

A stack of books is shown from a low angle, with the spines of several books visible. The books have various colored covers, including red, orange, and yellow. A semi-transparent gradient overlay in shades of red and orange is positioned over the right side of the books. The text 'Anaerobiosis' is written in white on the red part of the overlay, and 'Dr. Tanmay Mehta' is written in black on the orange part.

Anaerobiosis

Dr. Tanmay Mehta



Ananerobes

- Anaerobic bacteria do not have cytochrome system for oxygen metabolism and hence are unable to neutralize toxic oxygen metabolites
- Classification
- **Obligate anaerobes:** Cannot tolerate O₂. Completely lack superoxide dismutase and catalase enzymes
- **Aerotolerant anaerobes:** Do not utilize oxygen for growth, but tolerate its presence. Possess small amounts of superoxide dismutase and peroxidase (but lack catalase)



Special requirements to grow Anaerobes

- **Anaerobic condition:**
 - McIntosh and Filde's anaerobic jar
 - GasPak system
 - Anoxomat system
 - Anaerobic glove box
 - Pre-reduced anaerobically sterilized (PRAS) media.
- **Medium with low redox potential:** By adding reducing substances -unsaturated fatty acid, ascorbic acid, glutathione, cysteine, glucose, sulfites and metallic iron

A hand is shown pouring a yellow liquid from a beaker into a test tube. The background is a blurred laboratory setting with various glassware and equipment.

Anaerobic Culture Media

- Contain reducing substances which take-up oxygen and create lower redox potential and thus permit the growth of obligate anaerobes such as *Clostridium*.
- **Robertson's cooked meat (RCM) broth**
 - Contains chopped meat particles (beef heart), which provide glutathione (a sulfhydryl group containing reducing substance) and unsaturated fatty acids.
 - Most widely used anaerobic culture medium.
 - Also used for maintenance of stock cultures.



Anaerobic Culture Media (cont..)

- Other anaerobic media include
 - Thioglycollate broth
 - Anaerobic blood agar
 - BHIS agar-Brain heart infusion agar with supplements (vitamin K and hemin)
 - Neomycin blood agar
 - Egg yolk agar
 - Phenyl ethyl agar
 - *Bacteroides* bile esculin agar (BBE agar)

A laboratory setting with a petri dish and a pipette tip. The background is a blurred image of a laboratory bench with various glassware and equipment. The title 'ANAEROBIC CULTURE METHODS' is overlaid on a red banner at the top of the image.

ANAEROBIC CULTURE METHODS

- Obligate anaerobic bacteria can grow only in the absence of oxygen.
- Anaerobic culture methods includes:
 - Production of vacuum
 - By displacement and combustion of oxygen
 - Absorption of oxygen by chemical methods-
 - Anaerobic glove box
 - By reducing agents
 - PRAS (Pre-Reduced, Anaerobically Sterilized)



ANAEROBIC CULTURE METHODS

- **Production of vacuum-** Achieved by incubating cultures in a vacuum desiccator. It is not an effective method, not used.
- **By displacement and combustion of oxygen-** Involves evacuation of the air from jar and replacement with inert gas like hydrogen followed by removal of the residual oxygen by use of a catalyst. It is carried out by-
 - McIntosh and Filde's anaerobic jar
 - Anoxomat instrument

McIntosh and Filde's anaerobic jar

- Most effective and popular method.
- Consists of a metal or glass jar with a metal lid with a screw (to close airtight), pressure gauge and two openings (inlet and outlet).



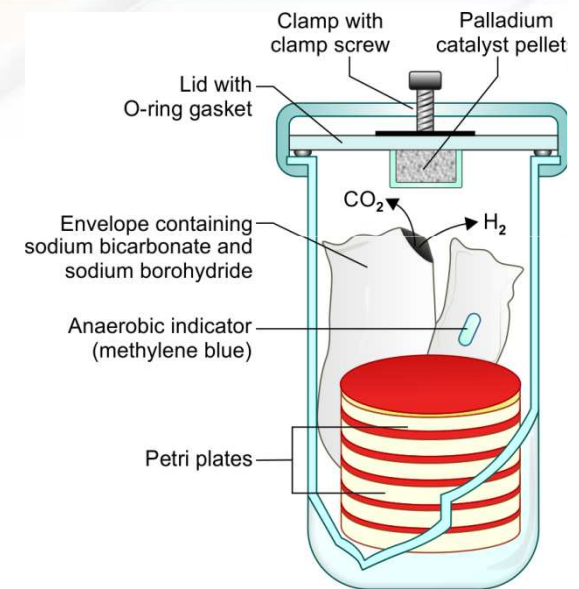
Anoxomat

- Automated equipment which evacuates the air from jar and replaces by hydrogen gas from a cylinder.
- Same catalyst is used here to remove the traces of oxygen.
- Easier to operate than McIntosh jar method and claims to be highly effective for creating anaerobiosis.



Absorption of oxygen by chemical methods

- **Principle-** Oxygen is removed by chemical reactions in contrast to evacuation and replacement technique used in McIntosh Filde's jar.
- **Gas-pak-** Most commonly used method for anaerobiosis, simple to perform and is perfect for a laboratory having less sample load.





Gas-pak

- Sachet containing sodium bicarbonate and sodium borohydride which react chemically in presence of water, to produce hydrogen and CO_2 gas.
- Traces of oxygen is removed by using same catalyst (aluminium pellets coated with palladium) placed below the jar.



Indicator of anaerobiosis

- ***Chemical indicator***- Reduced methylene blue remains colourless in anaerobic conditions, but turns blue on exposure to oxygen.
- ***Biological indicator***-Plate inoculated with *Pseudomonas* is incubated along with other inoculated plates for anaerobic culture. Absence of growth of *Pseudomonas* (which is an obligate aerobe) indicates that perfect anaerobiosis has been achieved.



Anaerobic glove box

- Also called anaerobic chamber.
- Self-contained anaerobic system that allows microbiologists to process the specimen and perform most bacteriological techniques for isolation and identification of anaerobic bacteria without exposure to oxygen.



By reducing agents

- Oxygen in culture media can be reduced by various reducing agents such as glucose, thioglycollate, cooked meat pieces, cysteine and ascorbic acid.
- Robertson cooked meat broth is the most widely employed anaerobic culture medium which uses chopped meat particles (beef heart) as reducing agent.

The background of the slide features a blurred image of a laboratory setting. On the left, there are several glass beakers containing liquids of different colors: yellow, red, and white. In the center, a person's hands are visible, one holding a white cylindrical container and the other holding a metal tray. The overall scene suggests a sterile laboratory environment where media preparation is taking place.

PRAS (Pre-Reduced, Anaerobically Sterilized)

- PRAS media are prepared entirely under oxygen-free conditions from initial sterilization to packaging in sealed foil packages.