B Pharm, 3rd Semester
Pharmaceutical Microbiology
BP 303 T Unit-1
Nutritional Requirements of Bacteria

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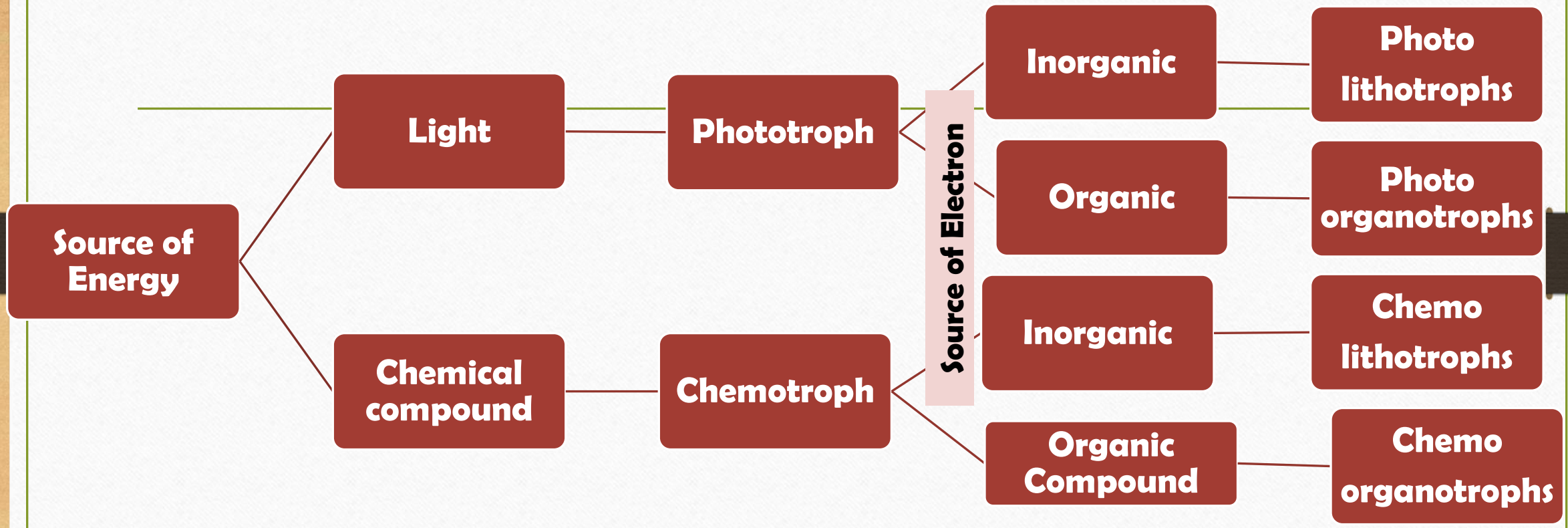
What is microbial growth?

- Microbial growth refers to an increase in cell number(population), not in cell size.
- Bacteria grow and divide by **binary fission**, a rapid and relatively simple process.
- Requirement for optimum Microbial Growth are-
  - Nutritional Requirement
  - Favorable Physical Condition

Nutritional Requirements:

- Nutrients are the chemical requirement essential for the growth of microbes.
- Extreme diversity is observer in bacteria and nutritional requirement varies widely. Therefore great difference in composition of culture media is there.
- All micro-organism require a source of energy, source of electron and source of carbon for their growth and development.
- Essential chemical required are carbon, nitrogen, sulphur, phosphorus, oxygen, metal ions and vitamins.

Nutritional Requirements:



Nutritional Requirements:

- **Autotrophs** make their own food by using sunlight, carbon dioxide, and water to form sugars which they can use for energy.
- **Heterotrophs** can not make their own food by using sunlight and therefore depend on other for carbon source.
- **Chemoheterotrophs**: Obtain carbon from their energy source form lipids, proteins, and carbohydrates.
- **Chemoautotrophs and Photoautotrophs**: Obtain carbon from carbon dioxide.
- **Facultative Autotrophs**
- **Fastidious Heterotrophs**

Nutritional Requirements:

Major Nutritional Types	Energy Source	Hydrogen/Electron Source	Carbon Source
Photolithotrophic autotrophs (algae, cynobacteria, purple and green sulphur bacteria)	Light	Inorganic H/e^- donor	CO_2
Photo-organotrophic heterotrophy(purple and green non-sulphur bactria	Light	Organic H/e^- donor	Organic carbon (CO_2 may also be used)
Chemolithotrophic autotrophy (sulphur oxidizing bacteria, hydrogen bacteria, nitrifying bacteria, iron bacteria)	Chemical(inorganic)	Inorganic H/e^- donor	CO_2
Chemo-organotrophic heterotrophy (protozoa, fungi, most of the non photosynthetic bacteria)	Chemical(inorganic)	Organic H/e^- donor	Organic carbon

http://www.simplynotes.in/microbiology/nutritional-types-of-microorganisms/

Chemical Requirements- Carbon

- **Makes up 50% of dry weight of cell and Structural backbone of all organic compounds.**
- **Obtain carbon from their energy source: lipids, proteins, and carbohydrates or from carbon dioxide.**

Chemical Requirements- Nitrogen

- Makes up 14% of dry cell weight.
- Used to form amino acids, DNA, and RNA.
- Sources of nitrogen: Protein, Ammonium & Nitrogen gas (N_2)
- Important nitrogen fixing bacteria, live free in soil or associated with legumes (peas, beans, alfalfa, clover, etc.).
- Legume cultivation is used to fertilize soil naturally.
- Nitrates: Salts that dissociate to give NO_3^-

Chemical Requirements- Sulphur and Phosphorus

- **Sulfur**: Used to for synthesis of amino acid, proteins and some vitamins (thiamin and biotin). Sources of sulfur is Protein, Hydrogen sulfide. Sulfates: Salts that dissociate to give SO_4^{2-}
- **Phosphorus**: Used to for nucleotides in DNA, RNA, ATP, and phospholipids. Sources: Mainly inorganic phosphate salts and buffers
- **Other Elements**: Potassium, magnesium, and calcium are often required as enzyme cofactors. Calcium is required for cell wall synthesis in Gram positive bacteria.

Chemical Requirements- Trace Elements

- Act as cofactors in various enzymes . Commonly found in tap water.
- Iron
- Copper
- Molybdenum
- Zinc
- Mn
- Ni
- B
- Co

Physical Requirements for Growth

- Temperature
- pH
- Osmotic Pressure
- Gaseous Requirement

Physical Requirements for Growth: Temperature

- **Psychrophiles:** "Cold-loving"- grow at 0 °C or low. Optimum growth at 15°C or below. Found in very cold environments (North pole, ocean depths). Seldom cause disease or food spoilage.
- **Mesophiles**: "Middle loving". Most of the bacteria lie in this group. Include most pathogens and common spoilage organisms. Optimum temperature commonly 37°C. Many have adapted to live in the bodies of animals.
- **Thermophiles**: "Heat loving" Optimum growth temperature lie between 50 to 60°C. Adapted to live in hot springs, compost piles, and sunlit soil. Some thermophiles form extremely heat resistant endospores. Extreme Thermophiles (Hyperthermophiles): can growth at 80°C or higher. Archaebacteria. Most live in volcanic and ocean vents.

Physical Requirements for Growth: pH

- Most bacteria prefer neutral pH (6.5-7.5)
- Molds and yeast grow in wider pH range, but prefer pH between 5 and 6. 4 Acidity inhibits most microbial growth and is used frequently for food preservation (e.g.: pickling).
- Alkalinity inhibits microbial growth, but not commonly used for food preservation. Acidic products of bacterial metabolism interfere with growth. Buffers can be used to stabilize pH.

Physical Requirements for Growth: pH

- **Acidophiles**: "Acid loving". Can grow at very low pH (0.1 to 5.4) Lactobacillus produces lactic acid, tolerates mild acidity.
- **Neutrophiles**: optimum pH for grow is 5.4 to 8.5. Includes all human pathogens.
- **Alkaliphiles**: "Alkali loving". Can grow at alkaline pH (7 to 12 or higher) Vibrio cholerae and Alkaligenes faecalis optimal pH 9.Soil bacterium Agrobacterium grows at pH 12.

Physical Requirements for Growth : Gaseous requirement

- **Aerobic Bacteria-** require oxygen for growth
- **Obligate aerobes-** grow only in the presence of oxygen Eg. Cholera bacillus.
- **Anaerobic Bacteria**
- **Facultative anaerobes-** are ordinarily aerobic but can grow in the absence of oxygen.
- **Obligate anaerobes** may even die on exposure to oxygen.
- **Microaerophilic** bacteria are those that grow best in the presence of low oxygen tension.

Why are anaerobes killed by oxygen?

- Singlet Oxygen: Extremely reactive form of oxygen.
- Superoxide Free Radicals ($O_2^{-}.$): is extremely toxic and reactive form of oxygen and can inactivate vital cell components.
- All organisms growing have an enzyme superoxide dismutase (SOD), to get rid of them. SOD is made by aerobes, facultative anaerobes, and aerotolerant anaerobes, but not by anaerobes or microaerophiles. Reaction:

$$O_2^{-}. + O_2^{-}. + 2H+ \xrightarrow{SOD} H_2O_2 + O_2$$

Why are anaerobes killed by oxygen?

- **Hydrogen Peroxide** (H_2O_2): Peroxide ion is also toxic and the active ingredient of several antimicrobials (e.g. benzoyl peroxide). There are two different enzymes that break down hydrogen peroxide
- **Catalase**: converts hydrogen peroxide into water and O_2 . Commonly produced by humans, as well as many bacteria.

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2.$$

- **Peroxidase**: Converts hydrogen peroxide into water.

$$H_2O_2 + 2H^+ \xrightarrow{\text{Peroxidase}} H_2O$$

Physical Requirements for Growth: Osmotic Pressure

- **Halophiles:** Require moderate to large salt concentrations. Ocean water contains 3.5% salt. Most bacteria in oceans.
- **Extreme or Obligate Halophiles**: Require very high salt concentrations (20 to 30%). Bacteria in Dead Sea, brine vats.
- **Facultative Halophiles:** Do not require high salt concentrations for growth, but tolerate 2% salt or more.

Culture media

- It is chemically defined system containing nutrients prepared for microbial growth in the laboratory.
- Culture media must be sterile and should contain appropriate nutrients.
- It must be incubated at appropriate temperature at which microbes that grow and multiply.

Culture media: Types

- **Chemically Defined Media** -Nutrient material whose exact chemical composition is known
- **Complex Media**-Nutrient material whose exact chemical composition is not known. Made of extracts from yeast, meat, plants, protein digests, etc. and composition may vary slightly from batch to batch. Widely used for heterotrophic bacteria and fungi.
- Energy, carbon, nitrogen, and sulfur requirements are primarily met by protein fragments (peptones). Vitamins and organic growth factors provided by meat and yeast extracts. Two forms of complex media:

  Nutrient broth: Liquid media

  Nutrient agar: Solid media

Culture media: Types

- **Nutrient Broth(Liquid Culture media)** **It is Liquid culture media**
- **Nutrient Agar(Solid culture media)** **It contains a solidifying agent agar for preparing agar plates, agar slants. Agar melts above 95°C and once melted, does not solidify until it reaches 40°C. It cannot be degraded by most bacteria.**
- **Selective Media: Used to encourage the growth of particular microbes and suppress the growth of unwanted bacteria.**
- **Saboraud's Dextrose Agar: pH of 5.6 discourages bacterial growth. Used to isolate fungi.**
- **Brilliant Green Agar: Green dye selectively inhibits gram-positive bacteria. Used to isolate gram-negative Salmonella .**
- **Bismuth Sulfite Agar: Used to isolate Salmonella typhi. Inhibits growth of most other bacteria.**

Culture media: Types

Differential Media: Used to distinguish colonies of a desired organism.

- **Blood Agar**: Used to distinguish hemolytic bacteria that destroy red blood cells (hemolysis)from nonhemolytic bacteria. Hemolysis appears as an area of clearing around colony. Example: Streptococcus pyogenes.

Selective and Differential Media: Used for to distinguish colonies of a desired organism and also inhibit the growth of other microbes.

- **Mannitol Salt Agar**: Used to distinguish and select for Staphylococcus aureus. High salt (7.5% NaCl) discourages growth of other organisms. pH indicator changes color when mannitol is fermented to acid.
- **MacConkey Agar**: Used to distinguish and select for Salmonella . Bile salts and crystal violet discourage growth of grampositive bacteria. Lactose plus pH indicator: Lactose fermenters produce pink or red colonies, nonfermenters are colorless.

Culture media: Types

- **Enrichment Culture**: favor the growth of a microbe that may be found in very small numbers. Unlike selective medium, does not necessarily suppress the growth of other microbes. Used mainly for fecal and soil samples. After incubation in enrichment medium, greater numbers of the organisms, increase the likelihood of positive identification.
- **Assay Media**-used to assay for vitamin, amino acid,antibiotics
- **Media for Enumeration**-used to determine bacterial count in water, milk etc
- **Media for characterization**
- **Maintenance Media**

Culture media: Types

Culture Media for Anaerobic Bacteria: anaerobes are being killed in presence of oxygen.

- **Reducing media contain ingredients that chemically combine with oxygen and remove it from the medium. Example: Sodium thioglycolate. Tubes are heated shortly before use to drive off oxygen. Plates must be grown in oxygen free containers (anaerobic chambers).**

Raw materials for culture media

- **Nutrient Broth**
  - Peptone-10 gm
  - Beef Extract-10 gm
  - Sodium Chloride-5 gm
  - Distilled Wated-1000 ml
- **Nutrient Agar-**Same composition as Nutrient broth but **also contain agar (1-2%).**
- **Peptone** contributes organic nitrogen in the form of amino acids and long-chained fatty acids.
- **Beef Extract** provides additional vitamins, carbohydrates, salts and other organic nitrogen compounds
- **Sodium chloride** is used to provide electrolytes and maintain the osmotic balance
- Water contributes as source of hydrogen and oxygen

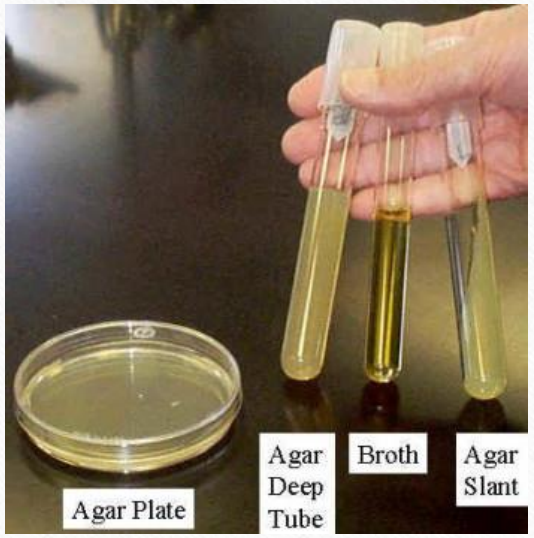
Raw materials for culture media

- **Meat extract, yeast extract – Protein degradation products/carbohydrates/Inorganic salts/Growth factors.**
- **Blood– It enriches media**

Cultivation of Aerobic Bacteria:

- **Small Scale**
- **Large Scale**

https://www.shutterstock.com/image-photo/flask-bacterial-culture-microbiology-laboratory-difference-564005815



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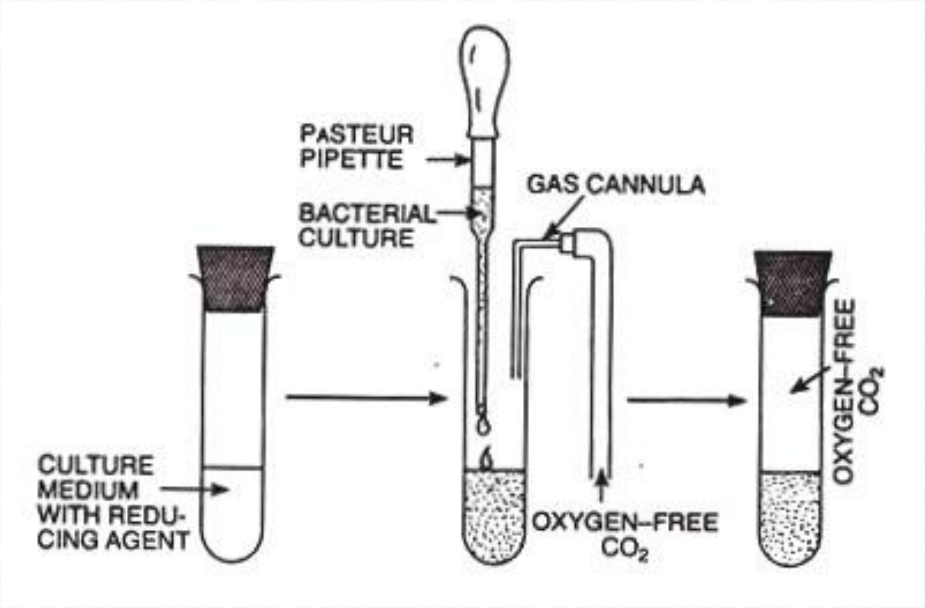
Cultivation of Anaerobes:

- **Pre-reduced Media**
- **Anaerobic Chamber**
- **Anaerobic Jars (or GasPak Anaerobic System)**
- **Oxyplates-**

Cultivation of Anaerobes: Pre-reduced Media

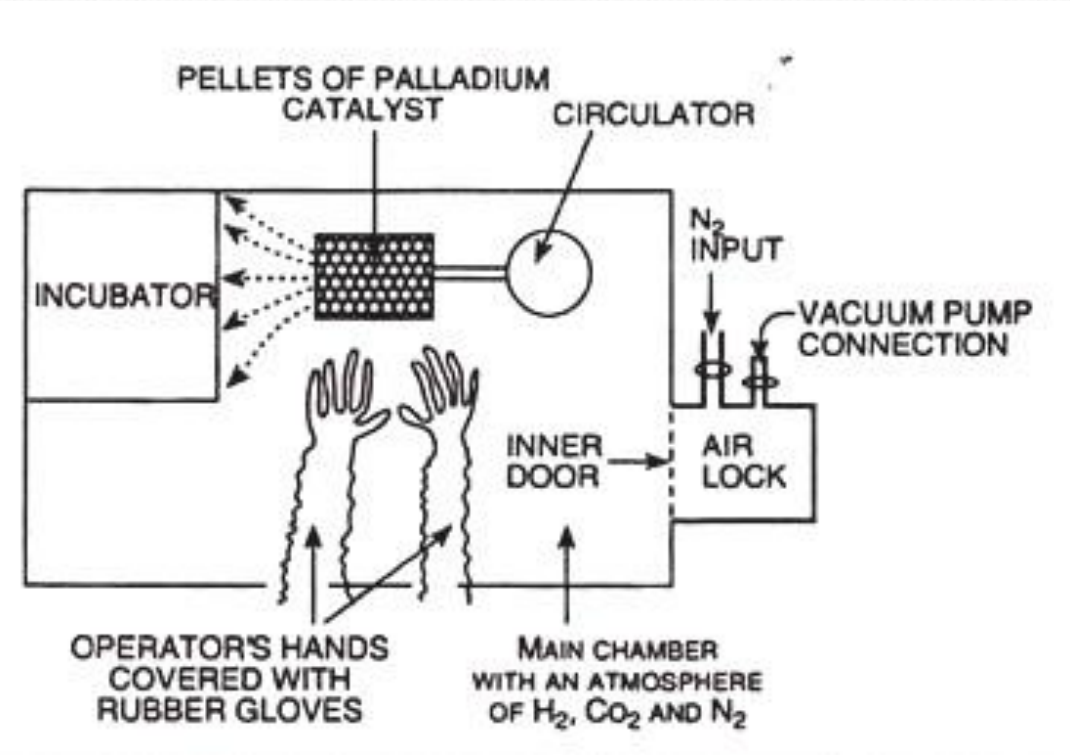
- elimination of oxygen from the culture medium is the simplest method.
- The liquid culture medium is boiled by holding for 10 minutes to drive off most of the dissolved oxygen
- Reducing agent like cysteine 0.1%, ascorbic acid 0.1%, sodium thioglycollate 0.1% etc is added to further lower the oxygen content.
- Oxygen-free N2 is bubbled through the medium to maintain anaerobic condition.



https://www.biologydiscussion.com/microorganisms/culture-media-for-cultivation-of-anaerobic-bacteria-4-types/55049

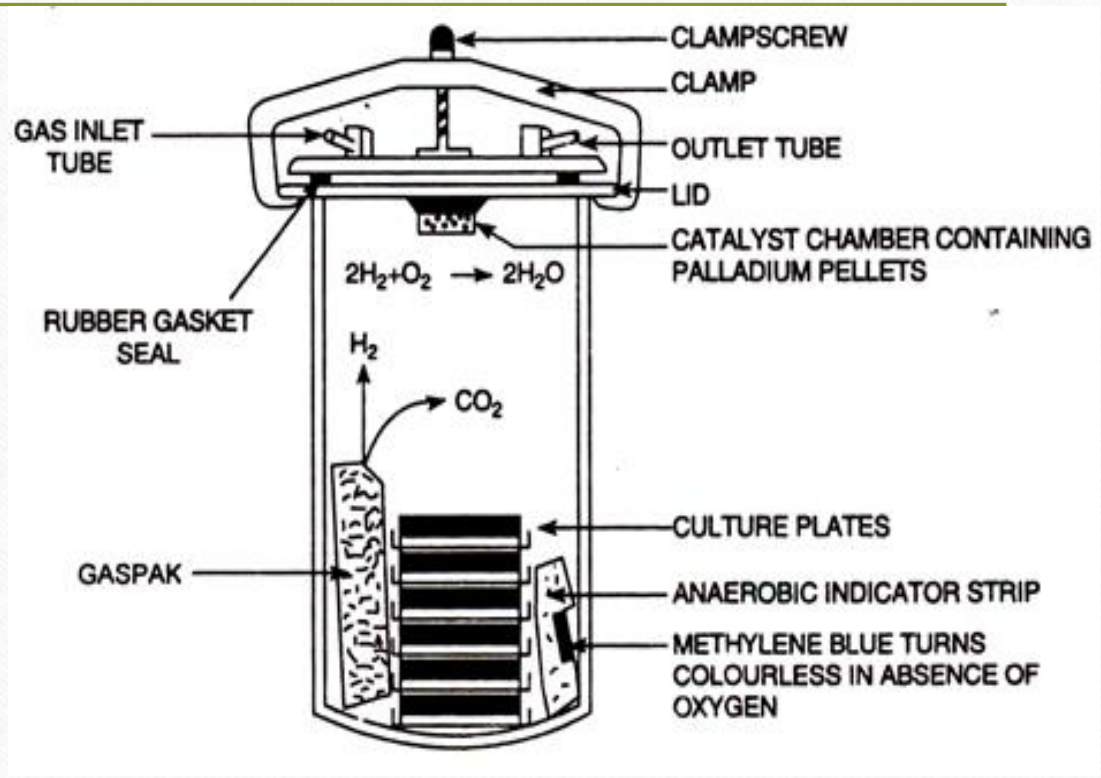
Cultivation of Anaerobes: Anaerobic Chamber

- Anaerobic chamber is an ideal anaerobic incubation system, which provides oxygen- free environment
- It is a plastic anaerobic glove box that contains an atmosphere of H_2, CO_2, and N_2
- Glove ports and rubber gloves are used by the operator to perform activities in the chamber.
- There is an air-lock with inner and outer doors.
- Air of the chamber is removed by a vacuum pump connection and replaced with N_2
- circulator fitted in the main chamber circulates the gas atmosphere
- pellets of palladium catalyst remove any residual O_2 present in the culture media by reaction with H_2



Cultivation of Anaerobes:Anaerobic Jars

- It is a cylindrical vessel made of glass or metal with a metal lid, which is held firmly in place by a clamp
- gauze sachet carry palladium pellets act as a catalyst for the conversion of hydrogen and oxygen into water.



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Cultivation of Anaerobes: Oxyplates

- New technique used for isolation of anaerobes in laboratories
- Use of Oxyrase enzyme that reduce O_2 into water
- There is a sealing ring in the lid of the plate

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