Laboratory Exercise # 3 Preparing Bacterial Media

Purpose:

The purpose of this exercise is for the student to experience the process of preparing bacterial media.

Introduction:

In this exercise we will be preparing a basic culture medium. This type of medium contains ingredients, which will support the growth of a large number of different bacteria, but will not support the growth of bacteria that are fastidious heterotrophs. The fastidious heterotrophs require special ingredients, like blood, amino acids or vitamins.

Trypticase soy agar has a protein base derived from soybean and agar, which is an extract of seaweed that has no nutritional value to bacteria. Agar is added to the medium for the sole purpose of producing a solid surface on which the bacteria can grow. The addition of agar to the media has other desirable attributes. It remains in a solid state even at high temperatures and does not liquefy until it reaches 100°C. This allows the growth of bacteria that are thermopiles (like temperatures > 40°C) within the laboratories. Agar solidifies at approximately 40°C. Also very few bacteria have to ability to decompose the agar so it remains stable even while bacteria grow upon or within it.

Buffers are put into the culture medium to keep the pH of the medium from changing drastically. Most bacteria prefer a pH around neutral. As the bacteria grow and reproduce on the medium, acidic and basic by-products of metabolism are produced. If buffers were not included in the medium, the bacteria would be quickly killed off by their own waste products.

Materials:

- Dehydrated Trypticase Soy agar
- Distilled water
- Hot plates
- Stir bars
- Graduate cylinders
- Digital balance
- Weigh paper
- Hot mitts
- Flasks
- Media bottles with lids
- Autoclave
Procedure:

**Weighing the media:**

1. Place weigh paper on the balance and touch the zero key. This negates the weight of the paper from the total weight on the balance.

2. Read the directions on the bottle of dehydrated media and determine the amount of agar to weigh out. Notice the amount is for one liter of agar. **You will not be making a liter of media**, so some calculation is required to determine the amount of agar to weigh out.

3. If you only wish to make 600 ml of agar and assuming the directions call for 40 grams of agar for a liter of media, the following calculation can be performed.

\[
\frac{40 \text{ grams of agar}}{1000 \text{ ml of water}} = \frac{X \text{ grams of agar}}{600 \text{ ml of water}}
\]

Cross multiply: \(1000X = 40 \times 600\)

\[
1000X = 24000
\]

\[
X = \frac{24000}{1000} = 24 \text{ grams of agar}
\]

4. Add media to the weigh paper until the required amount appears on the digital read out.

**Measuring the water:**

1. Using a graduate cylinder measure out the required amount of distilled water.

2. Remember that you read the meniscus at the lowest level and the cylinder needs to be held at eye level in order to be read.

**Heating and Dispensing the media:**

1. Pour the distilled water into the flask provided and add a magnetic stir bar to it.

2. Place the flask on the hot plate. Turn the stirrer on so the water mixes at a slow rate.

3. Turn the heat control knob to high.

4. Slowly add the media to the water.

5. Heat the media until it comes to a boil and the solution becomes clear.
6. Turn off the hot plate and using hot mitts carefully pour the media into the provided bottles using a funnel. **Never fill a media bottle totally full!** The agar bottles hold approximately 150 ml of agar. Place the caps on the jar. They should be left slightly loose. The bottles are now ready to be autoclaved.

**Autoclaving the media:**

1. The autoclave is an instrument used to sterilize media and equipment. Sterilization is the removal of all living organisms. This is accomplished by the use of high temperature, pressure and steam. It is similar to a pressure cooker used in the kitchen.

2. The media is placed inside the autoclave. The door is sealed and the instrument is set for a cycle of 20 minutes. During that time the air will be removed from the chamber and replaced with steam. A special valve increases the pressure within the chamber to 15 pounds per square inch above normal atmospheric pressure. The temperature increases to 121°C.

3. Superheated water molecules rapidly conduct heat into any microorganisms present. The timed required for killing bacteria, even those that produce spores is then reduced to approximately 15-20 minutes.

4. After the proper time elapses the steam is released from the chamber and cool air is returned. At this point the autoclave may be opened again and the media removed.

5. The bottles of media are then placed in a water bath that is set at 50°C in order that the media be partially cooled prior to pouring.

**Pouring the petri dishes:**

1. When the media bottles are cool enough to touch comfortably with your hand it is ready to be poured into the petri dishes.

2. Place 4 petri dishes lid up on the bench.

3. Light the Bunsen burner using the striker.

4. Unscrew the lid of the media bottle and flame the lip of the bottle.

5. Crack the lid of the petri dish and pour in enough media to just cover the bottom of the dish, then close the lid.

6. Flame the lip of the media bottle before pouring the next plate and between each plate.

7. Leave the petri dishes on the bench top without moving them until they are solidified. They will be refrigerated and then used in exercise # 4.
Questions:

1. What are the ingredients found within Trypticase soy agar?

2. At what temperature does agar solidify? _______________

3. At what temperature does agar liquefy? _________________

4. What is the function of the agar that is within this media?

5. Why are buffers added to the medium? (Note there are no buffers within Trypticase Soy agar)

6. At what temperature, pressure and time is media autoclaved?

7. If a media requires 19.5 grams of media in order to make a liter of the media, how much powdered media should be weighed out if you are only going to make 375 ml of the media?