

Cultivation Of Aerobic and Anaerobic Bacteria

Cultivation of Aerobic Bacteria:

Cultivating aerobic bacteria requires a continuous supply of free oxygen, standard nutrients (carbon, nitrogen, minerals), and a temperature of 35°C to 37°C. Growth is performed in normal atmospheric air using solid agar plates, slants, or liquid broths with active aeration.

- Atmospheric condition is generally satisfactory for the culture of aerobes or facultative anaerobes. However, for the growth of many aerobes, it is necessary to provide extensive aeration.
- Forced aeration of cultures is therefore frequently desirable. It is achieved either by vigorously shaking the flask/tube on a shaker or by bubbling sterilized air into the medium.
- When aerobic organisms are to be grown in large quantities, it is advantageous to increase the exposure of the medium to the atmosphere. This is accomplished by dispensing the medium in shallow layers or by providing aeration by constantly shaking the inoculated liquid cultures.

Cultivation of Anaerobic Bacteria:

Cultivating anaerobic bacteria requires removing oxygen and lowering the redox potential of culture media, because oxygen is toxic or inhibitory to strict anaerobes. Special equipment and chemical reducing agents are used to establish and maintain an oxygen-free environment.

Classification of Anaerobes by Oxygen Tolerance:

- **Obligate (Strict) Anaerobes:** Die in the presence of oxygen. They lack protective enzymes like catalase and superoxide dismutase.
- **Facultative Anaerobes:** Grow better with oxygen but can switch to anaerobic respiration or fermentation if oxygen is absent.

- **Aerotolerant Anaerobes:** Do not use oxygen for growth but are not harmed by its presence.
- **Microaerophiles:** Require low levels of oxygen to survive but are killed by normal atmospheric concentrations.

Methods of cultivation of Anaerobic Bacteria

I. Robertson's Cooked Meat Medium (RCM):

In RCM, the meat particles maintain anaerobic conditions passively throughout incubation, without requiring an anaerobic jar for the enrichment broth itself.

Robertson's cooked meat medium is unique among enrichment broths: it does not just support anaerobe growth, it also differentiates between *Clostridium* species by the reactions produced in the meat itself.



- Raw meat contains proteolytic enzymes that would continue to break down proteins in the medium during incubation, interfering with both the reducing action and the interpretation of results. Cooking denatures these enzymes and also denatures the muscle proteins, making reducing substances (glutathione, cysteine) more available for oxygen scavenging. This is why the medium is called "cooked meat" medium.
- Before inoculation, RCM/CMB medium is boiled to make it oxygen-free. After inoculation, it is covered with a layer of sterile liquid paraffin oil to prevent the entry of oxygen into the medium. The medium's ingredients help maintain the anaerobic (reduced) environment.

REACTION TYPE	APPEARANCE OF MEAT	ODOR	MECHANISM	REPRESENTATIVE ORGANISMS
Saccharolytic	 Meat turns pink/red	Rancid, sour	Carbohydrate fermentation produces acid and gas; acid pH causes reddening of meat due to pH indicator effect	<i>Clostridium perfringens</i> , <i>C. butyricum</i> , <i>C. tertium</i>
Proteolytic	Meat turns black	Foul 	Protein digestion releases H ₂ S, amines, indole; H ₂ S reacts with iron in meat → black iron sulphide	<i>Clostridium tetani</i> , <i>C. histolyticum</i> , <i>C. sporogenes</i>
Both saccharolytic and proteolytic	Meat blackens and may show reddening	Foul + rancid	Both carbohydrate and protein breakdown	<i>Clostridium bifermentans</i>

II. Anaerobic Jar (Gas Pak system):

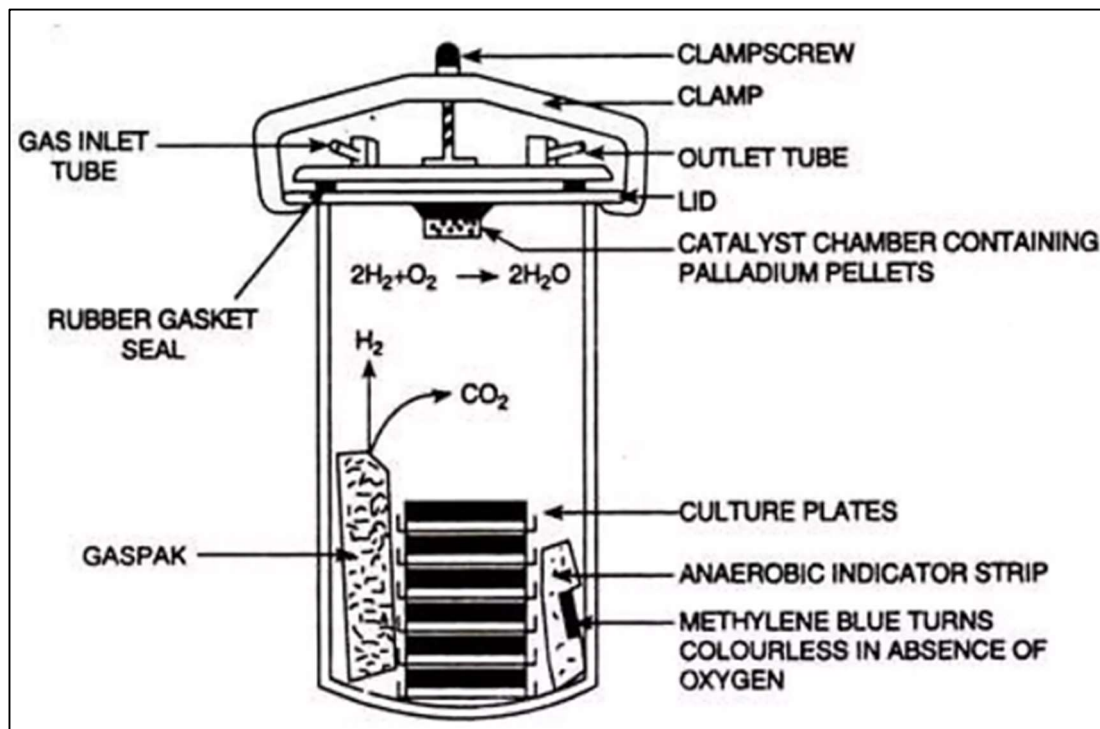
Inoculated plates or tubes are placed inside the anaerobic jar along with gas generator sachet and indicator strip and is sealed completely.

- In the presence of water, chemicals present inside the sachet (sodium borohydride, sodium bicarbonate, and citric acid) react chemically, producing hydrogen and carbon dioxide gas.
- The hydrogen thus produced reacts with oxygen present inside the jar producing water (which forms as condensation on the inside of the jar).



- This reaction is catalyzed by the element palladium, which is attached to the underside of the lid of the jar. The oxygen inside the jar is consumed as it combines with hydrogen to form water; this consumption creates the anaerobic environment.
- The carbon dioxide generated by the sachet enriches the atmosphere and supports the growth of capnophilic and many anaerobic organisms.

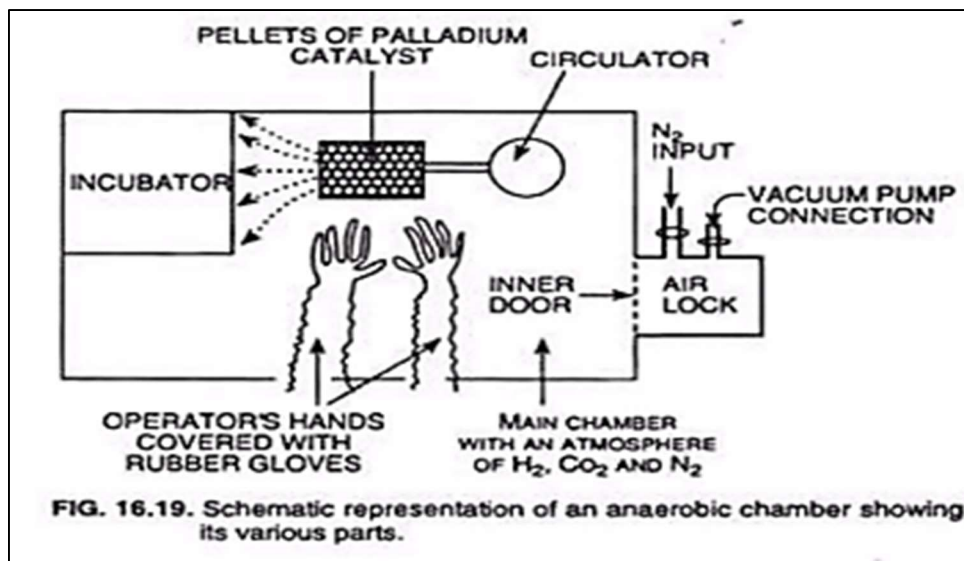
- Impregnated with methylene blue: blue in the presence of oxygen, colorless in anaerobic conditions.



III. Anaerobic Chamber: This refers to a plastic anaerobic glove box.

- Glove parts and rubber gloves allow the operator to perform manipulation within the chamber.
- Air lock with inner and outer doors. Media are placed within the air lock with the inner door remaining sealed; air is removed by a vacuum pump connection and replaced with N_2 through another pipe.
- The inner door is opened and the media are placed within the main chamber, which contains an atmosphere of $\text{H}_2 + \text{CO}_2 + \text{N}_2$.
- A circulator circulates the gas atmosphere through pellets of palladium catalyst, causing any residual O_2 in the media to be used up by reaction with H_2 .

- After media have become completely anaerobic they can be inoculated and placed in an incubator located within the chamber.

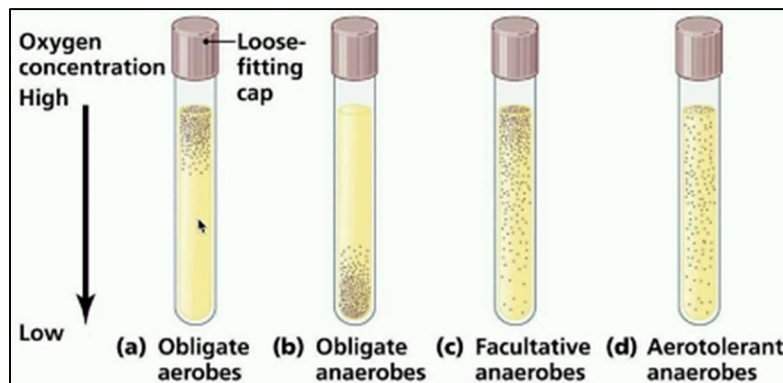


IV. Fluid Thioglycollate Medium (FTM):

FTM is a versatile, multi-purpose liquid medium that supports the growth of aerobic, microaerophilic, and anaerobic microorganisms by establishing an oxygen gradient from an aerobic zone at the top to an anaerobic zone at the bottom.

1. **Cystine** supply carbon and nitrogenous compounds, and **Dextrose** is added as another energy source.
2. **Sodium Thioglycollate:** It is a reducing agent which maintains a low oxygen tension by removing molecular oxygen from the environment i.e., it creates anaerobic conditions when it reduces molecular oxygen to water.
3. **Reducing Agents:** Sodium thioglycollate and L-cystine consume oxygen and lower the oxidation-reduction potential, providing an anaerobic environment at the bottom of the tube.
4. **Resazurin** is an oxidation-reduction indicator that turns pink when increased oxidation (presence of oxygen) has occurred, it is colorless when reduced (absence of oxygen).

5. **Agar:** The addition of a small amount of agar in the Thioglycollate medium aids in the initiation and growth of small inocula and anaerobes by impeding the diffusion of oxygen into the medium.



Microbial Growth Patterns in FTM

- **Obligate Anaerobes:** Grow exclusively in the lower, oxygen-depleted zone (colorless area) at the bottom of the tube.
- **Obligate Aerobes:** Gather strictly in the upper, oxygen-rich band near the surface (pink area).
- **Facultative anaerobes** can grow with or without oxygen because they can metabolize energy aerobically or anaerobically. They gather mostly at the top because aerobic respiration generates more ATP than either fermentation or anaerobic respiration.
- **Aerotolerant** organisms do not require oxygen as they metabolize energy anaerobically. Unlike obligate anaerobes, though, they are not poisoned by oxygen. They can be found evenly spread throughout the test tube.