suitable medium:

- Proper supply of food need for the growth of microorganisms
- Proper amount of moisture
- Essential minerals elements
- Growth promoting substances
- Optimum pH
- Proper supply of oxygen for growth
- Approximately isotonic solution
- Must not contain any toxic and growth inhibitor
- Must be sterilized without alteration the properties of composition

Classification of culture media

On the basis of physical state/ Consistency:

1. Solid media:

Solid media are made by adding a solidifying agent (agar at the concentration of 1.5-2.0% w/v) to the nutrients and water. This form of media is mainly used in petri dish as plate culture. They are used to observe the colony characteristics, size and shape of microorganisms and also for the isolation purpose. e.g. Nutrient Agar Medium.

2. Semisolid media:

Semi solid media are microbial culture media that are prepared to add less amount of solidifying agent (agar at the concentration of 0.4 to 0.5% w/v). These media are gelatinous in nature with jelly like consistency and used for motility test, different biochemical test etc. e.g. Peptone Water Agar Medium.

3. Liquid media:

Liquid media are a type of culture media where nutrients are dissolved in water without adding any solidifying agent. They are also referred to as culture broth. Generally, liquid media are used for the propagation and transfer of microorganisms. e.g. Nutrient Broth.

On the basis of chemical composition:

1. Natural media:

Those media whose chemical composition is not known are called natural media. It contains all necessary ingredients for growth of microorganisms but they are in crude form. They are more useful for cultivating unknown bacteria, as it usually provides full range of growing factors such as amino acids, polypeptides, vitamins and minerals etc. e.g. Oatmeal Agar Medium.

2. Semisynthetic media

The medium of which chemical composition is partially known that is called semisynthetic medium. e.g. PDA medium.

3. Synthetic/Artificial media:

Media prepared by adding precise amount of highly purified inorganic or organic chemicals to distilled water are known as synthetic media. So in this media the chemical composition is known. This media are great importance in studying the metabolic activity of microorganisms. e.g. Rose Bengal Streptomycin Sulfate Agar Medium.

Different steps for preparation of culture media

- Each ingredient/ complete dehydrate medium should be dissolved in distilled water.
- Determine the P^H and adjusted (Fungi 6-6.5 and Bacteria 7±0.2) by using HCl, H_2SO_4, $Na(OH)_2$, $K(OH)_2$ etc.
- In case of solid medium, agar should be added and dissolved by boiling.
- Dispensing the medium in flask or tube.
- Finally, sterilization the medium by autoclaving.
- In case of heat-labile medium or ingredients, sterilization should be done by filtration.

Potato Dextrose Agar (PDA) Medium

History: In the course of a comparative test on several culture media in 1938, Shadwick found that Potato Dextrose Agar gave good results for the enumeration of yeasts and molds in butter. PDA means Potato Dextrose Agar is very commonly used routine medium for plant pathology laboratory. A broad spectrum of fungus grows well on this medium.

Ingredients (g /L)

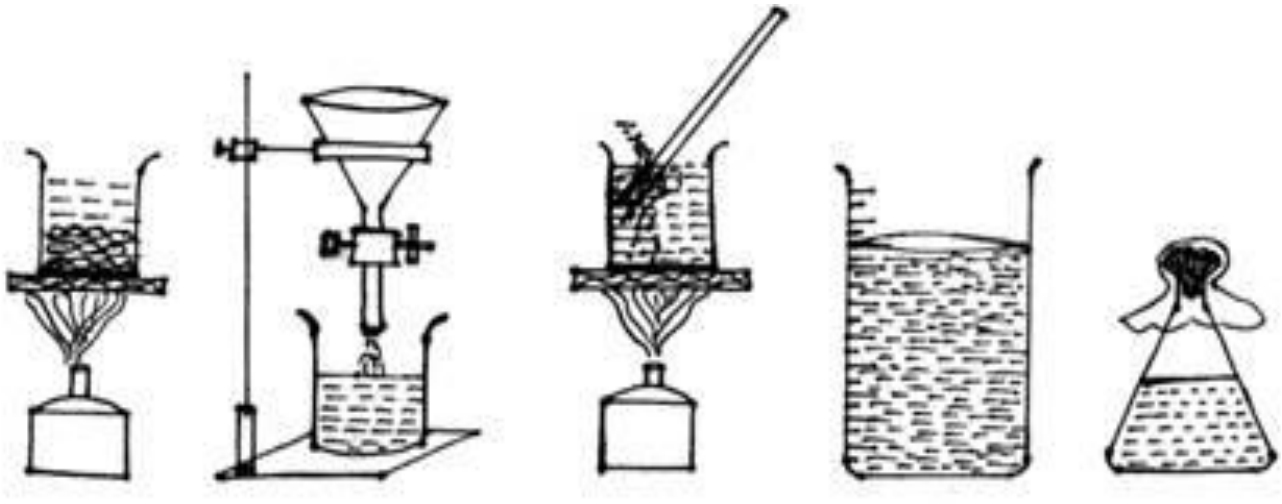
- Peeled potato 200 g
- Dextrose 20 g
- Agar 17 g
- Distilled water for 1L

Equipments:

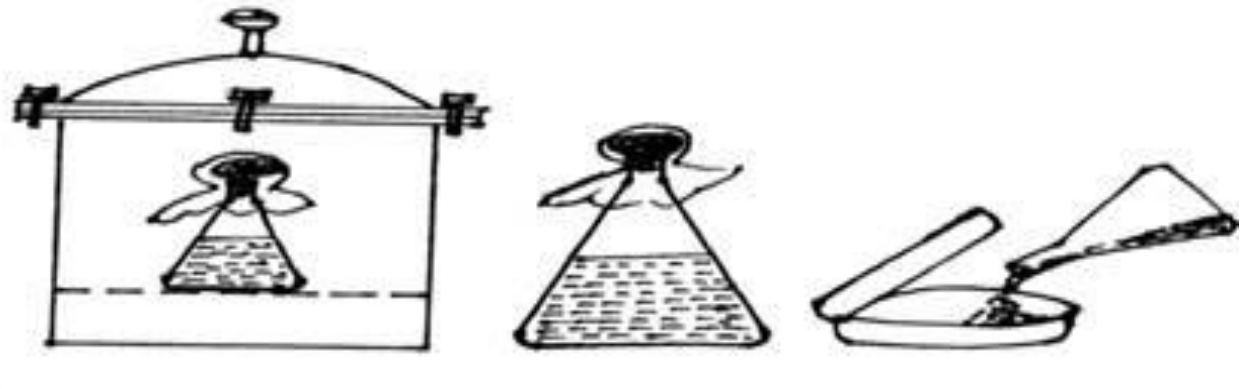
Sus pan, Beakers, Petri dishes, Test tubes, Flasks, measuring cylinders, Cheese cloth, P^H meter, Knife etc.

Procedure:

At first 200 gram potato was taken and washed with tap water to remove all sorts of dust and dirtiness. Then, tubers were cut into small slices and put in a sus pan with 500 ml of water. The mixture was melted through heating for about half hours on a gas burner. The melted mixture was then filtered by cheese cloth and collected the extract in a beaker. Seventeen gram melted agar and 20 gram Dextrose sugar were then added to the beaker. The volume of the mixture adjusted to 1 liter by adding adequate amount of distilled water. Then, agitated the mixture and allowed to soak for 5 minutes. Then, heated the mixture to boiling with frequent stirring with the glass rod or magnetic stirrer to dissolve and melt the agar. Adjusted the p^H of the mixture at 6.0. Finally kept the media in an autoclave at 121°C (~250°F) temperature under 1.1 kg/cm^2 (100 kPa/ 15 psi) pressure for 20 minutes. After sterilization the medium was stored at room temperature (25 °C) for future use.



Boiling potato slices **Filtering through cheese cloth** **Boiling Potato extract + Dextrose+ Agar** **PDA**



PDA Sterilization **PDA** **PDA pouring in petri-dish**