Media prepration and sterilization- 6th Sem(M)

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Introduction :

- Culture media are available commercially as powder; they require only the addition of water. Nutrient medium is a general purpose preparation for culturing microorganisms which are not nutritionally fastidious.

- The media contains:

- 1.5 g/L “Lab-lemco” powder (a beef extract)
  1.5 g/L yeast extract
  5.0 g/L peptone (a nitrogen source)
  5.0 g/L sodium chloride
  15.0 g/L agar powder

Autoclaving

- Autoclaving is a process that use moist heat and pressure so that all parts of the material to be sterilized reach 121 degree celcius for 15 minutes. An autoclave is, in essence, a large pressure cooker; a chamber which may be sealed off against surrounding air.
- Materials for sterilization are placed in the chamber, the door is sealed, and pressurized steam is forced into the chamber. The incoming steam displaces cooler air through an exhaust valve; this valve closes when the cell cooler air has been vented.
- Steam is continually forced into the chamber until the pressure reaches 103 kPa above atmospheric pressure; at sea level, this pushes the temperature in the chamber to 121 degree celcius. The high pressure prevents solutions from boiling over at this temperature. Larger volumes require longer than 15 minutes to heat up to 121 degree celcius throughout. After sterilization, the steam pressure is slowly decreased to atmospheric pressure. The sterilized objects can then be removed.
- **Objective:**
- To prepare sterile nutrient agar for culturing microorganisms.
- **Material and reagents:**
- Commercial nutrient agar,Balance,Distilled water,Scott bottles,Measuring cylinder Beaker,Forcep,Universal bottles
- **Procedure**
- 1. Appropriate amount of broth (with agar) powder is weighed into Scott bottles and dissolve
- 2. The bottles are loosely recap and set aside for sterilization
- 3. All the media are sterilized at 121 degree celcius for 15 minutes
- 4. After autoclaving, the media is removed. The broth preparation is allowed to cool and the cap of each bottle is tightened.

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Discussion:

- There are several precaution steps we need to take when handling the experiment.
- **1) Balance**
- The appropriate amount of broth powder and agar powder is weighed using electronic analytical balance which has the precision of one hundredth of a gram, ±0.01 or one ten-thousandth of a gram, ±0.0001 g.
- The proper receiver for the material must be selected. The receiver's weight plus the weight to be measured must not exceed the maximum load for the balance. The size and shape of the receiver should permit it to fit into the space and on the balance pan without interfering with any operation. It is important that the receiver is clean and in dry condition. Common receivers are weighing bottles,weighing funnels,flasks,and weighing paper. The correct receiver depends upon the quantity and type of material (liquid,solid,or powder)to be weighed.
- Make sure the surrounding of the pan and the pan of the balance is clean. Place the receiver on the center of the pan of the balance and close the balance door. Then, press the appropriate tare key on the balance to set the signal from the strain gauge to zero so that the weight of the receiver is no longer indicated. Carefully add the powdered material using a spatula until the desired amount is added. Handle with care to avoid spilling.
- If solids are spilled, remove the receiver and sweep out all of the spilled material from the balance using a brush.The spilled material must be properly disposed.
- To be effective the autoclave must reach and maintain a temperature of 121-123 degree celcius for at least 30 minutes. This is achieved by using saturated steam under at least 15 psi of pressure.

- **2) Autoclaving process**
- Check the drain screen at the bottom of the chamber before using the autoclave.
- Clean out any debris for efficient heat transfer as steam must flush out of the autoclave chamber. If the drain screen is blocked with debris, a layer of air may form at the bottom of the autoclave and prevent proper operation.
- Make sure that the water level is higher than the material in the autoclave
- Make sure the water level should between range of low and high. If there are too low water level, water should be added
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- Make sure the cap of the Scott bottles must not too tight to prevent breakage off the Scott bottles.
- Make sure the cap of the Scott bottles must not too loose to prevent the outflow of media inside the Scott bottles.

- Autoclave doors must be firmly locked into place before running the autoclave.
- Do not stack or store combustible material next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- Always use heat resistant gloves when removing materials after sterilization.
- Avoid touching the inner chamber surfaces after sterilization.
- **3) Agar**
- There are a few types of general nutrient agar plates.
- Luria Bertani (LB) agar is a common nutrient agar for the general routine growth of bacteria and is not preferentially suited toward a particular microbe type.
- Miller's LB agar is a variety of LB containing different proportions of the same components.
- Trypticase Soy agar (TSA) is another general purpose medium made with casein and soybean meal and is used as initial growth medium to observe bacterial morphology or increase bacterial growth for analysis or storage.
- Phenylethyl alcohol agar (PEA) is selective for species of Staphylococcus and inhibits Gram-negative bacteria.
- Brain Heart Infusion (BHI) agar is a general purpose medium suitable for the cultivation of a wide variety of organism types, including bacteria, yeasts and moulds. The BHI agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusion are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is the carbohydrate source that microorganisms utilize by fermentation action. The medium is buffered through the use of disodium phosphate

- **Other precautions:**
- When preparing commercial media, we must read the label and instruction on the container before use.
- In the progress of experiment, use distilled water to clean all the apparatus.
- Measuring cylinder is used to measure the volume of distilled water required accurately.
- Stir the mixture continuously to ensure that the nutrient powder dissolves completely.
- **Conclusion:**
- Different types of agar are needed for the cultivation of different types of microorganisms. Agar of the same composition with the commercial agar can be made by following the correct procedures. Preparation and sterilization of culture media should be done with great care to avoid contamination of unwanted microorganisms. We had learnt the preparation and sterilization of culture media via autoclaving process and the precaution steps that we need to take into consideration when handling this experiment.