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Unit-1st

Microbiology

Micro + Bio + logy

↓ ↓ ↓
Small living science

It is the branch of science in which we study about the small living organism which is less than 1 μ (micron) which we can't see with naked eye we use microscope to see them.

e.g. Viruses, Bacteria, Fungi, Algae etc.
we study about them

History of Microbiology

- Aristotle → father of Biology ← living
Non-living
→ Many living, we can't see;

- Roger Bacon → 13th century → Disease
(Anything which enter in body + sick w)

- fracastorius → 1544 - communicable disease
→ those disease which spread through communication.

- Louis Pasteur → father of Microbiology

- Introduced sterilization techniques
→ pasteurisation of milk
62.8° - 30min (milk) all bacteria kill.

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- Lord Joseph Lister → father of Antiseptic Surgery.

→ He instructed surgeons under his responsibility to wear clean gloves and wash their hand before and after operation with 5% carbolic acid soln.

- Alexander Fleming → first antibiotic → Penicillin (1928)
→ *Pennicillium notatum* (made from)

Branches

- i) Virology → Study of Viruses
- ii) Bacteriology → Study of Bacteria
- iii) Protistology → Study of Protists
- iv) Mycology → Study of fungi
- v) Parasitology → Study of parasites

→ Acellular (cell not complete) eg. Viruses.

→ Cellular ← Eukaryotic eg. fungi, Algae etc.

Prokaryotic eg. Bacteria, Protozoa

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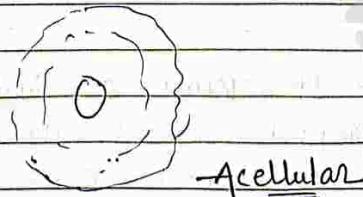
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Scope & its importance.

- i) Medical → Deals with diseases of human & animals.
- ii) Immunology → Study of the immune system that protects the body from pathogens.
- iii) Agricultural Microorganism → Impact of micro-organism on agriculture.
- iv) Industrial Microbiology → Using microorganism to make products such as antibiotics, vaccines, steroids, alcohols etc.
- v) Food & Dairy Microbiology → Use microorganism to make food such as cheeses, yogurts, pickles, beer, etc.

Classification →

- i) Acellular microorganism → Those in which cell does not developed properly.
eg → Virus.

Acellular

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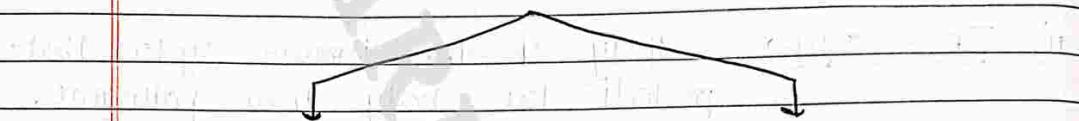
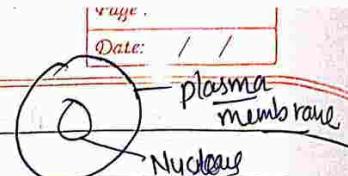
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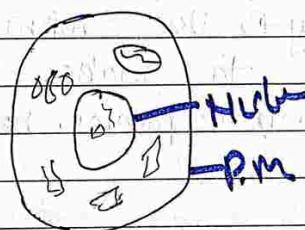
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11) Cellular → Cell developed.

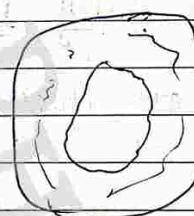
Those microorganism which cell was developed.



Eukaryotes
Those cells in which, cell membrane, Nucleus, their organelles are fully developed.



Prokaryotes
Those cells in which cell membrane developed, but Nucleus membrane does not developed, also some organelles not developed.



eg →

Fungi
Algae
Nematoda

eg →

Bacteria,
Protozoa,

- Bacteria: It forms a large group of unicellular prokaryotic that do not contain nucleus & other membrane organelles

Archaeobacteria → Ancient bacteria

Eubacteria → True bacteria

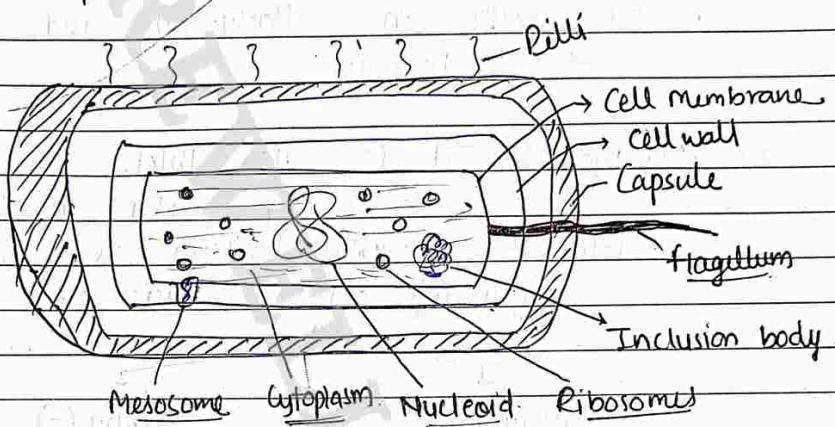
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• Structure of Bacteria →

On Ultra-microscopic view it look like as small capsule.



Parts of bacteria's

- 1) Capsules
- 2) Cellwall
- 3) Cell Membrane
- 4) flagella
- 5) Cytoplasm
- 6) Nucleoids
- 7) Inclusion body
- 8) Mesosomes
- 9) Ribosomes
- 10) Pilli

- i) Capsules → Outermost thick and slippery structure and rigid and flexible.
- Composition [98% water + 2% glycoprotein].
 - Basis of thickness
 - + Macro capsule → $> 0.2 \mu$
 - + Micro capsule → $< 0.2 \mu$.
 - It is identity of bacteria and decide its virulence (ETIOLOGY)

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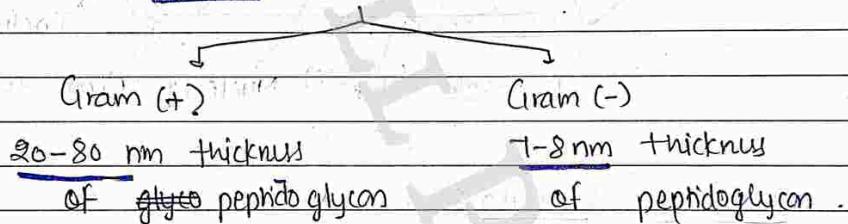
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- It gives protection to the bacteria, also give shape and size to bacteria, also prevent from phagocytosis.
- It causes disease in human body.
- It helps in attachment and repulsion with other bacteria.

2) Cell wall → It is also thick structure,

- made up with peptidoglycan layers.
- We can identify bacteria as gram (+) or gram (-) on the basis of cell wall



⇒ It provides protection, shape and identification to the cell bacteria.

3) Cell membrane → thin layer in prokaryotic cells or bacteria.

- Made up with phospholipid bilayer.
- It acts as semi-permeable membrane.

4) Cytoplasm : less developed Nucleus, so it is called nucleoid. NUCLEOID and Nucleoid contains, 60% DNA, 30% RNA, 10% protein.

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5) Inclusion Body: It stored food or food of bacteria is stored in the form of inclusion body.

6) Ribosomes → It helps in protein synthesis in bacteria.

In bacteria 7 types of ribosomes present.

7) Mesosomes → It is slightly inside in cell wall
 • It is extra chromosomal DNA.
 • It helps in cellular Respiration.

8) flagellum → It is 15-20 nm long thick hair like structure and 2-5 nm diameter.
 • It works like as antenna, which gives signal to bacteria for food, danger, locomotion etc and made up by flagellin protein.

9) Pili → It is small thin 8-10 nm hair like structure.

- It helps in attachment of bacteria with other bacteria.
- Also helpful in transfer in genetic material

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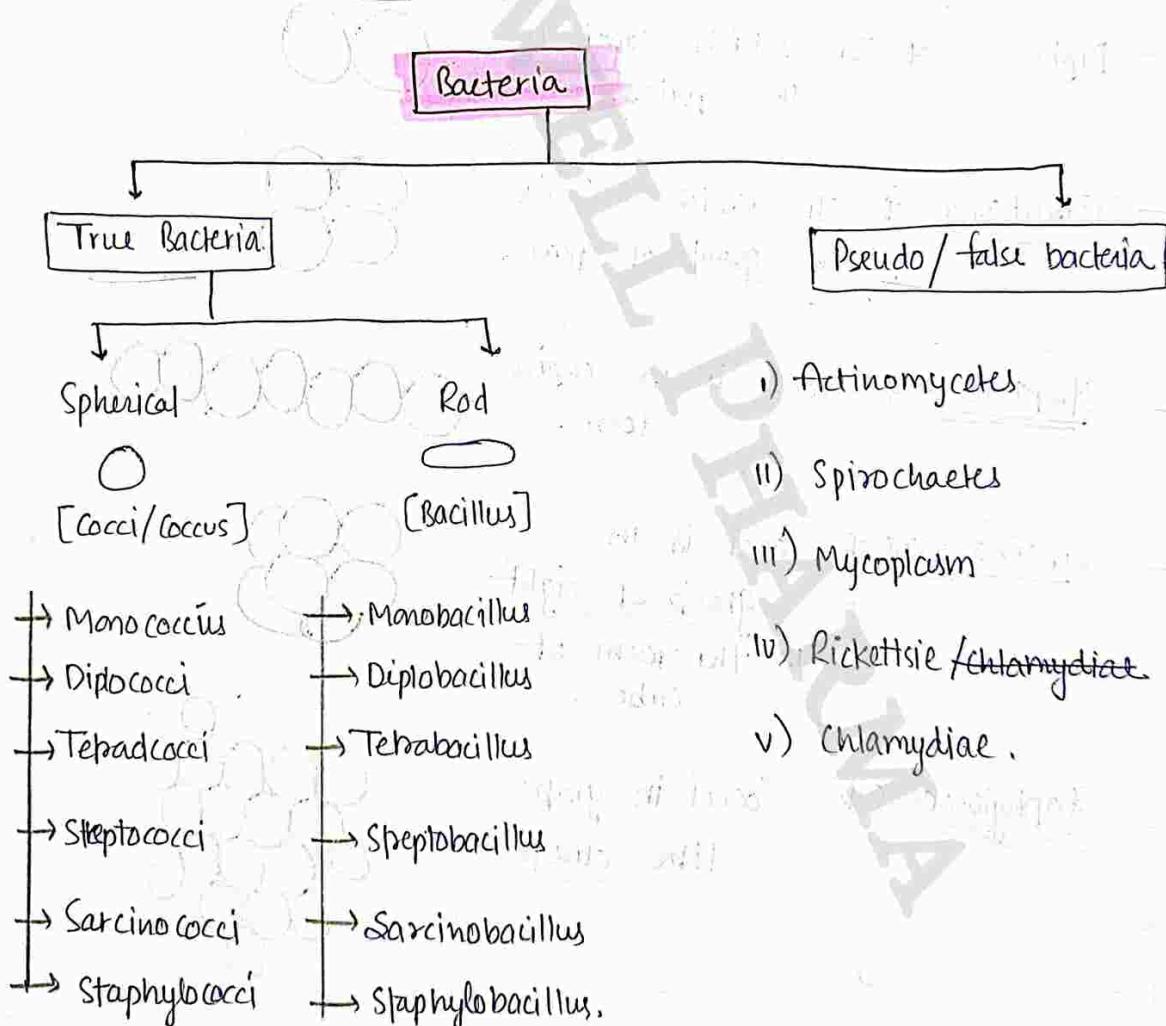
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Microbiology - Unit-1 | Lecture - 3rd

Morphological classification of Bacteria (According to external appearance)

On the basic of morphological, bacteria classified into six major groups.



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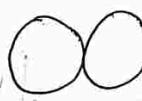
1) True bacteria & thus are real bacteria and their shape as same as bacteria.

- Cocci / Coccus → These are spherical or oval shaped. These are further classified as—

— Monococcus → In which cocci is in single form.
One



— Diplococcus → In which cocci is in pairs.
Two



— Tetradcoccus → In which cocci is in group of four
four



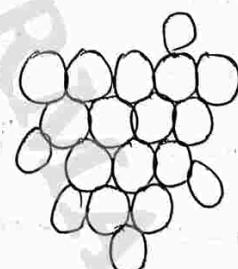
— Streptococcus → Cocci is in chain form.
chain



— Sarcinaoccus → Cocci is in group of eight or in the form of cube.
Cube form



— Staphylococcus → Cocci in grape-like clusters.
Grapes like cluster (grape)



• Pencil use → only for understand easily not for fair or exam

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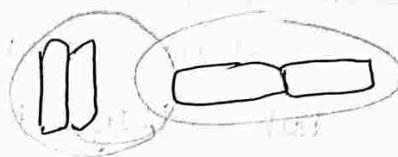
• Bacilli/Bacillus ⇒ These are rod shaped bacteria

thus are further classified as:-

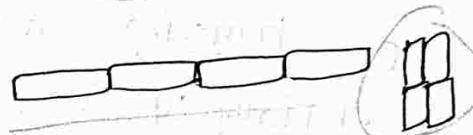
→ Monobacillus ⇒ In which bacilli is in single form.



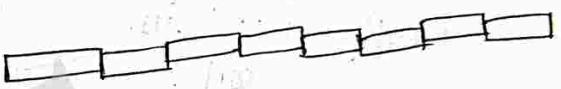
→ Diplobacillus ⇒ In which bacilli is in pairs.



→ Tetrad bacillus ⇒ In which bacilli is in group of four.



→ Spore bacillus ⇒ ~~Cocci~~ Bacillus is in chain form.



→ Sarcinabacillus ⇒ Bacillus is in group of eight or in the form of cube.



→ Staphylococcus ⇒ Bacillus is in the form of grapes like clusters.



cluster form

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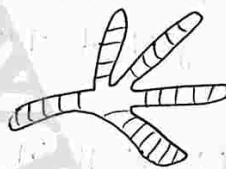
(2) **false/Pseudo Bacteria** \Rightarrow These are those bacteria, which are actually a bacteria, but their shape is different from bacteria.

1) **Actinomycetes** \Rightarrow (Actin-ray, Myces \rightarrow fungus)

These are rigid organism like true bacteria but they resemble fungi in that they exhibit branching and tend to form filaments.

- Basically it is gram (+)

- Mostly present in soil.



2) **Spirochates** \Rightarrow These are relatively longer, slender, non-branched microorganism of spiral shape.

- Basically it is gram (-)



- Double membrane



- Length \rightarrow 3 to 500 μ (micron)

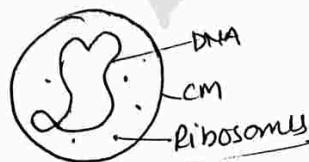
- It may be occurs on bacillus, but double membrane

3) **Mycoplasma** \Rightarrow These are in oval shaped and these are lack in rigid cell wall (cell wall lacking). And not developed properly.

- look like as virus
cell membrane not developed.

Some organelles and DNA present. [Smallest bacteria]

- caused disease in Animals



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4) Rickettsiae → These one look like as filaments in which no branched & no chain present.

→ these are non-motile (no movement)

→ Basically it is gram (-).

→ its diameter is 0.1-0.4 μ (micron)



5) Chlamydiae → It is oval-shaped.
peptidoglycan + protein are present in it.

→ It is mostly responsible

for the disease in human



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CULTURE MEDIA

That media (medium) in which bacteria is birth or grows. Culture media contain all essential material which is required for growth and development. So, it invite bacteria to grow.

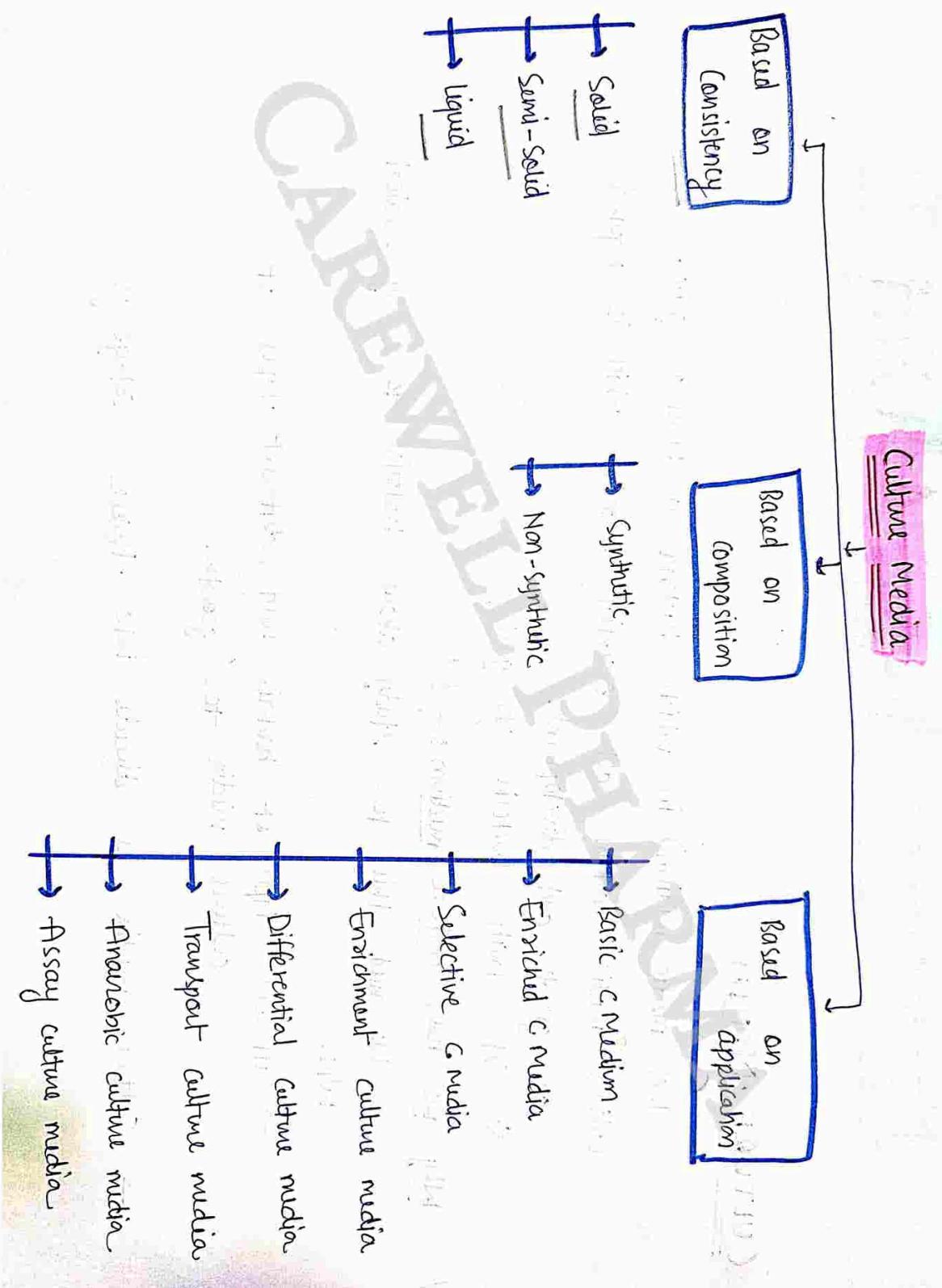
Why we need culture medium :- ? ?
We need this for study about bacteria for any medical helps.

→ Different types of bacteria need different types of culture media for growth

→ Culture media is divide into these categories:-

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Based on Consistency

It is of three types, Solid, semi-solid and liquid.

So, bacteria needs different media.

And, Agar is much more important for this. It is an solidifying agent.

→ Agar (1.2%) → Solid medium e.g. Nutrient Agar

→ Agar (less than 0.5%) → Semi solid e.g. Nutrient broth containing 0.5% agar

→ Agar (Absent) → Liquid

e.g. fluid thioglycollate broth

1) Based on Composition

It is depend upon the quality or quantity ratio of material which is used to prepare culture media.

material which is used in this is:

Nitrogen source, water, growth factors and salt etc.

+ Synthetic → (Highly pure) in which we know the composition of ingredients. e.g. Peptone water (1% peptone + 0.5% NaCl in water)

+ Non-Synthetic → (Impure) in which we don't know about the composition of ingredients. e.g. Yeast extract broth etc.

• Based on Application :-

In which we create culture media according to need of bacteria, on basis of applications of bacteria.

- i) Basic Culture medium → It is applicable for all bacteria, because it contain all types of essential material, so in which all types of bacteria grow.
- ii) Enriched Culture media → It is for those bacteria, which need extra nutritional material (extra material → blood, serum etc.) for extra growth.
- iii) Selective Culture media → these are specially designed culture medium made for special or any one bacteria.
- iv) Enrichment Culture media → It is designed for some specific type bacteria and in this media, antibiotic is used for reduce unwanted bacteria so that special type of bacteria grows easily.

v) Differential culture media :- (liquid medium)



→ In which metabolic dye is used to identify the different colonies by different color.

(dye is used for color) e.g. Blood agar

vi) Transport medium :- It is used when culture medium is made for

long time or not used frequently.
And it is prevent from drying.

→ It is used because, in culture media bacteria is died in around 4 days, but if we need this after 15-20 day
or after many days, so we prepare this transport

culture media.

vii) Anaerobic culture media :- It is used for those bacteria, which it

does not required Oxygen. And in

which we use Hemin or Nit. K and bacteria
will grow easily.

e.g. Robertson's cooked meat medium

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- Assay medium:— This culture media is used for study ~~method~~ ~~of~~ ~~different~~ ~~types~~ ~~of~~ ~~microorganisms~~ ~~in~~ ~~order~~ ~~to~~ ~~determine~~ ~~the~~ ~~concentration~~ ~~of~~ ~~material~~ ~~which~~ ~~is~~ ~~used~~ ~~to~~ ~~make~~ ~~culture~~ ~~media~~,
→ It is right, perfect or not.

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Nutritional Requirement :-

FOR BACTERIA

All living beings required nutrient for their growth. So, bacteria also need this for growth.

- 1) Essential elements (major macronutrients) :- these elements are important for bacteria and required in large amount.
 - Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, Sulphur etc—
 - Hydrogen & Oxygen ⇒ these are made available from water added to culture media.
- Carbon & Nitrogen ⇒ Carbohydrates is the principal source of carbon which is degraded by some bacteria - either by oxidation or by fermentation.
- Nitrogen ⇒ It is the main source of protein and nucleic acid for bacteria and its main source is ammonia.
- Water ⇒ Moisture is an essential requirements for bacterial growth as for 80% of the bacterial cell consists of water.

- Sulphur and phosphorus ⇒ Sulphur forms part of structure of several coenzymes and cysteine and methionyl side chain of protein.
Phosphorus is required as a component of nucleic acids, ATP, coenzymes, NAD and flavins.

ii) Mineral sources ⇒ Thus are Potassium, calcium, magnesium, iron, copper, cobalt, manganese and zinc required in little amount for enzyme functions.
and it can be provided in tap water or as contaminants of other medium ingredients.

iii) Energy sources ⇒ Carbon dioxide is required by all bacteria and much more important for cellular metabolism and it can be provided by atmosphere to bacteria.

And energy source depends upon the nature of bacteria.

- Aerobes → required oxygen for growth. (obligate aerobes) eg. Pseudomonas, Bacillus
- Anaerobes → which grown only in absence of oxygen. eg. clostridium, Bacteroids.

- Autotrophic Bacteria → capable to synthesized their own food.
 - Photosynthetic → Use sunlight as a source of energy ($\text{CO}_2 + \text{H}_2\text{O}$)
 - Chemosynthetic → without photosynthetic pigments. they use sulphur as a ($\text{CO}_2 + \text{H}_2\text{S}$) source of energy

• Heterotrophic Bacteria → unable to synthesized their own metabolites.

→ Photoheterotrophic → they obtain carbon from organic compound, made by other organism.

→ Chemo-heterotrophic → they take their food from another plants and animals, they obtain both energy and carbon source from organic compounds.

- (iv) Growth factor ⇒ for growth Bacteria need Purine & Pyrimidine, it is much more important for formation of DNA & RNA and also for cell division.
- And also required some amino acid and protein and Harmony for growth.

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- v) Vitaminus → In many cases, the growth factor are identical with vitamins.

Thiamine, Riboflavin, Nicotinic acid, Pyridoxin, Folic acid and Vitamin B₁₂.

Microbiology - Unit-Ist

contain and magnesium, which may be react with phosphate present in peptones, and may be inhibit the growth of bacteria.

Raw Material used for Culture Media

That material, which are used in making of culture media.

- i) Water
- ii) Peptone
- iii) Meat extract
- iv) Fat
- v) Yeast extract

Water :- In bacteria 70-80% of water is

present and it used as a vehicle for flow of nutrients.

So, water is necessary for preparation of culture media. But, distilled water is preferred in use, because tap water

ii) Peptone :-

It is hygroscopic in nature (which attract water from its surroundings).

It is ~~partially~~ complex mixture of partially

digestive protein obtain from meat, heart muscle, fibrin, soya meal etc.

It contain certain growth factor, imp. constituents like protease, amino acids, magnesium,

potassium which supply ribogenous material and also act as a buffer.

iii) Meat extract :-

It contain gelatin, protease, creatine, purines and growth factor etc. which is essential for bacteria and it prepared from fresh lean meat by hot water extraction process.

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ii) Agar:-

It is important material for culture media, and it is used for preparation of solid media.

It is long chain polysaccharide obtained from seaweed's algae.

- It act as a good solidifying agent.
- It is easily available.
- It has no nutritional value in media.
- Microorganism cannot eat and destroy it.

v) Yeast extract:-

It consists of the cell contents of the yeast without the cell walls, if contain carbohydrates, amino acid, growth factor, inorganic salts. Used mainly as a source of vitamins and substitute for meat extracts.

Physical parameter for growth

There are many physical conditions, which are essential for bacterial growth or it affect the growth of bacteria.

i) Temperature:-

It is one of the important factor that for growth.

- Optimum growth temp :- That temp, bacteria grow rapid and maximum.
- Different bacteria grow at different temp.

- The highest temp at which microbe shows growth is known as maximum growth temp.
- The lowest temp at which microbes shows growth is called minimum growth temp.
- Based on temp, they classified :-

- i) Psychrophiles ($0\text{--}15^\circ\text{C}$)
- ii) Mesophiles ($20\text{--}40^\circ\text{C}$)
- iii) Thermophiles ($45\text{--}70^\circ\text{C}$) or may be more

ii) pH :-

All bacteria grow in a certain pH range.

- Acidophiles ($\text{pH Range } 1-6.5$)

- Neutrophiles ($6.5-7.5$)

- Alkalophiles ($7.5-14$)

iii) Gaseous Requirement :-

The principal growth that affects growth

are O_2 and CO_2 .

Aerobic bacteria \rightarrow Required O_2 for growth

Photosynthetic and Chemosynthetic bacteria \rightarrow Required CO_2 for growth.

iv) Osmotic Pressure :-

Bacteria can tolerate osmotic variation due to mechanical strength of cell wall.

They can growing media with varying

contents of salts, sugar and other solutes.

v) Light :-

Bacteria are light sensitive (sensitive to UV light, direct light (sunlight) and other radiations, So, Darkness is usually favourable for growth of all microbes.

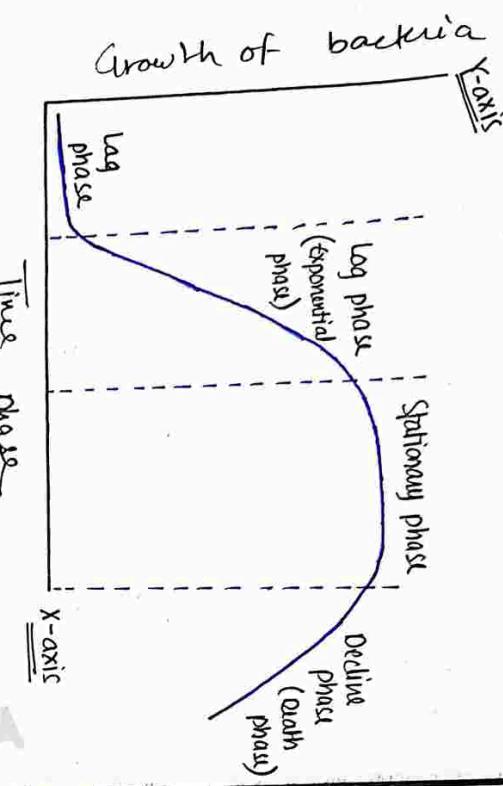
vi) Growth Curve

Bacterial growth curve :-

When a graph plot between the growth of bacteria and time phase is called growth curve.

The resulting curve has four distinct phases:-

- i) Lag phase
- ii) Log phase
- iii) Stationary phase
- iv) Decline phase (Death phase).



- i) Lag phase :- In this phase, there are no growth of bacteria, because culture media is just prepared, so upto 2-3 hour there are no growth of bacteria, only nutrition medium present.

- ii) Lag phase :- In this phase, there are maximum growth of bacteria, because after the germination of bacteria, they reproduce in fast rate and bacteria increases,

and there are nutritions are more than the bacteria, so bacteria grow maximum.
 - Also called as exponential phase

iii) Stationary phase :- In this phase, growth of bacteria stop, and it may be stationary, because no. of birth of bacteria is equal to the no. of death of bacteria.

- It is just happened due to lack of nutrition medium.

- iv) Decline phase :- Also known as death phase, In this phase, bacteria start dying, because no. of bacteria is more than nutrition medium.

- So due to lack of nutritions, bacteria start dying.

Isolation and Preservation method for
Pure Culture

V.V.I.M.P

• Isolation of Pure culture :-

⇒ A culture media, which contain more than one kind of microorganism is called

a Mixed culture media.

⇒ A culture that contain only one kind of microorganism is called a

Pure culture

⇒ Isolation is the separating of a particular microorganism from the Mixed culture.

• It is used to study about specially one species & for growth of any one species in their own pure culture.

⇒ The most commonly used method for pure culture:-

i) Streak-plate technique (imp).

ii) pour-plate technique

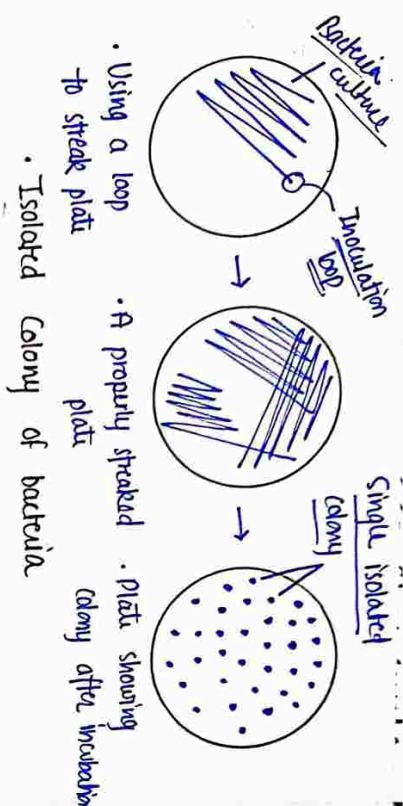
iii) Spread plate technique (imp)

iv) Serial dilution technique (imp).

v) Streak plate technique :-

In this method, the bacteria is isolated from (mixed culture media) by applying streak on the surface of culture media with the help of inoculation loop.

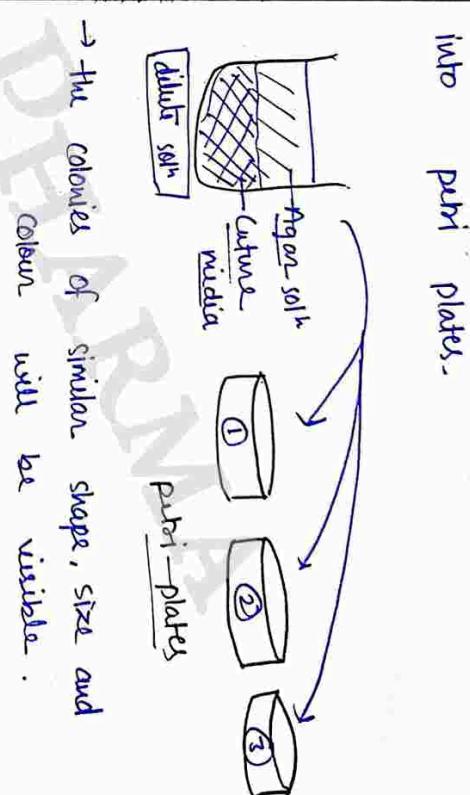
• firstly inoculation loop is heated for sterilization, and then streak on the surface of mixed culture, then it speak on new sterile culture media.



- Using a loop to streak plate
- A properly streaked plate showing colony after incubation
- Isolated colony of bacteria

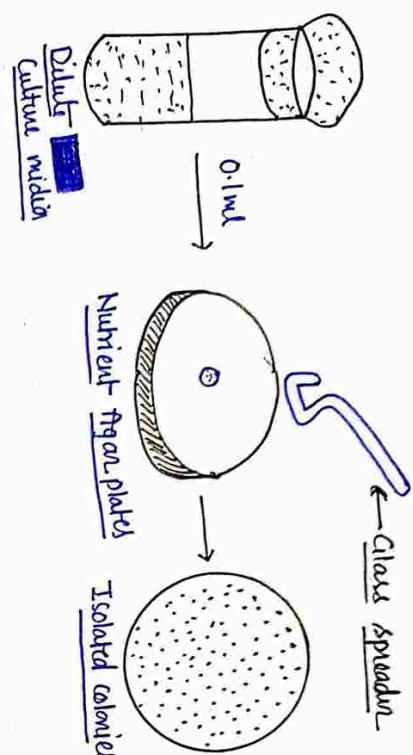
(Inoculation loop)

- Pour plate method :-
In this method, Dilute agar solution (Agar mix (dilute) with some water) is mixed in the mixed culture media, and dilute the mixed culture media.
- Now, these dilute culture media after proper mixing, is transferred (pour) into petri plates.



→ the colonies of similar shape, size and colour will be visible.

- Spread plate technique :- In this method, a mixed culture media is diluted with some sterile water or a saline (salt+water) soln.
- Now take 0.1ml of culture media drop and placed on the surface of agar plate, then spread it with the help of glass spreader, all over the surface. thus, allow bacteria to grow, and isolated colonies are observed after 24 hours and counted.



(iv) Serial dilution method :-

In this method, take five - seven

test tubes with filled 9 ml of sterile

distilled water (solution).

Now take 1 ml soln from original suspension
and diluted it in 1st test tube, then

take 1 ml from 1st and diluted it in 2nd.

Take 1 ml from 2nd and diluted it in 3rd.

Repeat this process until all cultures should be diluted.

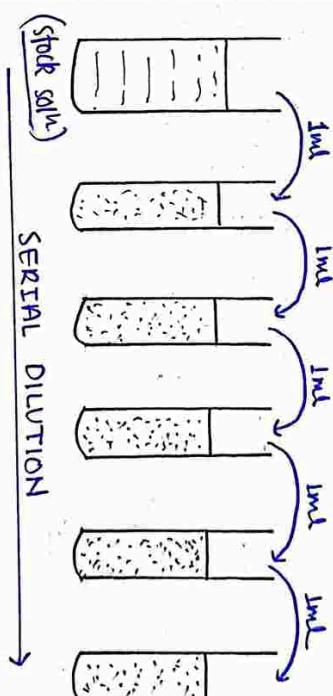
- After dilute all tubes, take 1ml of each

test tube and pour into nutrient agar

plates.

As number of test tube increase, the concentration of bacteria is decreases and the dilution is increases.

→ This method is used for actinomycetes bacteria (soil).



→ Take 1 ml of from each
and take it on
separate nutrient agar plate.

Preservation method for pure culture

[Preservation]:- In this, maintain pure

culture for long periods

in viable condition.

- for preservation, it is important that microbial growth stop or may be least.
- Avoid contamination in media.

- Methods:-

- Subculturing
- Refrigeration
- Paraffin method
- Cryopreservation

changed.

So, culture media should be pure.

Paraffin Method :- In this method, paraffin oil added in the culture

media, which inhibit the growth of bacteria, because it form a thin layer over the surface of culture medium.

iii) Refrigeration :- In this method, pure

culture is placed under refrigerator in which temperature is about (5°C - 4°C) for some days.

so, microbes not birth (growth) and others microbe will kill.

iv) Cryopreservation :- In this method, we put culture media at very low temp. (minus) (-196°C), then all the microbes were kill by dehydratation.

) Vippolization :-

In this method, culture put in very low temp. thus reduced the pressure.
So, microbial cells are dehydrated and their metabolic activities are stopped.
After it sealed and stored in the dark at 4°C in refrigerators.

- Also called freeze-dried pure cultures.

Quantitative Measurement of Bacterial Growth

After making pure culture media (isolated). we placed culture in incubators, in which bacteria grow rapidly.

- Now, we have to count (calculates) the no. of bacteria in culture media.

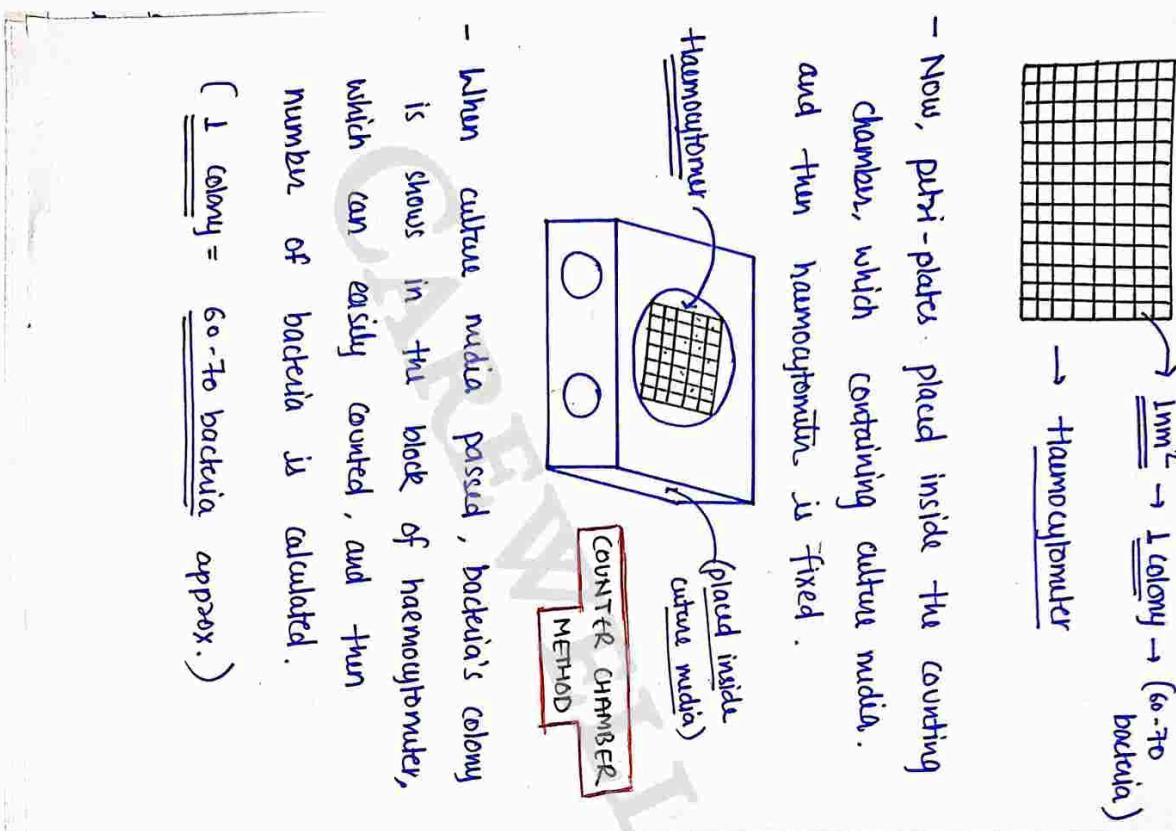
• Bacterial growth (no. of bacteria) is calculated in two terms:-

i) Total count (Direct method)

ii) Viable count (Indirect method)

iii) Total count & In this measurement, count all ~~bacteria~~ bacteria (either it live or dead).

- i) Counting chamber method :-
In this method, firstly we required colonies counting chamber in which, we pass the culture, and one lens is upper side, from where we watch the bacteria.
- ii) Microcytometer, it is sieve like but rectangular, in which size of one hole is 1mm², in which only one colony can identify.
- iii) It is also called direct method, because in which we directly count the no. of colonies of bacteria by using microscope.
- iv) Thus we count by two methods following:—
 - i) Counting chamber method
 - ii) Electron counter method



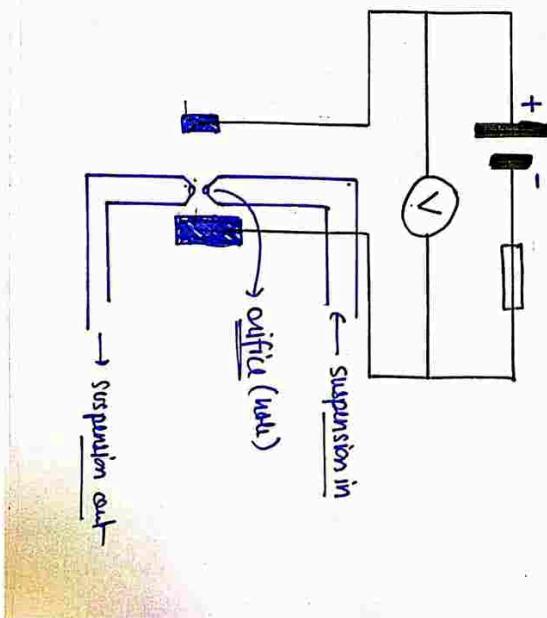
- When culture media passed, bacteria's colony is shows in the block of haemocytometer, which can easily counted, and thus number of bacteria is calculated.
- ($1\text{ colony} = 60-70 \text{ bacteria}$ approx.)

ii) Electron Counter Method :-

In this method, **Coulter Counter** is used for measurement,

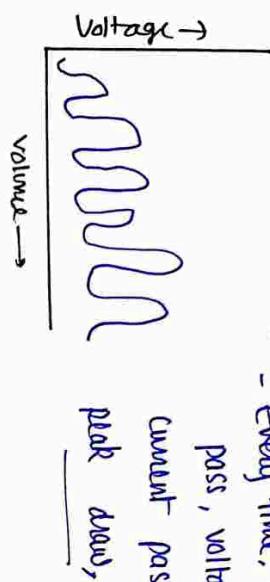
In this, bacterial suspension (culture media) is passed through a capillary tube, the (capillary) diameter of tube is so microscopic that it allow only one cell to pass at a time.

- When bacteria is passed between this, current pass and voltage generated and one peak is formed in chart.



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- Every time, when bacteria pass, voltage apply

current pass, and

- i) Plate count method
- ii) Membrane filter count method

i) plate count method :-

It measure the cell density and providing information regarding the living bacteria only (viable).

- In this method, culture media soln (in which we have to count bacteria) is diluted several times (serial dilution)

Here to write the serial dilution process
* Not viable in water in short with small diagram.

- After dilution take 1ml solution from each test-tube and put it on agar plate

individually.

- Also called indirect method because in which we also count bacteria indirectly.

→ following:-

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- After incubated, the number of colonies can be seen. (count colonies of each plates).
- Through this we know the quantity of bacteria.

ii) Membrane filter method :-

In this method, we take

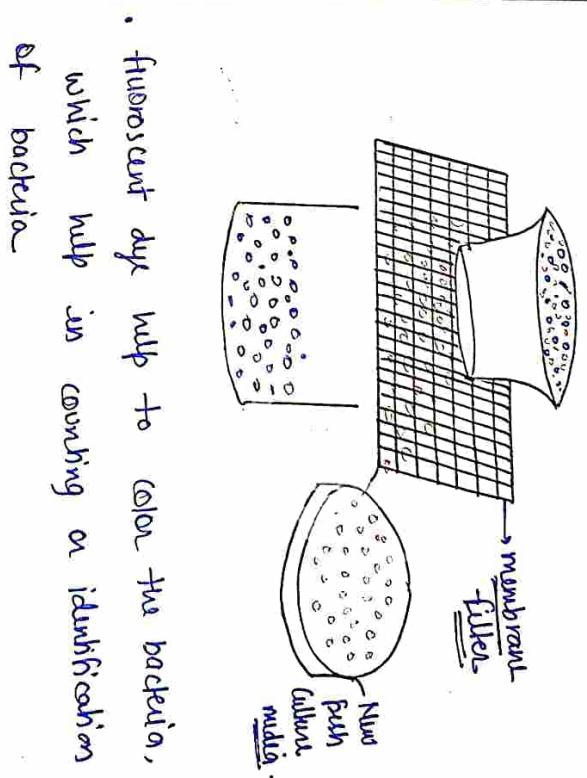
membrane filter, which does not

allow to pass the bacteria from membrane

- In which we also mix the fluorescent dye, which attached with bacteria's colony,

so we can easily identified bacteria.

- When culture media passes through membrane filter, all the material content of culture media pass down and bacteria are separated on membrane filter, which we further collected in a new fresh culture media.



Indirect method :-

(Turbidimetric method) → It is indirect method in which we use to measuring cell mass and no. by observing the light scattering capacity of sample or by their turbidity.

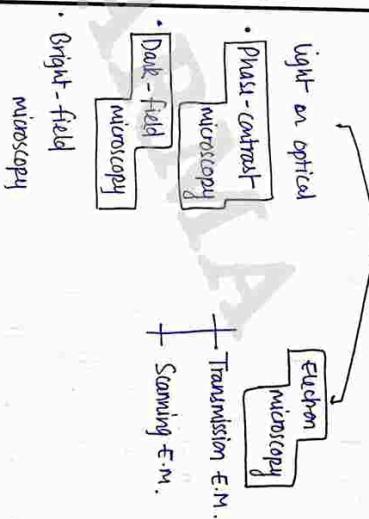
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- firstly bacteria solution make, solution should be produce turbidity due to presence of bacteria
- Turbidity measured by passing light through solution, And the more turbidity means the more bacteria and less turbidity means less bacteria.

- Study of different types of
- i) Phase Contrast microscopy
- ii) Dark field Microscopy.
- iii) Electron microscopy (very important)



Microscope ⇒ A microscope may be defined as it enlarge objects on our naked eye, And, This phenomenon is known as Microscopy

Depending on source

- light or optical microscope
- Electron microscope

- 1) Phase Contrast Microscopy :-
— discovered by Fritz Zernike in 1953 and awarded for nobel prize for this.
- It produces high-contrast images of transparent specimens. (such as living cell).
- The condenser has a special diaphragm consisting an annular stop, which allow

only a hollow cone of light ray to pass through the condenser.

The phase plate is special optical disc located in the real focal plane of the objective, which retard the direct rays.

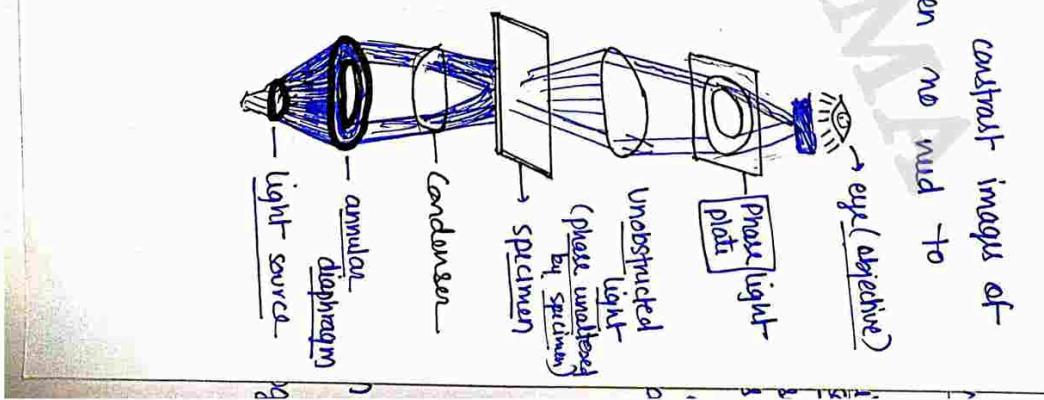
This phase plate emerged the light in single phase (phase changed) and help to contrast the image.

Principle :-

- When light passes, condenser annulus modifies the light beam.
- Then, when sample (specimen) is inserted it Scattered parts of light which is then refocused to the detector (image formed when).
 - to distinguish direct light from the scattered by the sample, a phase plate is inserted
 - light crosses a thicker part of the plate, this shift its phase

Advantages →

- thus, this scattered light interferes with direct light which creates a phase contrast.
- thus allow to see high contrast images of transparent specimens, even no need to color them.



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ii) Dark field microscopy :-

The microscope which forms a bright image against a dark background is called field microscopy.

Principle :- The effect produced by the dark field technique is that of a dark background against which object are brilliantly illuminated. The condenser

has a stop plate which blocks the light

over the centre of the field where

specimen lies

→ Used to see unstained samples.

→ stop plate (opaque disc) is placed under

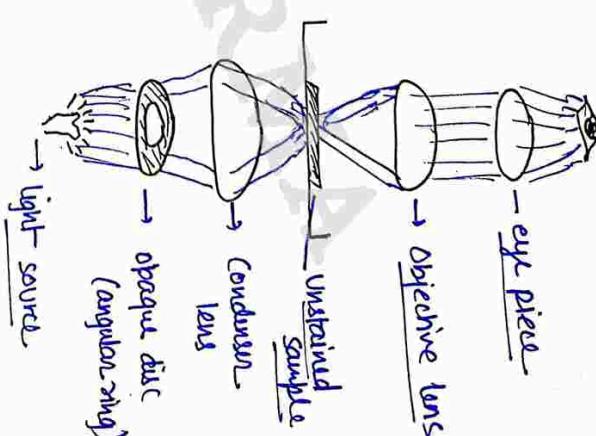
condenser lens due to which the

light scattered by object on slide

reached to eyes.

Advantages :-

- ideal for viewing object that are unstained, transparent and absorb light or no light.
- Simple and effective

iii) Electron Microscopy [em]

- It is a technique used to obtain high resolution ultrastructure of biological and non-biological specimen.

- In this, electromagnetic field used to form electron optical lens system.

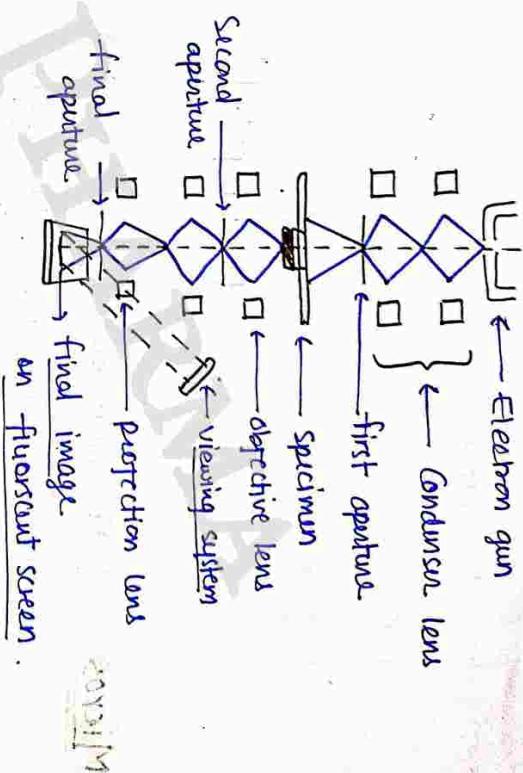
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- In this source of illumination is Beam of e⁻ used.
- It gives very clear high resolution images, because it has very short wavelength (10000 shorter) than visible light photons.
- In this fluorescent scan was used for final image
- Types →
 - 1) Transmission electron microscopy (TEM)
 - 2) Scanning electron microscopy (SEM)
- 1) TEM → It gives complete image, because in which, electron cross the specimen,
- Used thin specimen for better result.
- 2) SEM → It produce an 3D structure of the surface of specimen.
 - In TEM electrons are passed through a specimen, in SEM electron are scattered or emitted from the specimen's surface, only surface feature were evident.
 - SEM gives the structure of surface only while TEM gives the internal structure.



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Cultivation of Anaerobes

- Also called as growth of anaerobes.
 - [Anaerobes]** \Rightarrow Those microorganism or bacteria which does not required oxygen for its survival or growth.
- or
- Those microorganism which grow in the absence of oxygen.
- for cultivation, we have to create that system in which oxygen not present or we create it by reducing oxygen.
 - there are following two method for this
 - Candle Jar method.
 - Gas Pak Jar method.

i) Candle Jar method:

It consist of petri-plates in which culture media created,
- One ~~the~~ candle (burns)

Working Enclose the inoculated plates in an

closed jar (tight closed) with a burning

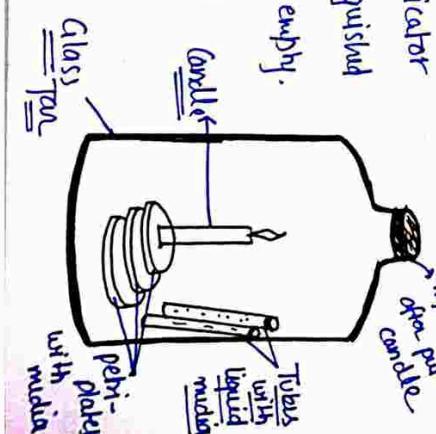
candle,

As we know than red candle required O₂ for burning, so as the candle burns,

the oxygen reduced and CO₂ rich + O₂ poor atmosphere create in Jar

Candle act as indicator when it gets extinguished shows that oxygen empty.

and jar is ready for growth of anaerobes.



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ii) Gas Pak Jar method :-

It consist of

- Polycarbonate jar.
 - a lid with a gasket to prevent airflow
 - Indicator strip
 - Disposable gas generating pouch (a pouch containing sodium borohydride and sodium bicarbonate).
 - a palladium catalyst.
- WORKING**
- Inoculated plates or tubes are placed inside the jar with gas generator pouch and indicator strip and sealed completely.
 - In presence of water (H_2O) chemicals present inside pouch (sodium bicarbonate, $NaHCO_3$ & sodium borohydride, $NaBH_4$)

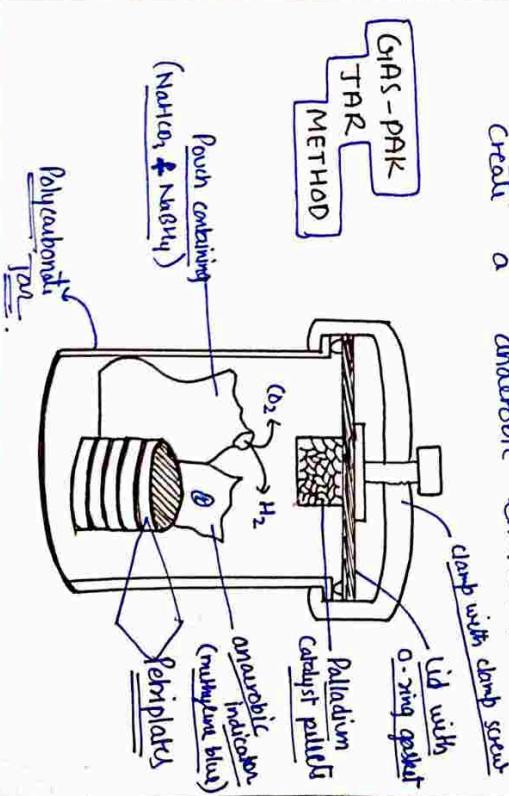
start chemically producing hydrogen (H_2) and carbon dioxide (CO_2) gas.

- Then hydrogen (H_2) react with O_2 (oxygen) present inside the jar producing water (which forms as condensation on inside of the jar).



- It happened with the help of catalyst palladium (attached on lid of the jar).

- finally CO_2 replaced the removed O_2 and create a anaerobic environment.



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