PW 04: ATMOSPHERIC OXYGEN REQUIREMENT FACTOR

1. Introduction

Bacterial growth is dependent on the presence of oxygen in the environment, as different bacteria have different oxygen requirements depending on the types of enzymes they possess. The major bacterial oxygen classes are:

Aerobes: require atmospheric O_2 (20%), and use O_2 as the final electron acceptor in the electron transport system.

Microaerophiles: require O_2 at below atmospheric concentrations, typically 2-10%. Microaerophiles have a limited ability to neutralize toxic oxygen, so excess O_2 will kill the bacteria. However, microaerophiles do use O_2 as final electron acceptor in the electron transport system.

Obligate Anaerobes: cannot survive in the presence of any oxygen. Obligate anaerobes lack the enzymes necessary to break down the toxic by-products of oxygen. In these bacteria, the final electron acceptor in the electron transport system is a molecule other than O_2.

Aerotolerant anaerobes: grow equally well in the presence or absence of oxygen. They do possess enzymes necessary to neutralize toxic oxygen by-products, but they never use O_2 as a final electron acceptor.

Facultative anaerobes are also able to live either in the presence or absence of oxygen, but they prefer oxygen so they can carry out aerobic respiration with O_2 as final electron acceptor to maximize ATP yields. These organisms can use other electron acceptors if O_2 is not available, such as fumarate and nitrate. They can also utilize fermentative metabolism in the absence of oxygen.

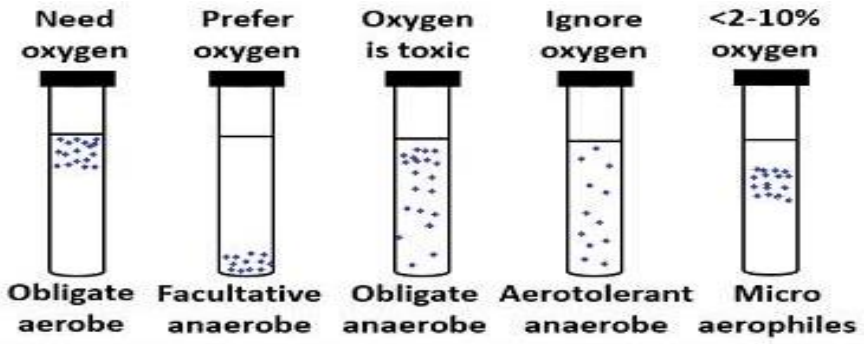
One simple way to determine the oxygen requirements of a bacterium is to inoculate the bacterial culture into a melted agar deep tube of Liver Meat Agar (LMA) media, inoculate well to evenly distribute the bacteria throughout the liquefied agar. Once the agar solidifies, an oxygen gradient is created, with atmospheric oxygen concentrations at the top of the tube and little to no oxygen present at the bottom of the tube. The bacteria present in the agar deep tube will only be able to grow where their oxygen requirements are met.

2. Media needed

4 deep tubs of Liver Meat Agar (LMA) medium.

3. Cultures needed

Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus sp.



4. Procedure

1. Inoculation and Incubation: at the moment of use, melt the medium in a boiling water-bath and regenerate for 20 minutes. Leave it to cool to 44°C.

2. Detection of respiratory mode: immerse the tip of a flame-sterilized and closed Pasteur pipette in the bacterial test culture. Drain the tip, then place the inoculum in the bottom of the tube, executing several twisting up-and-down movements (Or circular movement), taking care not to create bubbles or aerate the agar. You need to work quickly so the agar doesn't solidify!

3. Isolation of anaerobes: on completion of the preceding operation, and to use up all the inoculum, continue with a 2^{nd}, 3^{rd} and 4^{th} tube. Immediately immerse the inoculated tubes in cold water in a vertical position. Then place them in an incubator at 37°C and observe for 24h.

5. Results

Examine the LMA deep tubes for presence/absence of microbial growth and location of growth. Record results in the table below.

Species	**Distribution of growth**	**Oxygen requirement classification**
Staphylococcus aureus		
Escherichia coli		
Pseudomonas aeruginosa		
Bacillus sp.		

6. Study questions

1. Why is it important to avoid creating bubbles or aerating the liquefied BHI tubes when mixing the bacteria into the agar?

2. What do the following classes of bacteria have in common?
   a. Obligate aerobes and Microaerophiles?
   b. Aerotolerant and Obligate anaerobes?
   c. Facultative and Aerotolerant?