DATE
4/9/2019

Day - Wednesday

UNIT - 1st

Introduction

[A] The term of microbiology is derrived from 2 words -

Micro + Biology

[B] It means the study of life cycle of micro- organism is called microbiology.

[C] Those living organism whose size is less than 1 micron, unable to seen with naked eye.

[D] They are very small organism like bacteria virus, protozoa etc.

[E] These micro- organism may be unicellular or multicellular, and micro- organism found in diffrent - diffrent shapes like - spherical cylindrical, and rod shaped and comma shaped.

[F] These micro-organism canbe seen only with microscope.

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[G] These micro-organism are responsible for diffrent - diffrent disease to human body.

History of microbiology

[A] 1st time 'Aristotle' gave the concept of living and Non-living organism.

[B] In 13th century 'Roger Becon' gave the term disease and it is caused by micro-organism.

[C] Fracastorius in 1546 gave the concept of communicable disease, which ~~is~~ affect the healthy person when they comes in contact with infected person

[D] In 1st concept of living micro-organism was given by the Antony-Van-Leewenhock in 1675, so he is known as Founder of microbiology.

[E] He gave the term Animal cules for protozoa and bacteria.

[F] In 1729 Splanzini prepare the 1st culture media in which bacteria and virus canbe grow.

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[E] Scientist Thon Tyndall tell that the micro-organism are kills at high temperature, this is called Tyndall effect.

[G] The term microbiology was given by Lewis Pasture and he is also known as father of microbiology.

Lewis pasture gave the term aerobic and Anaerobic bacteria.

Lewis pasture explain that when milk is heated at 62.8° for 30 minutes then all microbes are kill and milk becomes pure, this process is known as pausterisation.

[H] Lord Joseph Lister gave the concept of use of antiseptic before surgery.

He is also known as Father of antiseptic Surgery.

[I] Alexender flamming discover the 1st ~~antiseptic~~ antibiotic Penicilline from fungus Pencillium Notatum, which was isalated from Tobacco leaf.

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Branches of Microbiology

On the basis of their taxonomy and Research work and the field where it is applied microbiology can be devided into following part :-

- Microbiology
  - Taxonomy
    - Bacteria - Bacteriology
    - Fungus - Mycology
    - Nematods - Nematology
    - Parasite - Paragitiology
    - Algae - Phycology
    - Protozoa - Protozology
    - Virus - Virology.
  - Research
    - Astromicrobiology -
    - Evolutionary microbiology -
    - Microbial cytology -
    - Microbial physiology
    - Microbial genetic
    - Microbial Ecology
  - Applied
    - Medical Microbiology
    - Pharmaceutical Microbiology
    - Industrial Microbiology
    - Biotechnology
    - Food Microbiology
    - Dairy Microbiology
    - Agriculture Microbiology
    - Veterinary Microbiology
    - Enviromental Microbiology

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Scope and importance of Microbiology:

[1] Production of antibiotic

Antibiotic are capable of inhibiting the growth of micro-organism.

Antibiotic mainly 2 type -

[A] Bacteriostatics [Inhibit the growth of microorganism]
[B] Bacteriocidal [Kill the bacteria].

[2] Production of enzyme vaccine and alcohal -

List of micro-organism, which are helping in the production of ezyme, vaccine, and alcohal.

[3] Use the production of dairy product -

Dairy products such as manufactured bacterial activity.

Cheese - Lactobacillus lactis.
Yoghurt - Lactobacillus vulgaricus.
Curd - Lactobacillus, Streptococcus lactis.

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Butter - Streptococcus lactis.
Butter milk - Streptococcus lactis, Streptococcus Cremoris.

[4] Cosmetic & Perfumes -

Some species use for making perfume and soap like aromatina.

[5] Agriculture and soil fertility - Nitrogen is an essential for the synthesis of protein, nucleic acid and other nitrogen containing compound.

[6] Industrial microbiology - Using microorganism to make products such as antibiotics, vaccines, steriods alcohals & other solvents, vitamins, amino acids, enzymes etc.

[7] Genetic Engineering - Engineered microorganisms used to make harmones, antibiobiotic, vaccines and other products.

[6] Sterilization

[7] Production of microorganism
[8] Testing of pharmaceutical.

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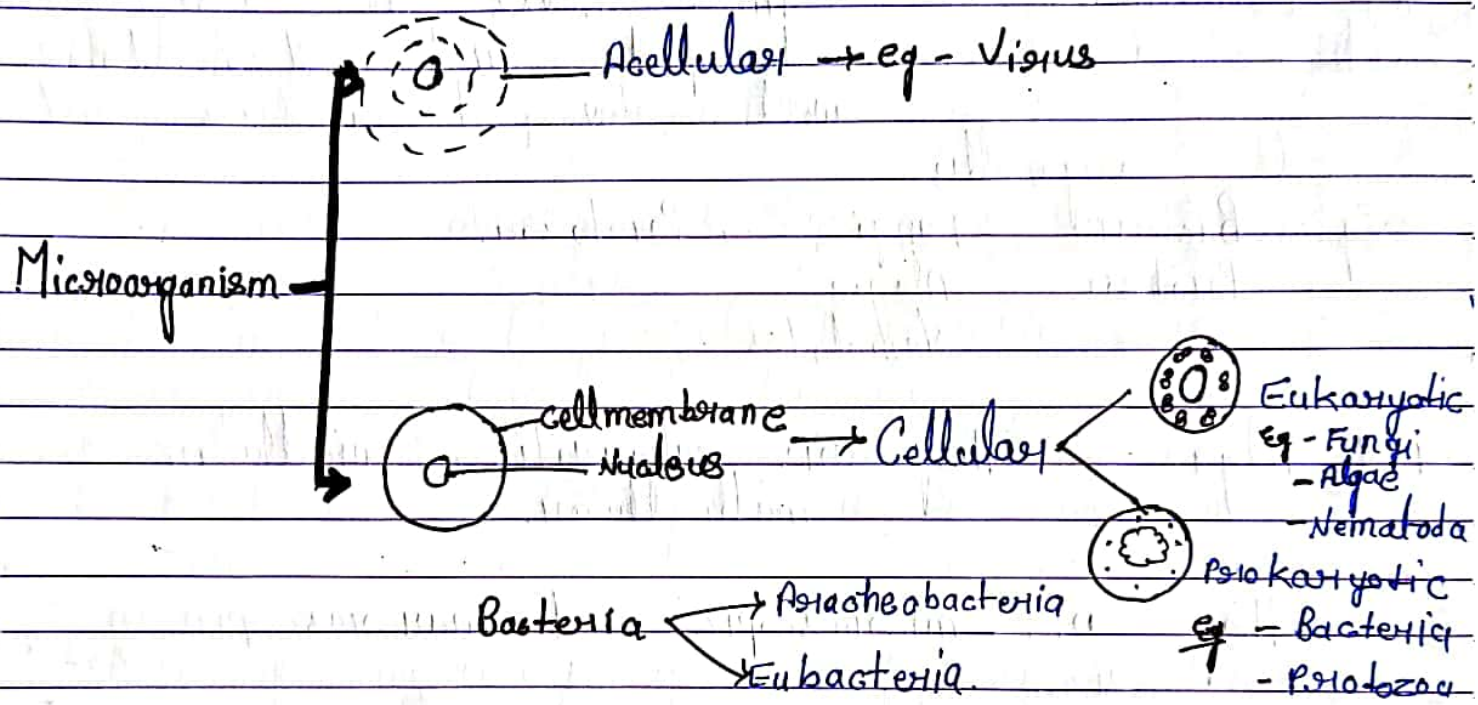
Classification of Microbiology

On the basis of cellular level microbial can be classified as following -



[A] Acellular - In this type of micro-organism single poorly developed cell is present, in which cell membrane and nucleus is absent.
eg - Virus

[B] Cellular - This is the complete cell structure in which the cell membrane and nucleus is develop, it further can be devided into 2 types -

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[A] **Prokaryotic cell** - Cell membrane is devlaped but cell organellis and nucleus is absent.

Eg - Bacteria, ~~protozoa~~. Archaea.

[B] **Eukaryotic cell** - These cells are well devlaped, and they have well devlaped nucleus and cell organellis.

Eg - ~~Bacteria~~ Fungi, Protozoa.
~~Protozoa~~ Algae
Nematodes.

[1] **Algae** - They are eukaryotic, unicellular or multicellular.

They are microscopic and macroscopin in size.

They are motile or non motile.

They obtain their energy from photosynthetic autotrophs.

[2] **Fungi** - They are eukaryotic
They are unicellular or multicellular (Yeasts are unicellular, molds are multicellular).

They are non - motile.

They obtain their energy from outside source of organic molecules.

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They acts as scavengers, they live off dead matter and thus, decompose it.

[3] Protozoa - They are eukaryotic.
They are unicellular.
They are motile or non motile.
They obtain energy from outside source of organic molecules.

[4] Viruses

They are acellular and obligate intracellular parasites.

Structure of virus -

- A pieces of nucleic acid [RNA or DNA] enclosed by a protein coat [Capsid] passess no nucleus, organelles, cell membrane or cytoplasm.

- Size 1/10 to 1/1000 the size of an ordinary bacterial cell.

- They are non - motile.

Bacteria

Bacteria form a large group of unicellular

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prokaryotics that donot contain nucleus & other membrane bound organells.

⇒ They were first absorb by Antony van Leeuwen hoek in 1676 using a single lence microscope.

⇒ Microscope of his own design he called "animal cules".

⇒ Mainly two groups of bacteria are -
Archaeobacteria - Ancient bacteria
Eubacteria - True bacteria.

⇒ They are various shapes like bacillus (rod), coccus (spherical), spirillum (spiral), Vibrio (curved rod).

⇒ They are mortile or non - mortile.

Structure of Bacteria

In ultra microscopic structure of bacteria it is seen that it is a small capsule like structure.

In the structure of bacteria following part is present -

(1) Capsule (2) Cellwall (3) Cellmembrane
(4) Flagella (5) Cytoplasm (6) Nucleoid
(7) Inclusion Body (8) Mesosome (9) Ribosome
(10) Pilli

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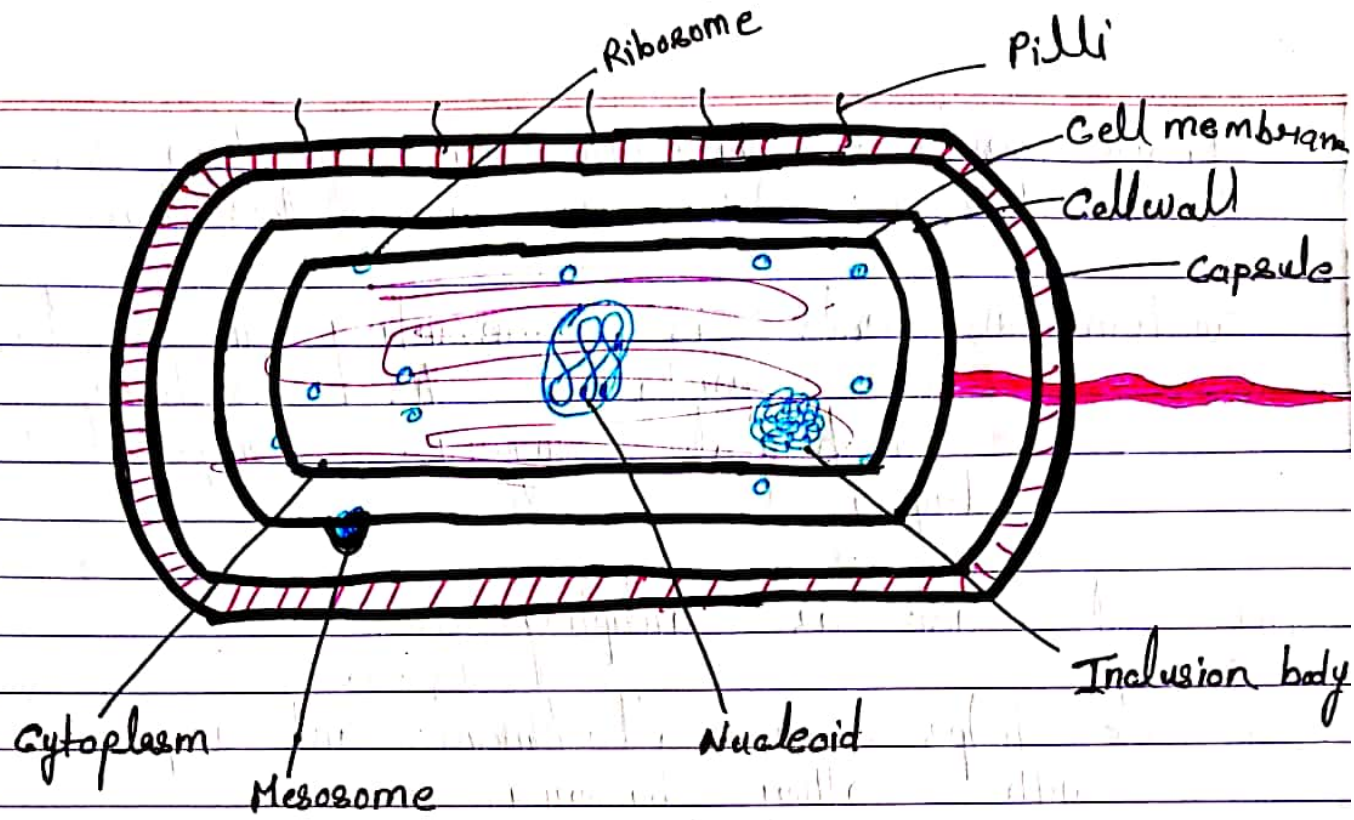

[1] Capsule - It is the outer most thick and slippery structure and it is rigid and flexible.

The composition of capsule is about 98 % water and 2 % glycoprotein.

This glycoprotein is of diffrent type in diffrent bacteria like homoplaysaccaride, Hemipoly-saccaride and Hetropolysaccaride.

On the basis of thickness capsule is of 2 types -

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(A) Macro capsule - Thickness more than 0.2 μ
(B) Micro capsule - Thickness ~~more~~ less than 0.2 μ

Funation - (A) Capsule is preventing in nature and protect the bacteria from mechanical injury, Temperature and drying.

[B] It act as antiphagocytic.

[C] It helps in attachment and repulsion -दूर भगाना with other bacteria.

[2] Cell wall - [A] It is a thick structure present bellow the capsule and it is madeup of peptidoglycon layers.

[B] On the basis of cell wall bacteria is identified as gram positive or gram negative

[C] In gram positive bacteria about 20-80 nm thickness of peptidoglycon is present and in gram negative bacteria its thickness is 7-8 nm.

[D] Peptidoglycon is a polymer of 2 sugar molecule NAG and NAM.

NAG - [N-acetyl glucosamine]
NAM - [N-acetyl Muramice acid]

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Function The main function of cell wall to provide shape to bacteria and identification.

[3] Cell membrane - Cell membrane is are thin layer in prokaryotic cell or bacteria, it is composed of 20 - 30 % Phospholipid and 60 - 70 % protein.

Function - The main function of cell membrane is act like SPM.

[4] Flagella - It is long thick hair like structure.

Which is surrounded by sheath - ~~ycd~~

Its diameter is 20 nm and length is 15 - 20 um.

Function - It act like antena ~~wit~~ which helps in searching food and danger.

It helps in locomotion.

It is made by flagellin protein.

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[5] **Inclusion Body** - Various type of organic and Inorganic food material is store in inclusion body.

[6] **Nucleoid** - The less developed nucleus without nucleoplasm and nuclear membrane is called nucleoid.

In the nucleoid of bacteria about 60 % DNA 30 % RNA and 10 % protein is present.

[7] **Mesosome** - Mesosome are present in the cell membrane of bacterial cell which helps in cellular respiration.

[8] **Ribosome** - In bacteria 70 S type of ribosome is present which helps in protein synthesise.

[9] **Pilli** - It is small thin 8 - 10 hair like structure which helps in the attachment of bacteria with other bacteria and transfer in genetic material.

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Morphological classification of Bacteria

On the basis of structure, shape, size and apperance bacteria canbe classified into 2 Categories -

☆ **Bacteria**

- True Bacteria
  - Spherical (O) — Coccus
  - Rod (▯) — Bacillus
- False / Pseudo Bacteria
  - (A) Actinomycetes
  - (B) Spirochates
  - (C) Mycoplasm
  - (D) Rickettsia
  - (E) Chlamydrae

[1] **True Bacteria** - They are the group of bacteria which shows the real characteristics of bacteria

It is of 2 types -

[1] **Coccus Bacteria** - It is spherical in shape

(1) O [Monococcus]
(2) OO [Diplococcus]

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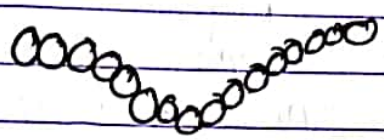
(3) [Tetradcoccus]

(6)

[Staphylococcus]

(4)  [Streptococcus]

(5) [Sarcinococcus]

[2] Bacillus - It is rod in shape

(A) [MonoBacillus]

(B) [Diplobacillus]

(C) [TetradBacillus]

(D) [Streptobacillus]

[F]

[Staphylobacillus]

[E] [Sarcinobacillus]

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[2] **False / Pseudo Bacteria** - These bacteria shows diffrent charectoristics than other bacteria like fungi, virus and protozoa.

They canbe further catagorried as -

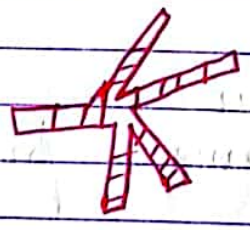
(A) **Actinomycetes** - They are rigid bacteria like a fungus.

They are filamentous in shape and ~~ba~~ branching is present.

They are gram +ve in nature.

They are present in soil, they are heterotrophic aerobic and mesophilic bacteria.

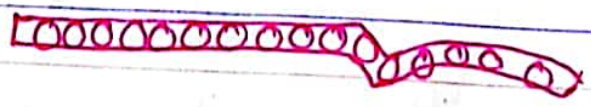
Eg - Mycobacteriaceae



- g+v
- Fillamentous

(B) **Spirochates** - They are long chain structure, non branched and consist of double membrane.

They are chemohetrophic in nature and gram negative bacteria.



- g +ve

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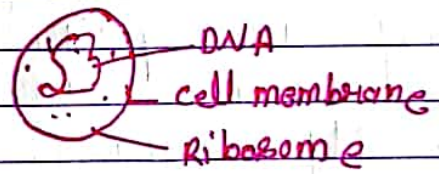
The length of this bacteria is between 3 to 500 um.

Eg Leptospirainterrogans.

[2] Mycoplasma - They are smallest bacteria and donot have rigid cell membrane.

They looks like virus and pathogenic in nature.

Eg - Mycoplasma - pneumoniae.
mycoplasma - genitalium.



[3] Rickettsia - They are non motile, gram -ve non spore forming bacteria.

They are present in cocous, bacillus and thread shaped.

This bacteria is pathogens and responsible for diffrent fever.

Eg - Rickettsia Conorui
R - Typhi

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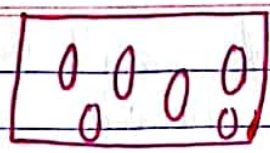

- Non moritile
- -g-ve

[5] **Chlamydiae-** They are ovoid in shape and gram negative bacteria, its cell membrane is consist of peptidoglycon and other proteins.

They are very small obligate parasite.

They commonly effect the human eye.

Eg- C- pneumonea
C- Psittaci

- ovoid shaped

Culture Media

Culture media is that nutrition medium in which bacteria is grow.

Culture media contains all essential supliments which is required for growth and devlopment.

All different types of bacteria cannot be grow in a single culture medium

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So different types of culture medium are used for growth of different bacteria.

Types of culture media

- Culture media
  - Based on consistency
    - Solid
    - Semi solid
    - Liquid
  - Based on composition
    - Synthetic
    - Non synthetic
  - Based on application
    - Basic medium
    - Enriched medium
    - Selective medium
    - Enrichment medium
    - Differential medium
    - Transport medium
    - Assay medium
    - Anerobic medium

[1] Based on consistency -

Based on the consistency culture media is of 3 types -

Solid, Semi solid, liquid.

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Agar is used as ~~Saliding~~ Solidifying agent if agar is use 1.522 % then it is solid medium, if agar is used less than 0.5 % then medium is semi solid, if agar is absent then medium is liquid.

[2] Based on Composition

For the growth of bacteria different composition is required like energy source, carbon source, nitrogen source, water, growth factors and salt.

The synthetic medium is prepared by using pure ingredient whose composition is known, where as in nonsynthetic medium, the composition is not known and impure.

[3] Based on application -

(A) Basic culture medium - This is the gernal culture medium which contains all essential element and for growth of all types of primary bacteria.

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[B] Enriched medium - In this culture medium extra nutritional material like Blood, serum, Yolk is added for the extra growth of bacteria.

[C] Enrichment medium - In this type of culture medium certain antibiotics are used to reduced the growth of unwanted bacteria and the concentration of desired bacteria is increase.

[D] Selective medium - This culture media is specific for any bacteria and only 1 type of bacteria can grow in this medium.

Eg (1) Thayer martin agar - It is used to recover N- gonorrhoeae. contains antibiotics vancomycin, colistin and nystatin.

(2) Wilson and Blair's Agar - It is used to recovering S. typhi rendered selective by the addition of dye brilliant green.

(3) Potassium tellurite - This medium is used to recover C. diphtheriae, contains 0.04 % potassium tellurite.

[E] Differential medium - This medium is a liquid medium, in this metabolic dye is use to

identify the different colonies by different colour.

Eg - Manitol salt Agar
Blood Agar
Mac conkey Agar.

[F] Transport medium - This nutrition medium is used when the ~~(sample)~~ specimen is not used very soon

This media is prevent from drying.

Eg - Caribalium medium
Ameir medium
Stuared medium.

[G] Assay medium - This medium is used for the assay of antibiotics, proteins / enzyme and hormones.

[H] Anaerobic medium - This medium requires for those bacteria is grow in anaerobic medium,

Hemin & vitamin K is the main source of energy in this medium.

This medium also contains 0.1% glucose, 0.1% ~~thyoc~~ thyoglycolate, 0.1% aericorbic acid

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0.05 % aystein.

Nutritional Requirement for Bacterial culture medium

For the preparation of culture medium for bacteria following requirements are necessary.

(1) Major (macro) Nutrients
(2) Major micronutrients
(3) Carbon - Energy source
(4) Growth factor
(5) Vitamin

[1] Major micro nutrients - These elements are required in large amount.

सीकोकिन्स केपी मईती गुड
C H O P K N S Ca Fe mg.

(A) Carbon - Carbon is the main source is organic compound and CO_2. It is main component of cellular material.

(B) Hydrogen - The main source of organic compound. It is the main component of cell water.

(C) Oxygen - It is the main consituent of cell

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material and cell water.

(D) **Sulfur** – It is the main constituent of some amino acids like cysteine, methionine, glutathione and several coenzymes.

(E) **Potassium** – It is the main component of cellular inorganic cation and cofactor for certain enzymes

(F) **Phosphorus** – It is the main component of nucleic acid, nucleotides, phospholipids etc

(G) **Nitrogen** – It is the main constituent of amino acid nucleic acid, nucleotides and coenzyme.

(H) **Calcium** – It is the main component of inorganic cellular cation, cofactor for certain enzymes and a component of endospores.

[2] **Major micronutrients** – This elements are required in small amount

बोरन — B, Cu
मैंगनीज — Mn, Zn
मोलिब्डेनम — Mo, Cl

[A] **Manganese [Mn]** – Assists in carbohydrate, amino acid and cholesterol metabolism.

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[2] **Zinc** – ~~Ness~~ Necessary for normal growth immune function and wound healing.

[C] **Copper** – Required for connective tissue formation, as well as normal brain and nervous system function.

[D] **Chloride** – Often found in combination with sodium. Helps maintain fluid balance and is used to make digestive juices.

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[3] Carban - energy Source - Carban is the main source of energy in the form of CO_2 source.

The photosynthetic bacteria is required CO_2 and sunlight, chemosynthetic bacteria requires CO_2 and chemicals as a source of energy. like ammonia, Nitrate, H_2S, etc.

[4] Growth Factor - All the bacteria require Small amount of organic compount for growth because they are essential as growth factors.

Eg - Purine and pyrimidine - This is essential for DNA synthesise.

Amino acid and proteins.
Harmones.

[5] Vitamins - Vitamins act as co-enzymes for the growth of purine, pyrimidine, vitamins, proteins and harmones in the body of bacteria.

There are different enzymes required for bacterial growth. -

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Vitamin	Coenzyme form	Function
[1] Falic acid	Tetrahydrofalate	Transfer of one carbon units and required for synthesis of thymine, purine bases, serine, methionine and pantothenate
[2] Biotin	Biotin	Bio Synthetic reaction that require CO_2 fixation.
[3] Lipoic acid	Lipoamide	Transfer of acyl groups in oxidation of keto acids.
[4] Nicotinic acid	NAD [Nicotinamide adenine dinucleotide] and NADP.	Electron carrier in dehydrogenations rxn.
[5] Pyridoxine [B6]	Pyridoxal phosphate	Transamination, deamination, decarboxylation and racemation of amino acids.
[6] Riboflavin [B2]	FMN [Flavin mononucleotide] and FAD [flavin adenine dinucleotide]	Oxidoreduction reaction.
[7] Vitamin [B12]	Carbalamine coupled to adenine nucleoside	Transfer of methyl groups.
[8] Vitamin [K]	Quinones and napthoquinones	Electron transport process.

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Factors affecting Bacterial growth

[1] Nutritional concentration – Bacteria requires different nutritional suppliment like purine, pyrimide vitamine. micro and macro nutrients in a certain limit.

When the conc. of nutritional suppliment is increase then the growth of bacteria is also increase but after a certain limit the growth of bacteria is remain constant.

[2] Temperature – The bacteria show maximum growth in the temp. rays 45°C to 70°C maximum growth

The lowest temp. (30°C) where bacteria allow to grow is called minimum temp. and the highest temp. after that (75°C)

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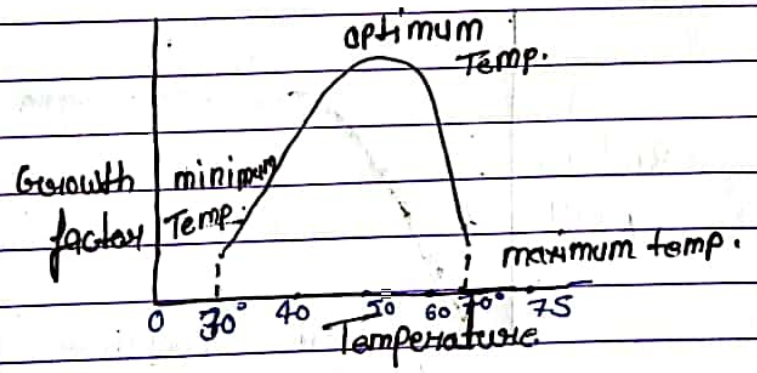
bacteria started to kill is called maximum temperature.

In the graph the line grows straight in increasing manner upto optimum temperature. Then after the growth is decrease.

Some bacteria's are growth in very high temp. 70°C to 110°C is called hyperthermophile bacteria, and some bacteria are grow in very low temp. (-5°C to 20°C) they are called psychrophilas bacteria.



[3] **PH** - All bacteria grow in a certain pH range.

Acidophyll bacteria show maximum growth in the range of 3 to 5 basophyll shows at 8 to 10 and nutrophyll bacteria in 6 to 8.

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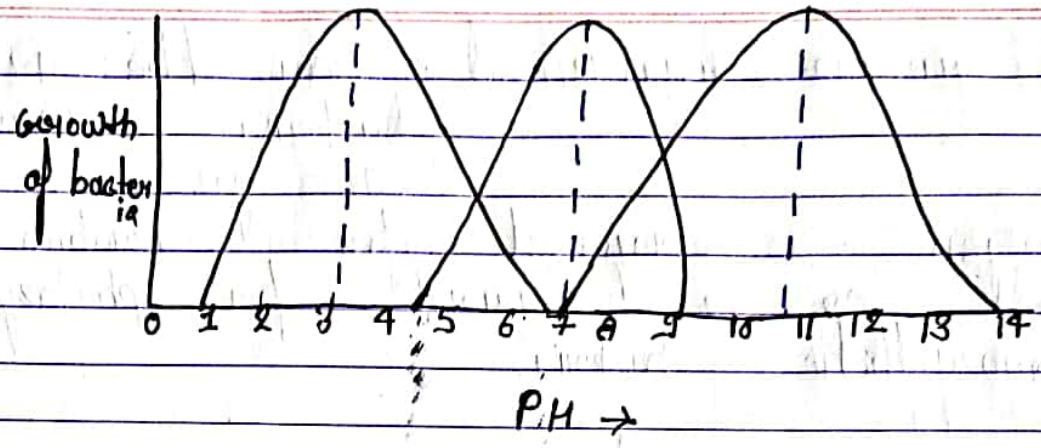
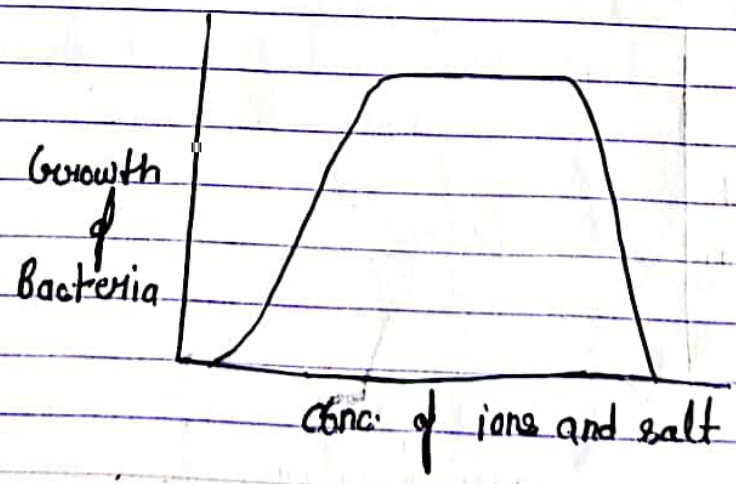


[4] **Ions and salt** - All bacteria require small amount of ions K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+} etc. for the synthesis of proteins and enzymes.

And bacteria can also ~~talor~~ tolerate some amount of salt.

These ions and salts helps in osmosis, but at higher concentration due to reverse osmosis bacteria's kills.

Some bacteria requires high concentration of salt. called Halophiles bacteria.



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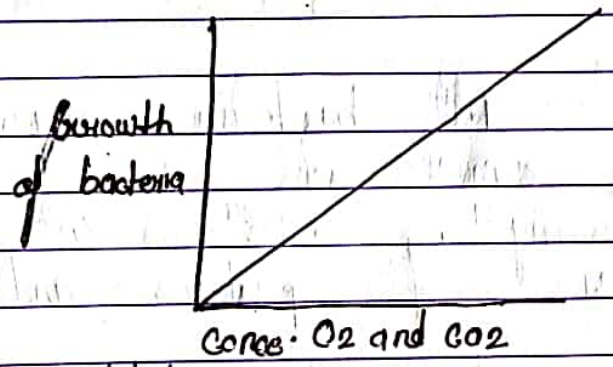
[5] Gaseous requirement - For the growth of bacteria oxygen and CO_2 gas is required.

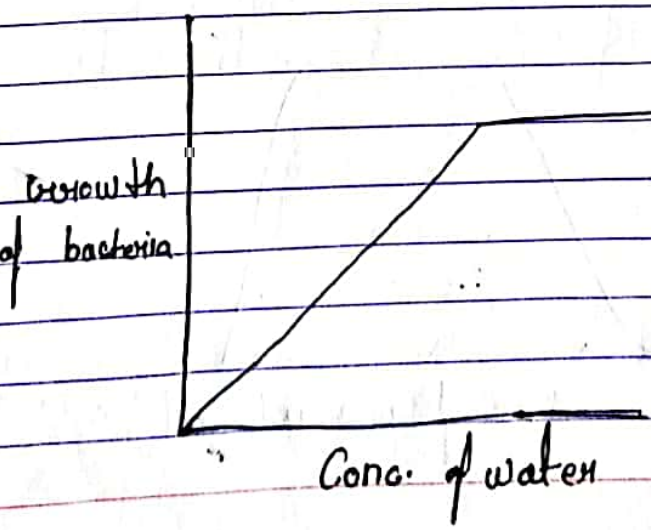
Oxygen is required for aerobic bacteria and CO_2 is required for photosynthetic and chemosynthetic bacteria.



[6] Water - Water is very essential factor for growth of bacteria.

The water in culture media decides the metabolism and physiological activities of bacteria.

All nutrients sugar and salt are dissolve in water which is made available for bacteria.



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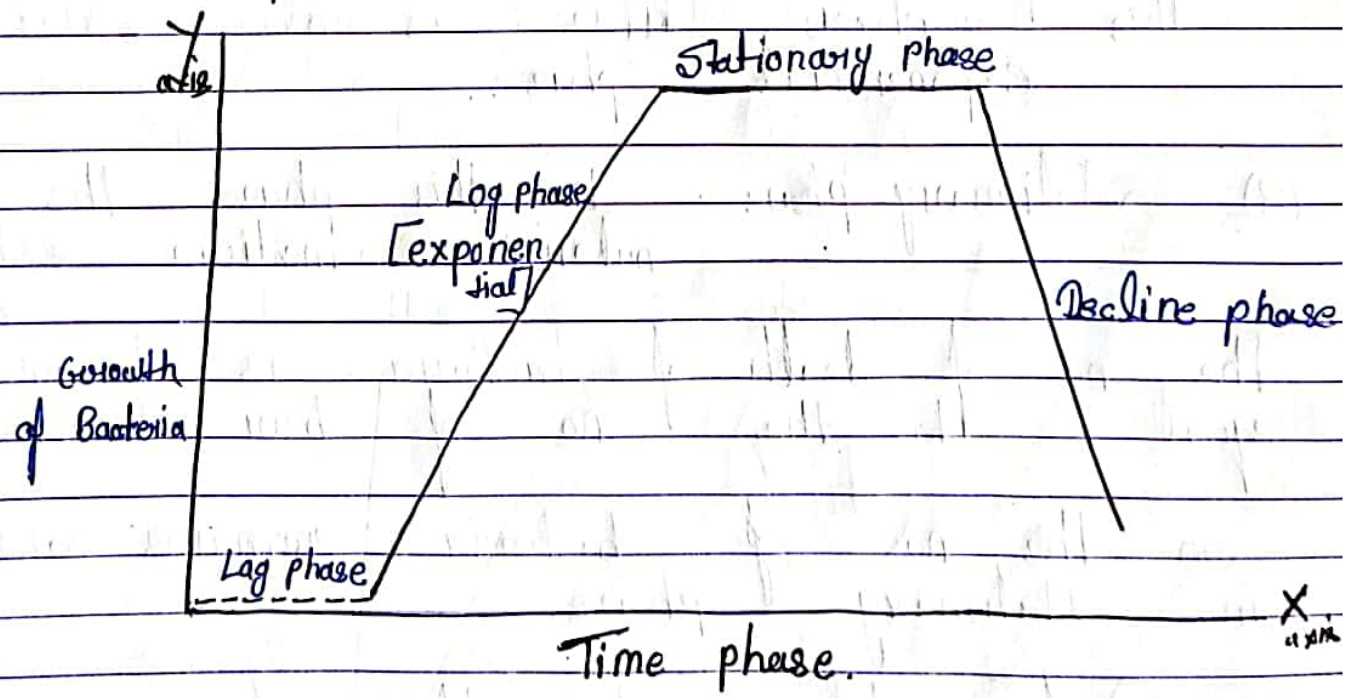
Bacterial Growth curve

When the graph is plotted b/w Time phase at X axis and growth of bacteria on Y axis, then the curve is obtain is called bacterial growth curve.

In this curve following 4 phases are present.

[1] Lag phase
[2] Log phase

[3] Stationary Phase
[4] Decline phase



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(1) **Lag phase** – When culture medium is prepare then upto 2 to 3 hours their is no growth of bacteria only sterile nutrition medium is present.

In lag phase their is no growth of bacteria.

(2) **Lag phase** – After the germination of bacteria they reproduce in fast rate and the no. of bacteria is increase.

In lag phase the conc. of nutrition medium is more than the bacteria so the growth of bacteria is maximum in this phase, ithus is also called exponential phase.

(3) **Stationary phase** – In this phase the nutrition medium exhausted
खत्म होना

the no. of death of bacteria is becomes equal to the no. of ~~bur~~ birth.

So the no. of bacteria remains constant in stationary phase.

(4) **Decline phase** – In this phase no. of bacteria is increase but the nutrition medium is very less.

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So due to lack of nutrients, bacteria started to death in decline phase.

Isolation of pure culture

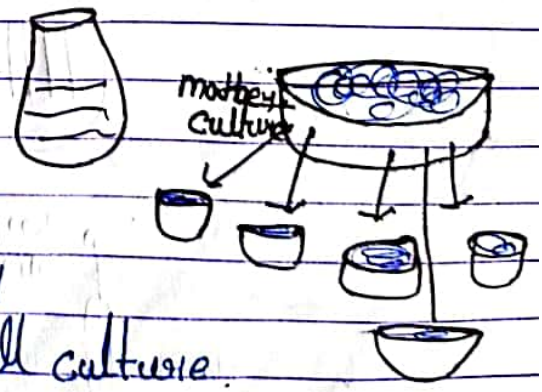
In a mother culture medium different types of micro-organisms are grown and the culture medium becomes impure.

The transfer of bacteria from mother culture to new sterile culture medium is called isolation of pure culture.

Advantage - (A) The desired bacteria is obtain
(B) Bacteria get more nutrition and space for growth and development.
(C) Easy in study.

Types of method of isolation of pure culture

(1) Streak plate method
(2) Pour plate method
(3) Spread plate method
(4) Serial dilution method
(5) Special method — Enriched, Single cell culture.



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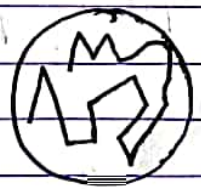
[1] Streak plate method -

In this method the bacteria is isolated from mother culture by applying streak on the surface of culture media.

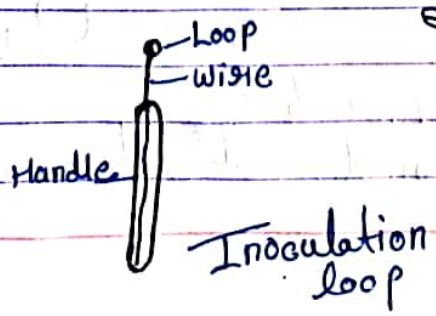
This is done by Inoculation loop. the inoculation loop is heated in ~~the~~ flame for sterilization and then streak on the surface of mother culture after streaky place the inoculation loop in ~~an~~ new sterile culture media.

Streaking is done by 2 methods -

(A) Contineous method



(B) Discontineous method



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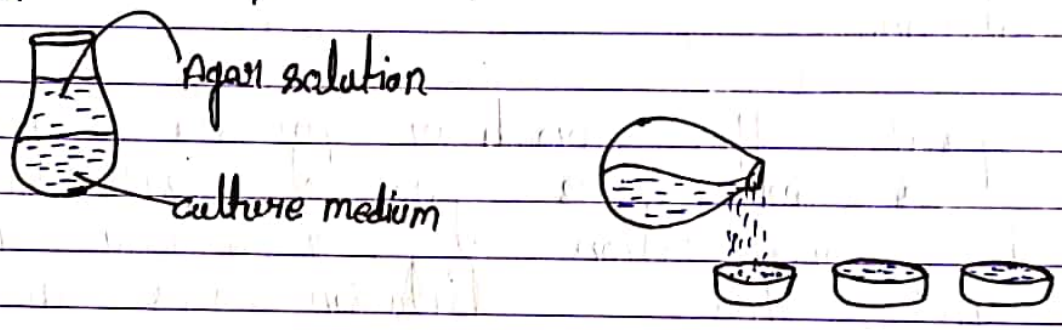
[2] **Pour Plate method** - In this method the diluted agar solution is added into culture medium and dilute the medium.

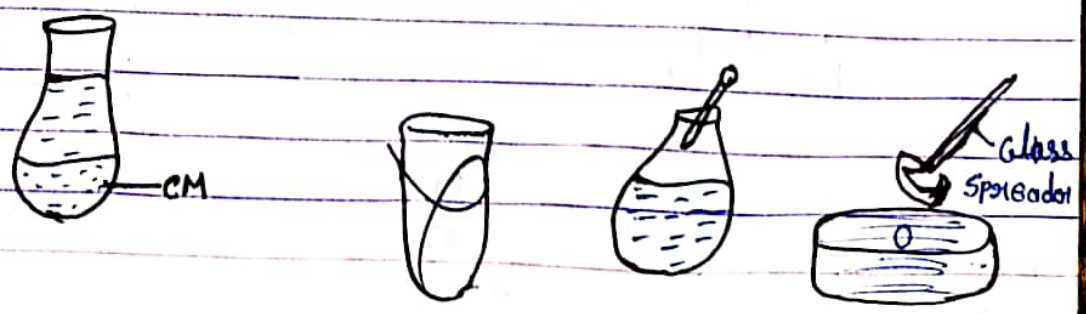
Now this diluted culture medium is pore into diffrent pettri-dish.



[3] **Spread plate method** - In this method the culture medium is diluted with sterile saline solution.

Now take 1 ml of culture medium and placed on the surface of new sterile culture medium.

Spread this drop with glass spreader in all over the surface and allow to grow the bacteria.



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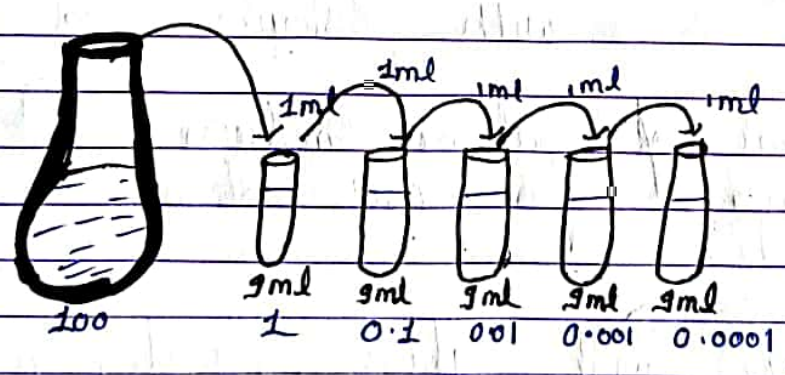
[4] **Serial dilution Method** - In Serial dilution method the culture medium is added into 1 ml of 9 ml sterile solution., now take 1 ml again from this 10 ml culture medium solution and add into another 9 ml solution.

Repeat this ~~procedure~~ procedure in the serial as well as no. of test tube increase the concentration of bacteria is decrease and the dilution is increase.

This method is used for actinomycetes bacteria. [Soil]



Preservation method For pure culture

[A] Preservation is a process to maintain pure culture for long period in ~~var viabilit~~ viable condition.

[B] Once in any culture medium ~~is~~ ~~per~~ bacteria is grow then it placed for long time and their is lot of chances to be contaminated.

So the preservation is important.

[C] The main objective of perservation to increase the viability of bacteria.

[D] To avoid contamination.

[E] The peservation of pure culture canbe acheived by following methods -

(1) Subculturing
(2) Storage in sterile condition
(3) Saline Suspension
(4) Refrigeration
(5) Paraffin method
(6) Cryopreservation
(7) Lypophillization.

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[1] Subculturing - In this method the culture medium solution is regularly changed.

In fresh culture medium the chances of contamination is less.

[2] Storage in sterile condition - In this method calcium carbonate solution is added and the container is closed with cotton plug and placed under incubator.

[3] Saline solution - suspension - Bacteria cannot grow in saline solution so 1 % Nacl solution is added into culture medium so the growth of bacterial will stop.

[4] Refrigeration - In this method the culture medium the culture medium is placed under refrigeration add 0 to 4°C for 2 to 3 days. So the microbes will kill.

[5] Paraffin method - In this method the paraffin oil is added in culture medium, it form a thin layer over the surface of culture medium so the aerobic bacteria cannot grow.

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Quantitative measurement of Bacterial growth

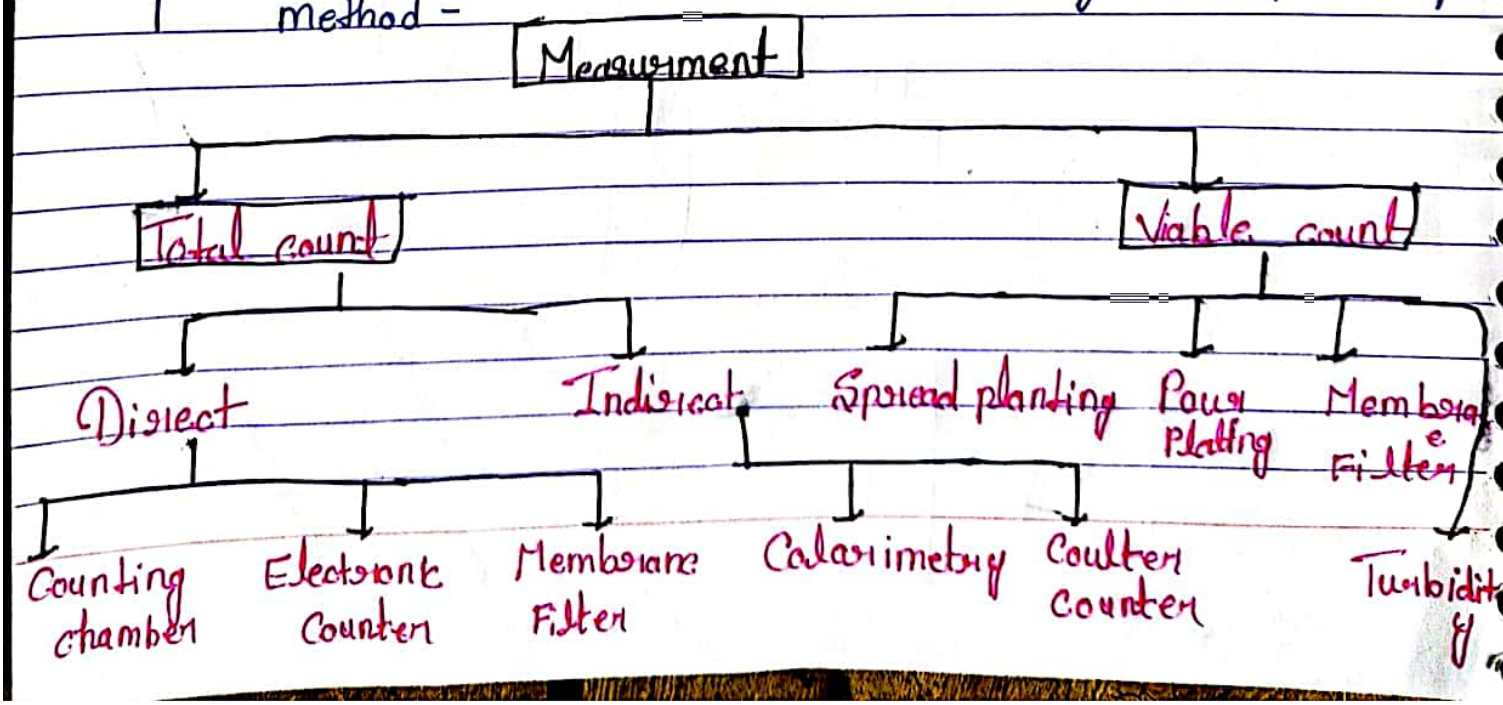
Bacteria is grow in culture medium after placing in incubator.
The growth of bacteria is culture medium and its totale no. canbe calculated.

The growth of bacteria is calculated in 2 terms -

[1] Total count
[2] Viable count.

Total count means total no. of living and non living bacteria, and viable count means totale no. of living bacteria only.

The quantitative measurment of no. and growth of bacteria is determine by following method -



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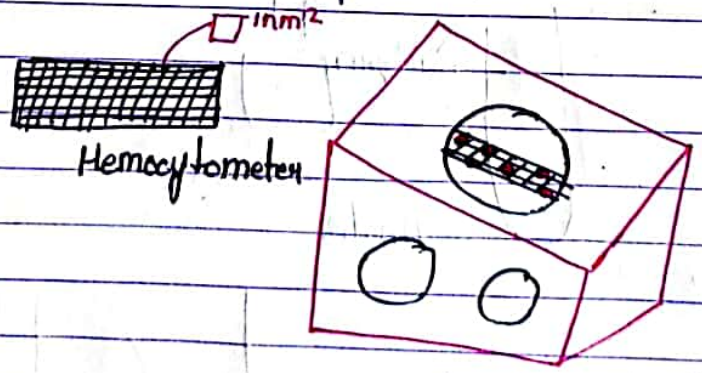
[4] Direct method - In this method the total no. of bacteria and its growth is calculated directly counting the no. of colonies by using diffrent technique.

(A) Counting chamber method - This is the simple and less time consuming method and this requires colonie counting chamber and heamocytometer.

In heamocytometer diffrent scales are made and each chamber 62 to 70 bacteria are present.

The petridish containing culture medium is placed inside the counting chamber and heamocytometer is fixed.

The total no. of colonies of bacteria canbe count and no. of bacteria is calculated.



Counter chamber method

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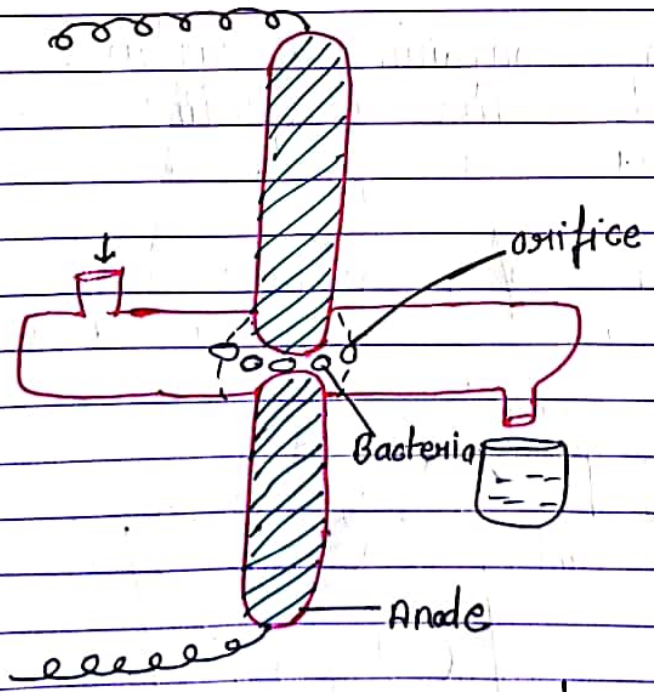
[B] Electron counter method - This method is also known as flow cytometry method

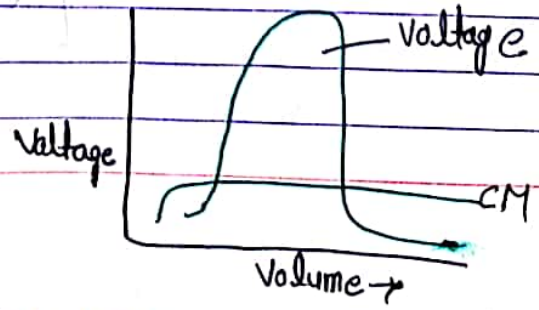
In this method the culture medium containing bacteria is pass through the electrodes by a small orifice.

When bacteria is pass between the electrodes then the voltage is generated.

By counting peakes the total no. of bacteria is calculated.

This method is also known as Coulter Counter method.





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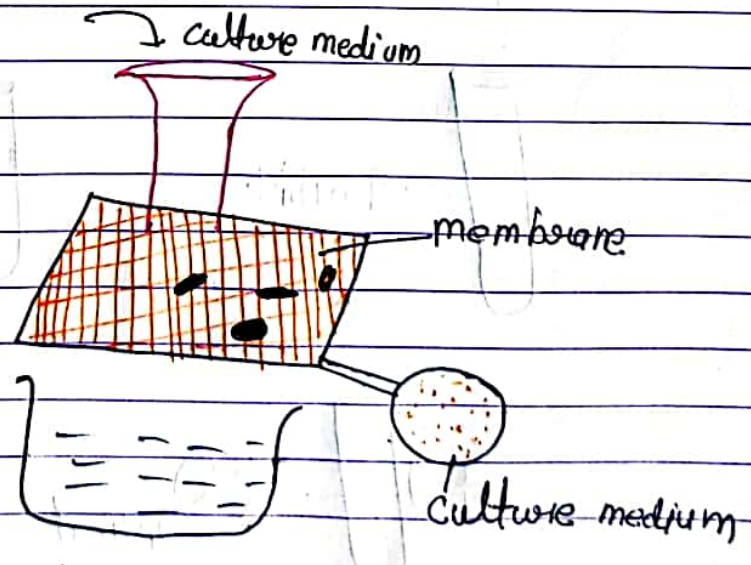
[3] Membrane filter method - This is a simplest and easy method this require fluorescent dye and the membrane filter which donot allow the bacteria to pass.

When the culture medium containing bacteria is pass through filter media then bacteria are separated and collected in a fresh culture medium.

After incubation the bacteria are grow which appear on the surface of culture medium



[4] Indirect count method -

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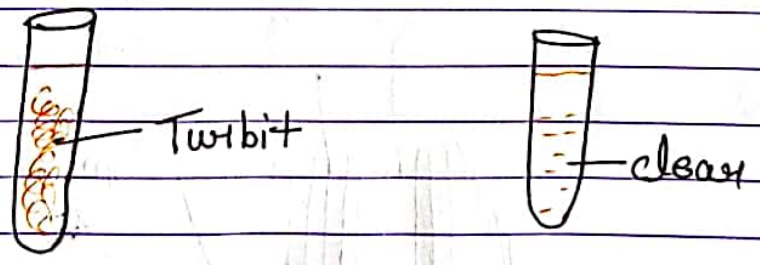
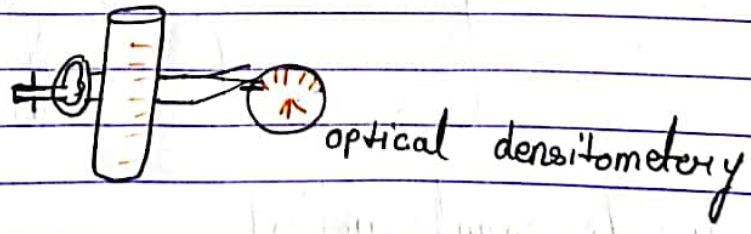
[2] Indirect method

In the indirect method the optical density of light is calculated and co-relat with no. of bacteria.

The culture media is added in clear solution and if bacteria is present then solution becomes turbit.

The turbidity is measured by passing the light from the medium.

More turbidity means more bacteria present.