Shri Swami Vivekanand Shikshan Sanstha's

Vivekanand College, Kolhapur (Autonomous)

Department of Microbiology

PPT Bank (2018-2023)

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3	Types of microorganisms	B.Sc I	DSC1010A Introduction to Microbiology
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ULTRA STRUCTURE OF PROKARYOTIC & EUKARYOTIC CELL

Presented by
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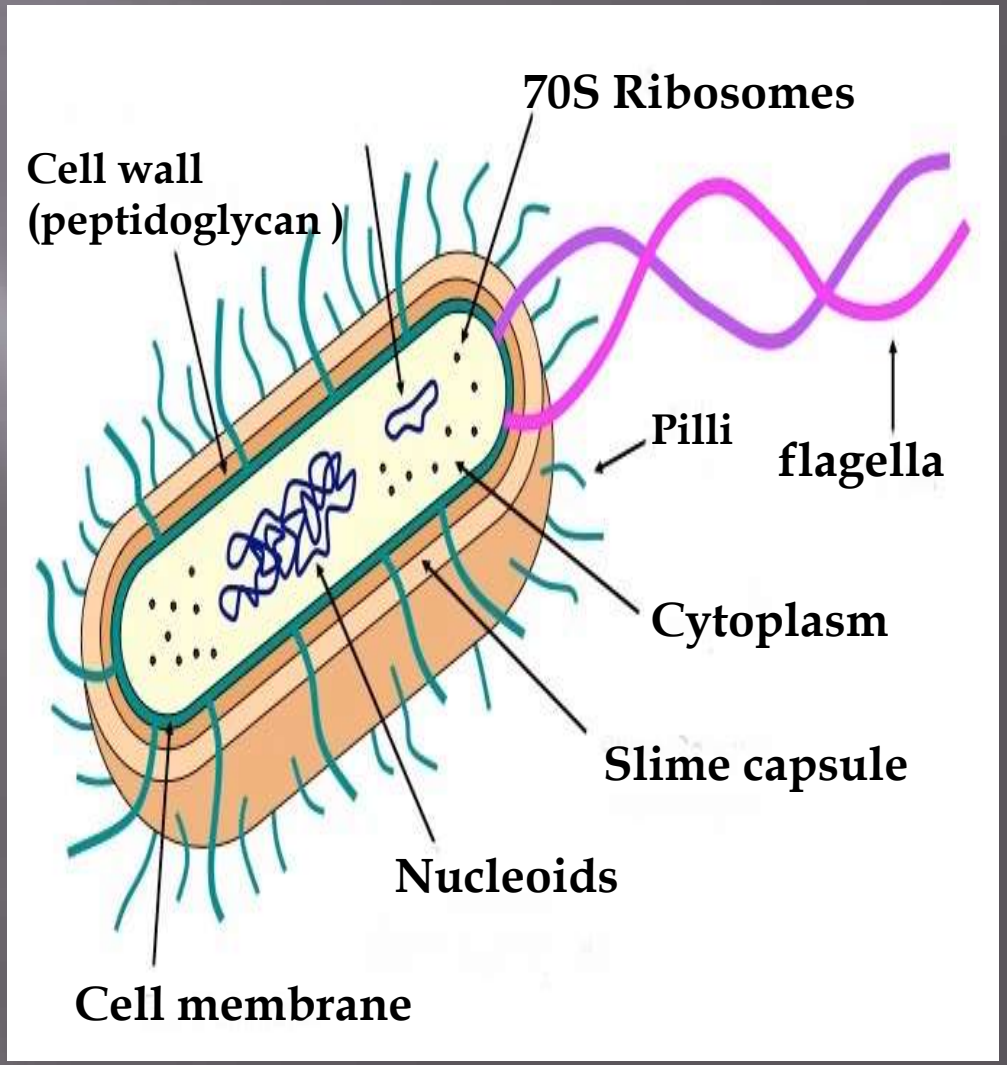
ULTRA STRUCTURE OF PROKARYOTIC & EUKARYOTIC CELL

- Scientists Charles Dougherty (1921-1965) classified the living organism into two groups Prokaryotic & Eukaryotic.
- This classification is based on presence or absence of a well defined nucleus.
- The prokaryotic organisms has primitive type of nucleus.
- Their genetic material is not bound by a nuclear membrane.
- The eukaryotic organisms possess well defined nucleus.
- Their genetic material is enclosed in a nuclear membrane.

Prokaryotes

(Greek,Pro- Primitive; karyon-nucleus)

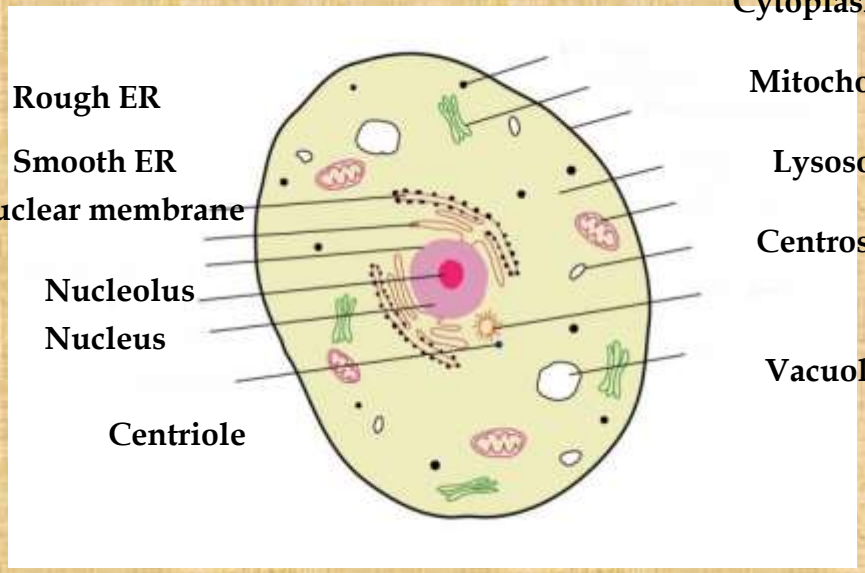
- Unicellular
- Have simple cellular organisation
- Doesn't possess well defined nuclei.
- Genetic material is circular & not enclosed by nuclear membrane.
- Genetic material of prokaryotes is called as Nucleiod.
- Many prokaryotes possess a rigid cell wall
- Cell wall made up of peptidoglycan & determines the shape & gives protection to the cell.
- Below cell wall is present cell membrane which holds cell cytoplasm.
- Possess 70S type of ribosome.
- Motile organisms possess flagella for motility



- Cell membrane is a made up of two layers of phospholipids which proteins and each layer contributing half of complete structure making it as whole unit . Cell membrane is specialized and contains respiratory electron transport chain & photosynthetic apparatus.
- Cytoplasm contains genetic material , ribosomes ,enzymes.
- Histone like proteins(i.e. HU proteins) are involved in organization of DNA.
- Prokaryotic genes are very compactly packed because they don't have introns (non coding region within a gene)
- Cytoplasm doesn't have organelle. They possess prokaryotic organelle but not considered as true organelles like in eukaryotes.
- Prokaryotes are small in size than eukaryotes. They have large surface area to volume. This facilitates the rapid transport of nutrients, fluids & waste across the membrane. As a result cell shows higher metabolic activity & shorter generation time than eukaryotes.

Eukaryotes
(Greek , Eu–well defined; karyon-nucleus)

- More complex in cellular organization than prokaryotes.
- Show well defined nucleus i.e. their genetic material is enclosed in a membrane called nuclear membrane.
- May have cell wall or may not have cell wall.
- Cell membrane made up of protein & phospholipids is present below cell wall which holds cytoplasm.
- Genes are not compactly packed because they have non-coding regions called introns.
- Genetic material i.e. DNA is linear & wrapped around special proteins-histone to form chromatin fibers which further get coiled & forms visible structure called as Chromosomes.
- Cytoplasm is filled with different organelles designed for special functionsE.g.chloroplast,mitochondria,endoplasmic reticulum,golgi apparatus,lysosomes,vacuoles. Etc.



Plant and fungi possess cell wall in which plant cell wall is made up of cellulose and fungal cell wall is made up of chitin.

Cell membrane do not contain ETC or photosynthetic apparatus. Instead cell has separate organelles to bring respiration(mitochondria)&photosynthesis(chloroplast).

Ribosome of a eukaryotic cell are of an 80S type.

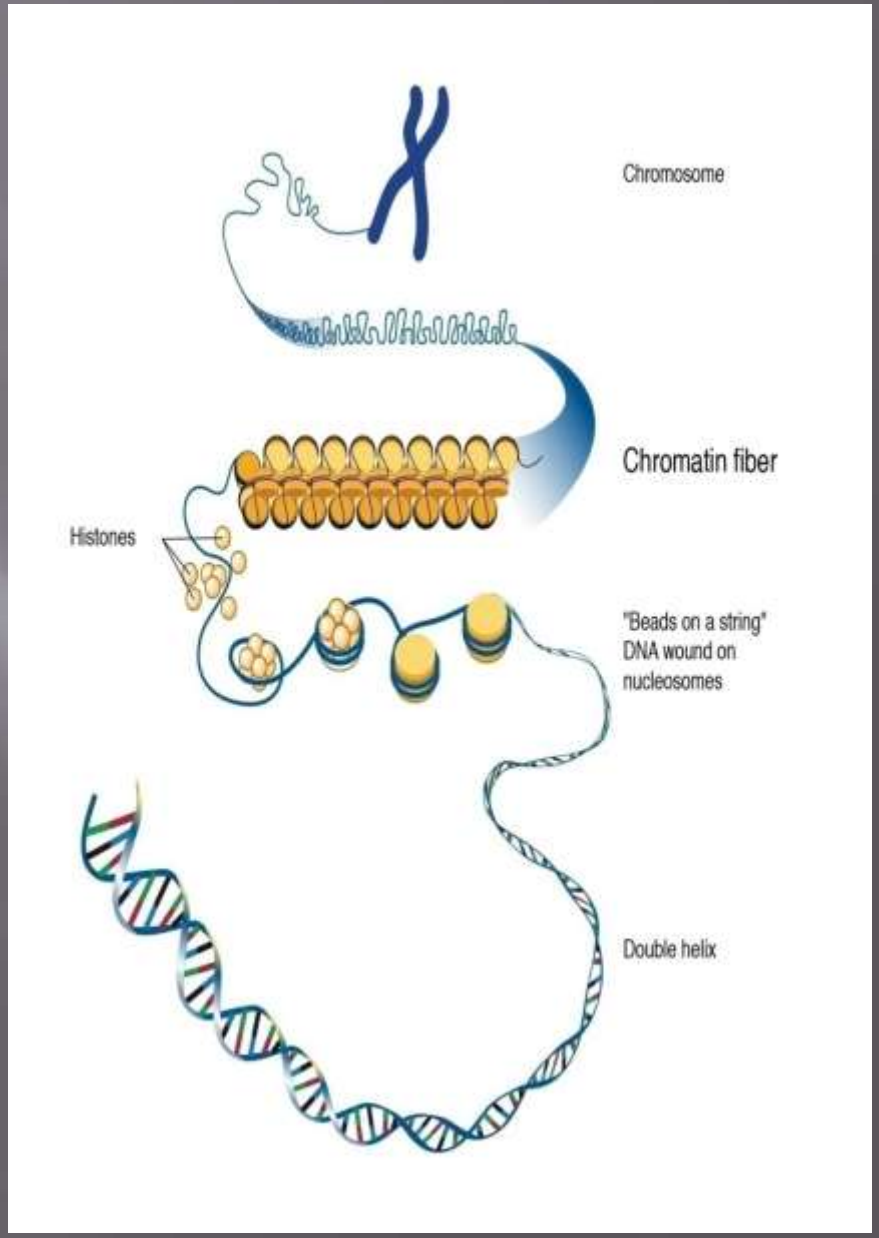
The mitochondria and chloroplast contain 70S type of ribosome .

Motile cell has flagella with 9+2 microtubules architecture.

These larger in size than prokaryotes.

Thet have surface area to volume ration .As a result , cell shows less metabolic activity and longer generation time than prokaryotes.

Examples :-Plant cell, animal cell ,protozoa and fungal cell.



Difference between Prokaryotic & Eukaryotic cell

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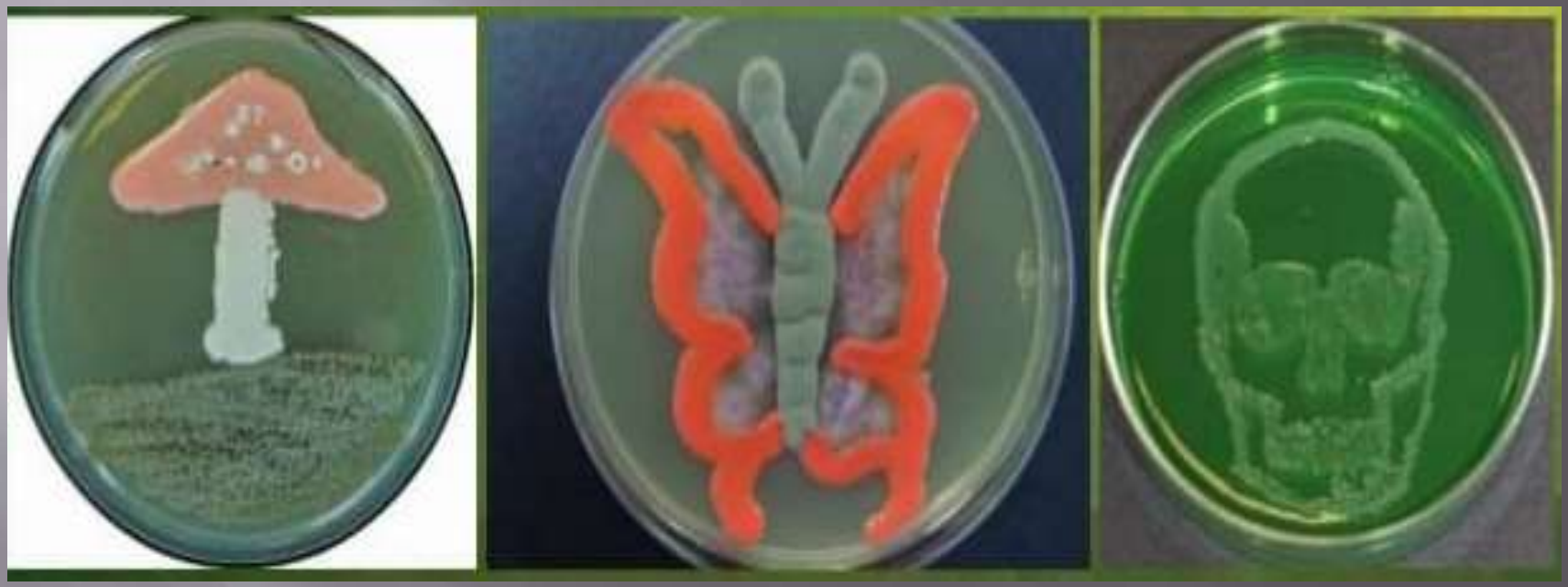
Sr.no.	Character	Prokaryotic cell	Eukaryotic cell
1	Size	1-10µm	10-100µm
2	Surface to volume ratio	Large	Small
3	Genetic material	dsDNA	dsDNA
4	Well defined nucleus	Absent	Present
5	Nuclear membrane	Absent	Present
6	Extrachromosomal material	Present (Plasmid)	Absent
7	Nucleolus	Absent	Present
8	Chromosomes	Not true chromosomes	Present
9	DNA wrapping proteins	Multiple proteins are involved in the wrapping of DNA,the histone like protein HU protein are involved in organization.	Present ,

Sr. no.	Character	Prokaryotic cell	Eukaryotic cell
10	Intron non-coding region in DNA	Absent	Present
11	True organelles - mitochondria,chroloplast ,endoplasmic reticulum,Golgi complex,vesicles,lysosomes etc.	Absent	Present
12.	Respiratory ETC & photosynthetic ETC in cell membrane	Present	Absent
13	Sterols in membrane	Absent	Present
14	Cell wall	Present, made up of peptidoglycan	May or may not be present,found only in the plant & fungal cell.
15	Flagella	Made up of flagellin protein	Made up pf microtubules with 9+2 architecture

Sr.no.	Character	Prokaryotic cell	Eukaryotic cell
16	Ribosomes	70S type ribosome	80S in the cytoplasm &70S type ribosome in mitochondria & chloroplast
17	Site of protein synthesis	On free ribosomes in the cytoplasm	On free ribosomes in the cytoplasm,on ribosome.on ribosomes present on endoplasmic reticulum,on ribosome present in the chloroplast& mitochondria
18	Cell division	Binary fission	By mitosis & meiosis
19	Genetic recombination	Conjugation , trasformation or transduction	Meiosis
20	Examples	Bacteria, Archaebacteria& Cyanobacteria	Plant cel l, Animal cell, Protozoa & fungal cell wall

Culture Medium



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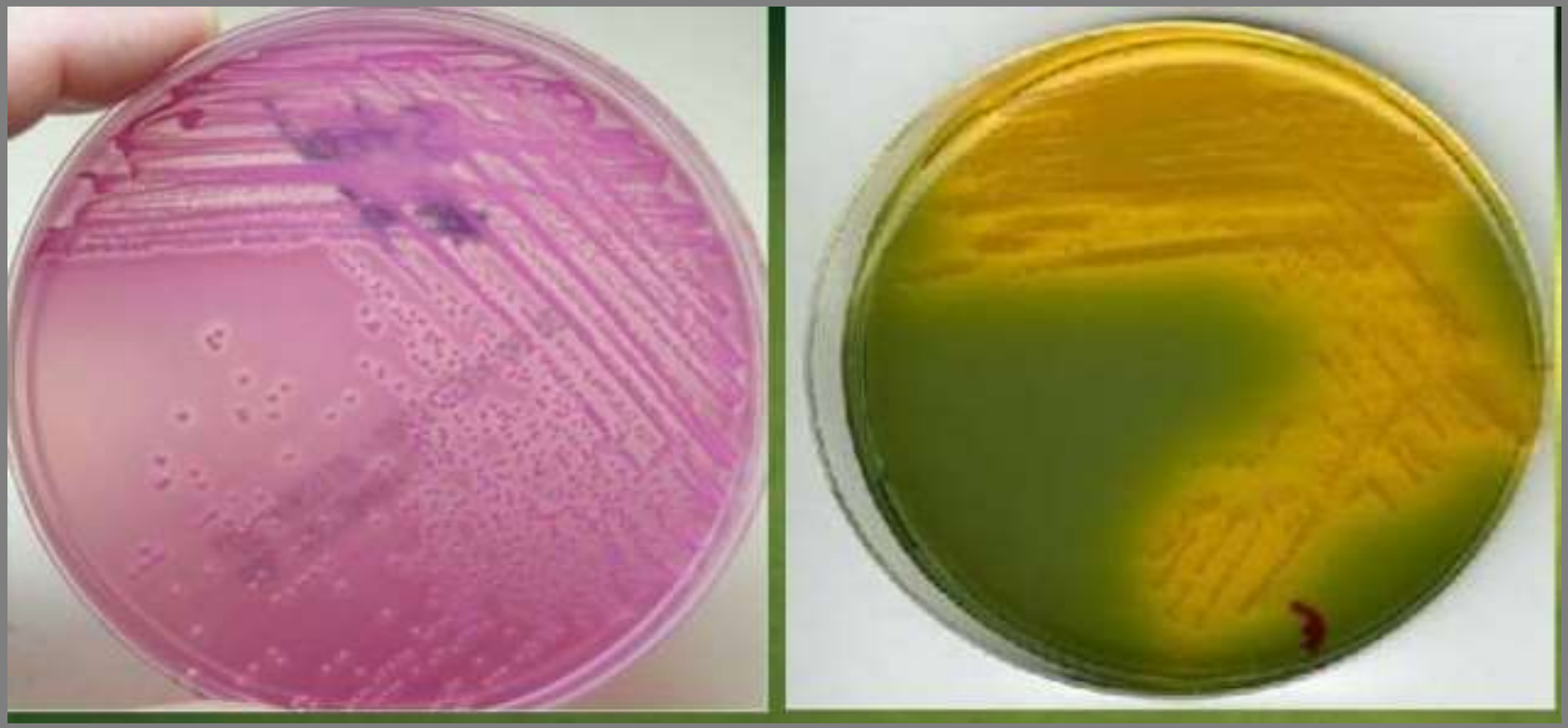
What is Culture Medium

The solid or liquid nutrients preparation used to grow & maintain microorganisms



Satisfactory Culture medium

- Have proper moisture content.
- *Contain readily available nutrients*.
- Have Correct pH .
- Sterile on microbiological sense.
- Provide desired physical properties such as clarity ,solid or liquid.

Types of media

Nature of ingredients

A. Living media

-embryonated chicken eggs

-tissue cultures

-lab animals

B. Nonliving media

-natural or empirical media

- semi synthetic media (complex media)

-Synthetic (chemically defined) media

Application & function

A. Enriched media

B. Enrichment media

C. Selective media

D. Differential media

Living media-

Media containing living cell

a) **Embryonated chicken egg:- fertile chicken egg incubated for 5-10 days contain embryonic tissue for cultivation of intracellular parasite.**

Technique involve-

1.locate air sac by candling .

2.Drill a small hole aseptically.

3.Inoculate material through opening.

4.Close opening with paraffin wax.

5.Incubate egg at 36degree celsius for desired period.

Tissue culture:-In vitro cultivation of tissue cells in laboratory medium is called tissue culture.

Today cell lines are best choice for growth of many microorganisms.

Cell line cultivation advantages-

1.most convenient to handle.

2.relatively economical as compared to live animal.

3.cytopathic effect can be easily detected visually.

4.can select type of tissue depending upon type of organism that has to be cultivated .

On basis of origin & characteristics of tissue culture, the cells are classified into 3 main type:-

1.primary cell culture

2.diploid cell strains.

3.Continuous cell culture

Live animal

- some microorganisms cannot be cultivated in egg embryos or even in cell lines ,but they require live animal for thier growth.
- Guinea pigs,rabbits,rat,mice etc animal are used.
- Organisms can be injected into the brain ,blood,body cavity,skin,footopads of animals.
- Costly method

❑ COMPLEX MEDIA

- Media other than basal media.
- Added complex ingredients (Yeast extract) Provide special nutrients

❑ SYNTHETIC OR DEFINED MEDIA

* Prepared from pure chemical substances

* Used for special studies,

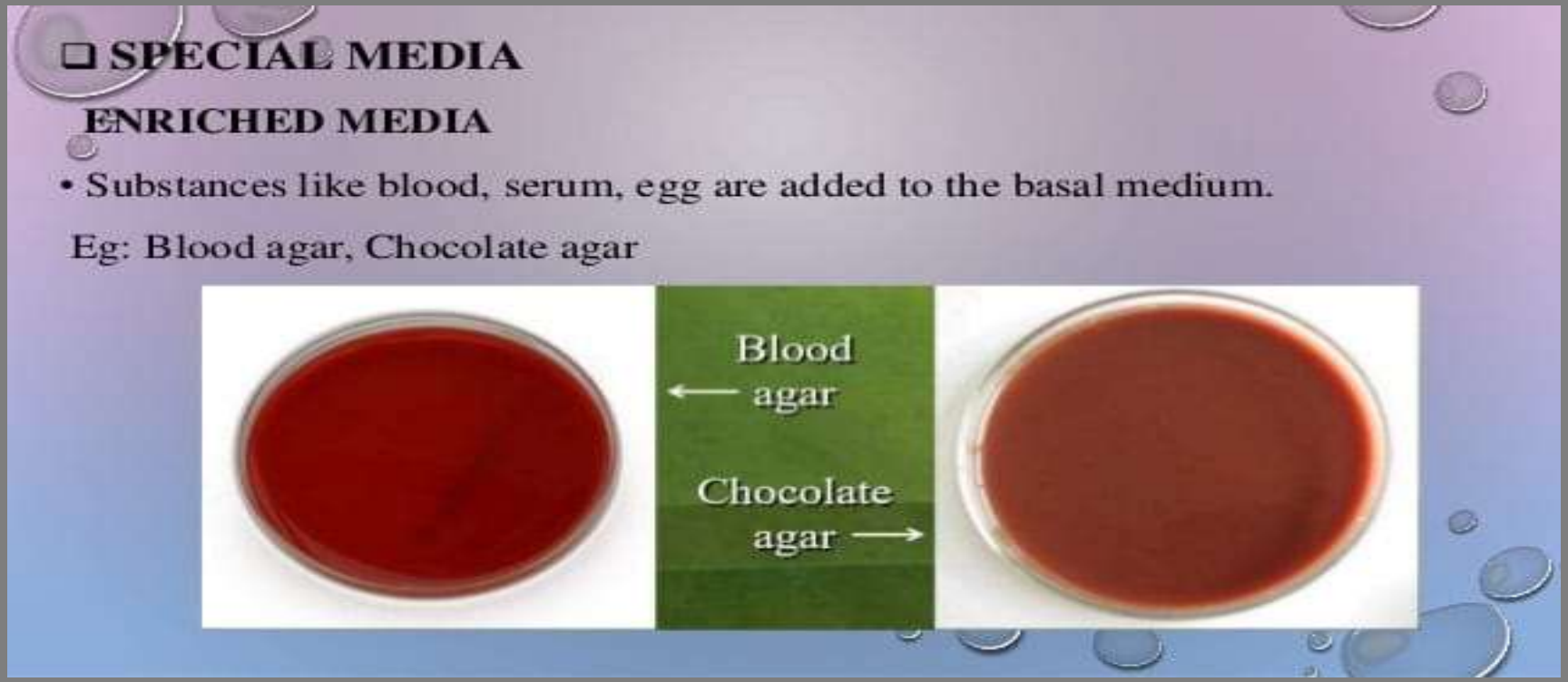
Eg. Metabolic requirements

Enrichment media

- Liquid media used to isolate pathogens from a mixed culture.
- Stimulate growth of desired bacterium
  Inhibit growth of unwanted bacterium
- Media is incorporated with inhibitory substances to suppress the unwanted organism → increase in numbers of desired bacteria
- Eg:
  Selenite F Broth – for the isolation of *Salmonella*, *Shigella*
  Tetrathionate Broth – inhibit coliforms
  Alkaline Peptone Water – for *Vibrio cholerae*

Enriched Medium

The medium that contain nutritionally rich ingredients such as blood,serum,vitamins,plants or animal tissues extract in addition to basal medium like Nutrient agar.



Selective medium

-The medium that contain selective component, which permit growth of desired organism and prevent growth of other organisms.

-MacConkeys agar, Mannitol salt agar, EMB agar ,Wilson & Blairs medium etc.are examples of selective medium.

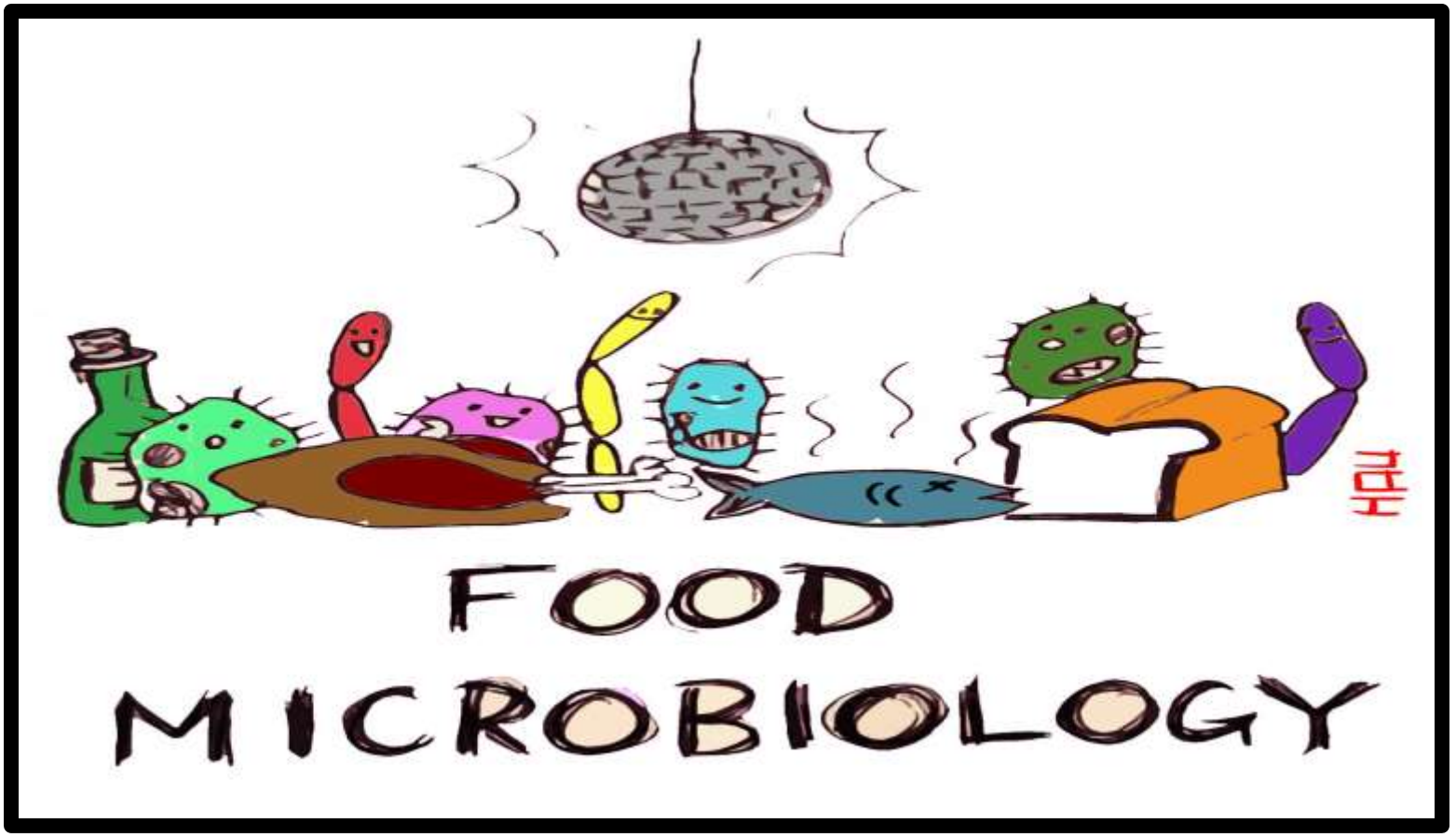
-E.g:-1) MacConkeys agar - sodium taurocholate (bile salt) – selective agent – inhibite growth of non-intestinal organisms.

2)Azide blood agar – azide as selective agent – inhibite growth of Gram negative bacteria & allow growth of *Staphylococcus* & *Streptococcus* species.

❑SELECTIVE MEDIA

- The inhibitory substance is added to a solid media
- Increase in number of colonies of desired bacterium

EMB – E.coli



FOOD MICROBIOLOGY

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FOOD MICROBIOLOGY

The branch of microbiology that deals with study of microorganisms that inhibit , create or contaminate of food.

TYPES OF FOOD

- **Based on origin**:

  a) food from plant origin

  b) food from animal origin

- **Based on stability (ease of spoilage) :**

  a)Perishable food

  b)Semi perishable food

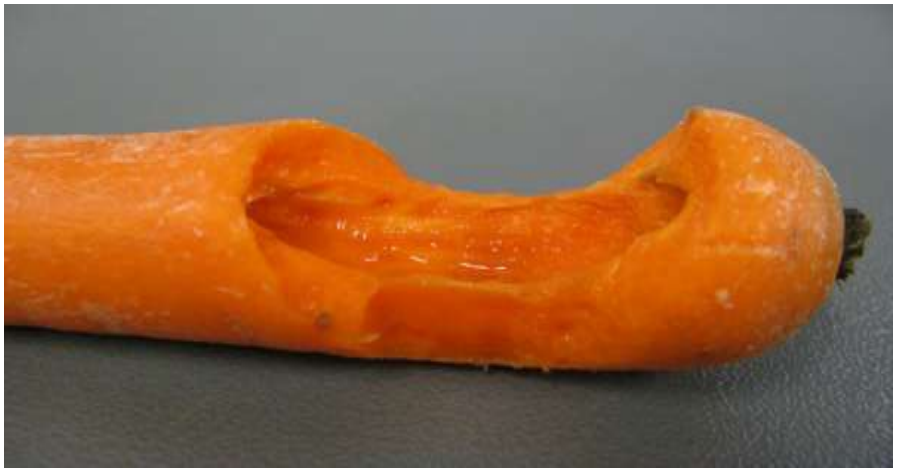
  c)Non-perishable (stable) food

SPOILAGE OF FRUITS

- pH of fruit is always below the favorable pH range for growth of microorganisms
- Wider pH range of Yeast and Molds allow them as spoilage agent for fruits
- Commons spoilage types vary with kind and variety of fruits
- Primary causative agent :- Bacteria, Yeast and Molds.

BACTERIAL SOFT ROT

- Caused by *Erwinia caratovora*
- Act on pectin in fruit skin
- Result in water soaked appearance, soft, mushy consistency & often bad odor

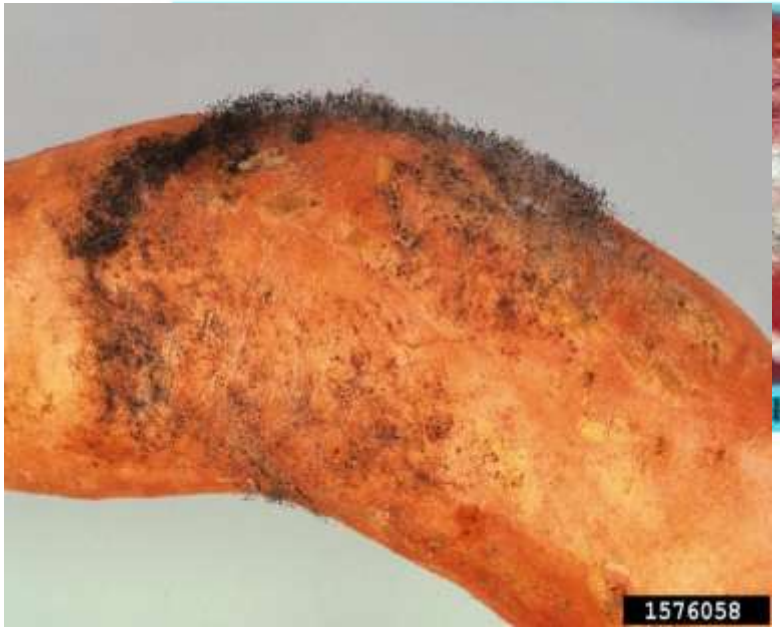


Gray mold rot

- **Gray mold rot** caused by species of Botrytis, e.g., *B.cinerea*. It is favored by high humidity and a warm temperature.

RHIZOPUS SOFT ROT

- **Rhizopus soft rot,** caused by species of *Rhizopus stolonifer*. A rot results that often is soft and mushy. The cottony growth of the mold with small, black dots of sporangia often covers masses of the foods.



ANTHRACNOSE

- **Anthracnose**, usually caused by *Colletotrichum lindemuthianum*, *C. coccodes*, and other species. The defect is a spotting of leaves and fruit.

ALTERNARIA ROT

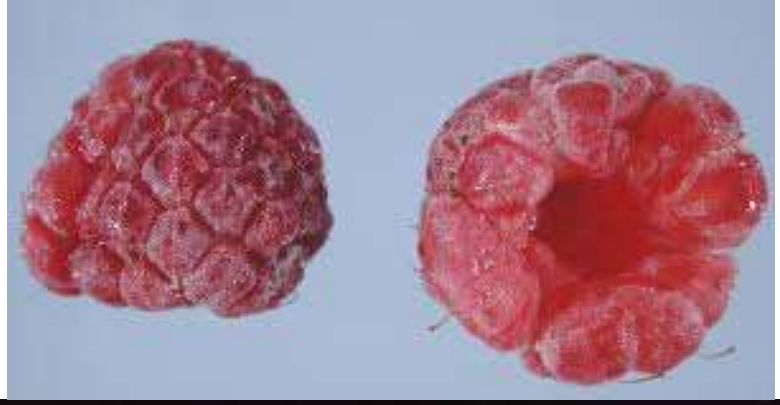
- **Alternaria rot,** caused by Alternaria tenuis and other species. Areas become greenish-brown early in the growth of the mold and later turn to brown or black spots.

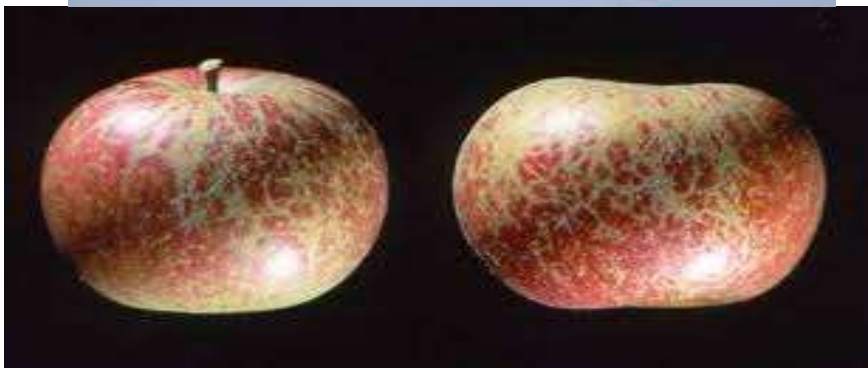
BLUE MOLD ROT

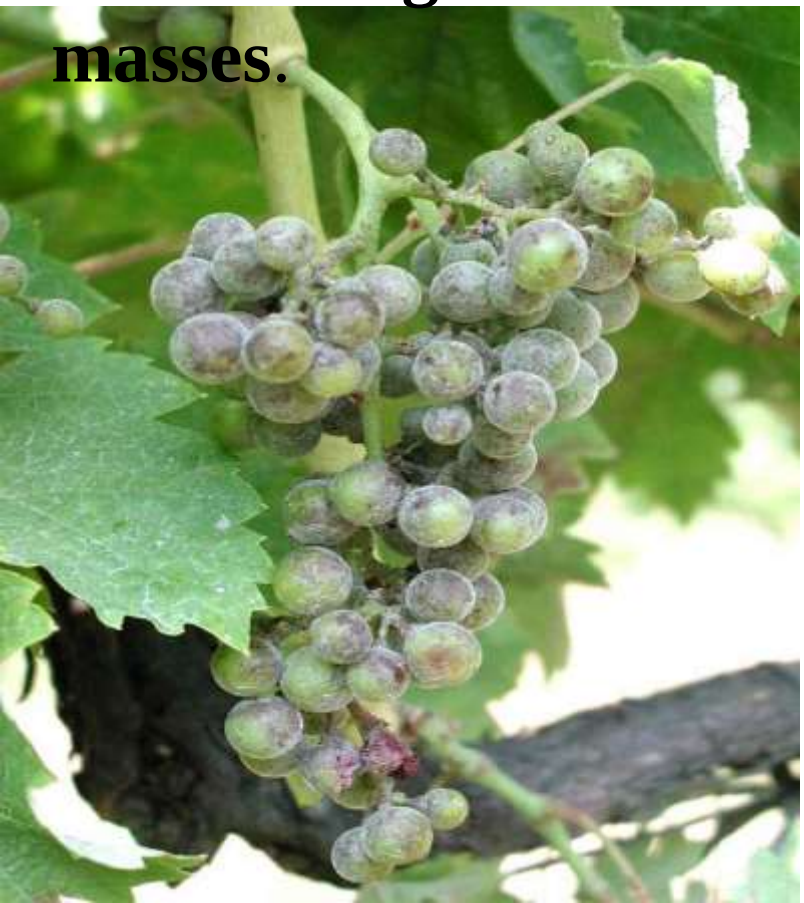
- **Blue mold rot**, caused by species of *Penicillum digitatum* and other species. The bluish-green color that gives the rot its name results from the masses of spores of the mold

DOWNY MILDEW

- **Caused by *Phytophtora,Bremia* & of other genera.**
- **The mold grow in white masses.**







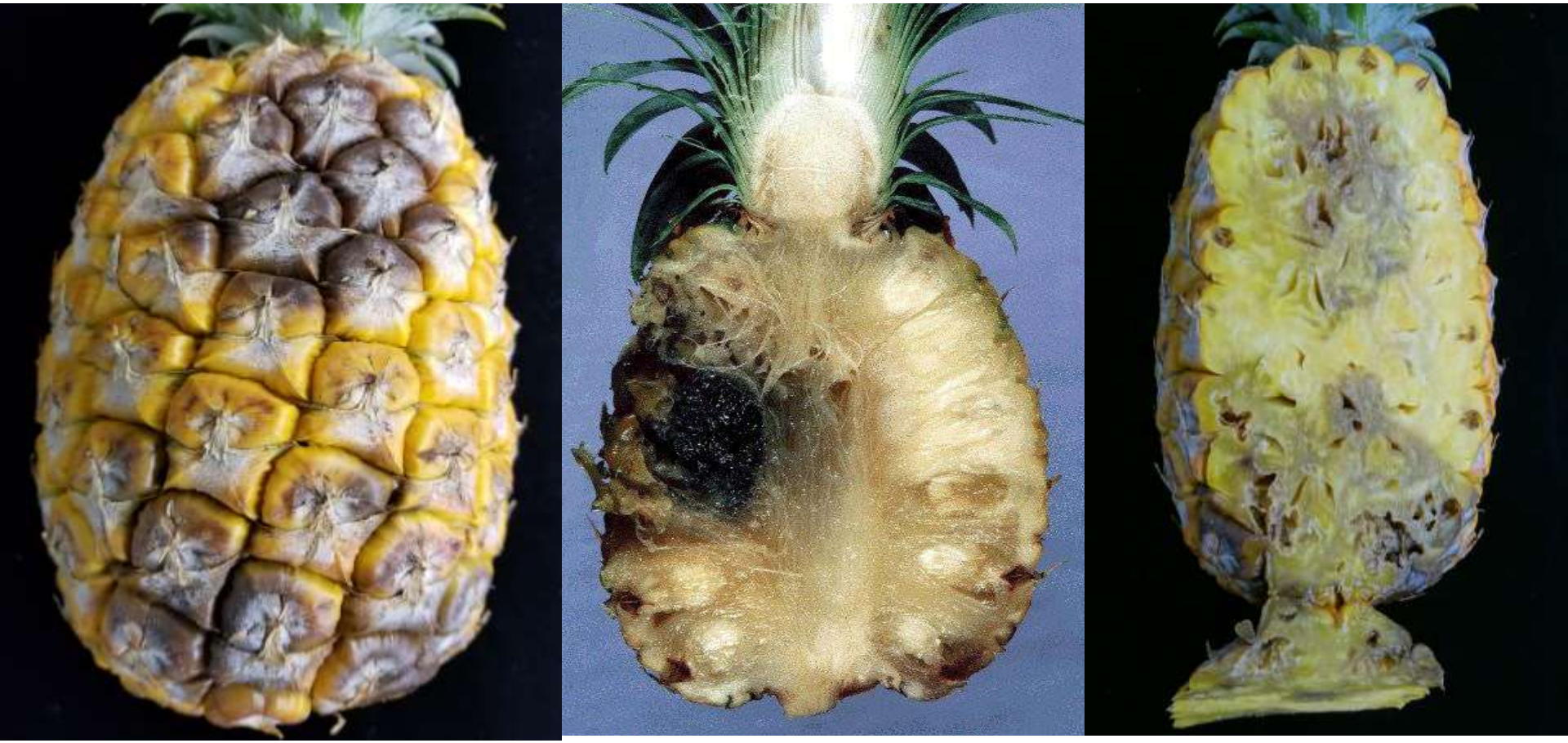
STEM END ROT

- Caused by *Diplodia,Phomosis, Fusarium* & other.
- It affect stem end.

BLACK MOLD ROT

- **Black mold rot**, caused by Aspergillus niger. The rot gets its name from the dark-brown to black masses of spores of the mold, termed "smut" by the layperson.

PINEAPPLE BLACK ROT(SMUT)



PINK MOLD ROT

- **Pink mold rot**, caused by pink-spored *Trichothecium roseum.*

FUSARIUM ROT

- **Fusarium rots**, a variety types of rots caused by species of *Fusarium*.

GREEN MOLD ROT

- **Caused by species of *Cladosporium & Trichoderma***

BROWN ROT

Caused by *Sclerotinia* species

LINTICEL ROT

Microbial Spoilage Of Bread

During baking process, most of vegetative forms of yeast ,mold , bacteria are killed. But heat resistant bacterial spores and conidia of molds can survive. Thus the growth of unwanted bacteria and molds causes the spoilage of bread.

ROPY BREAD

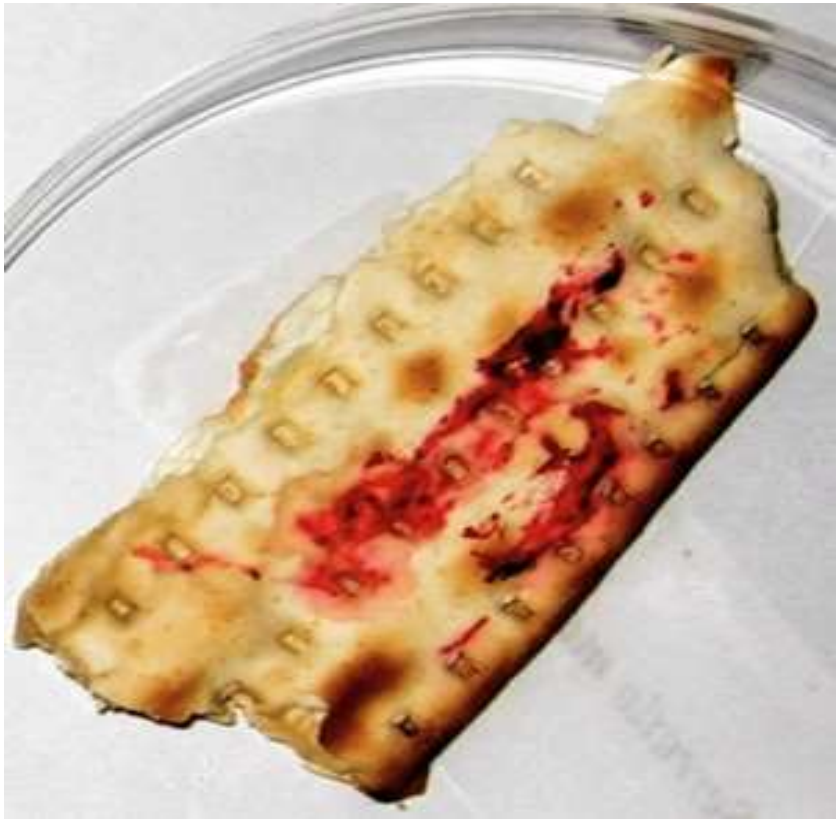
Rapiness - Due to proteolytic activity of micro- organisms.

Due to this bread becomes

Yellow to brown in color, more soft and sticky.

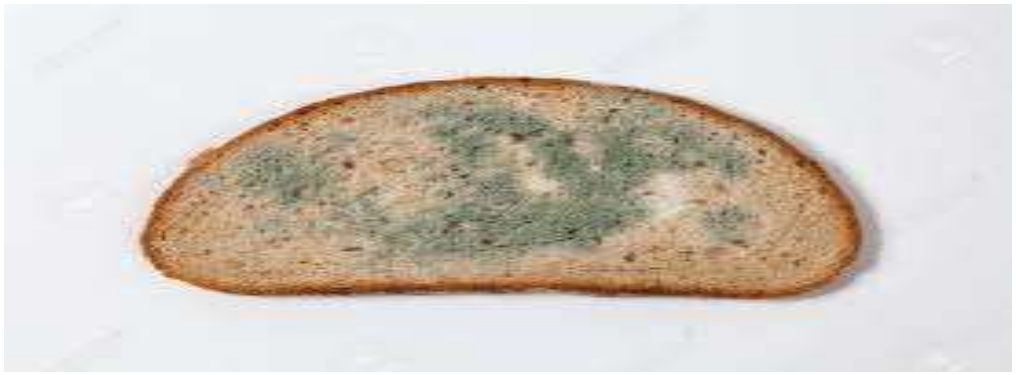
RED OR BLOODY BREAD

- Here bread surface becomes red.
- Due to growth of red pigment producing organism *Serratia mercescens*.



MOLDY BREAD

- Here visible growth of mold appear on surface of bread.
- Primary mold involved are *Rhizopus nigricus* and species of *Penicillium , Aspergillus , Mucor* etc.
- Some Aspergillus species ar



IMMUNOLOGY

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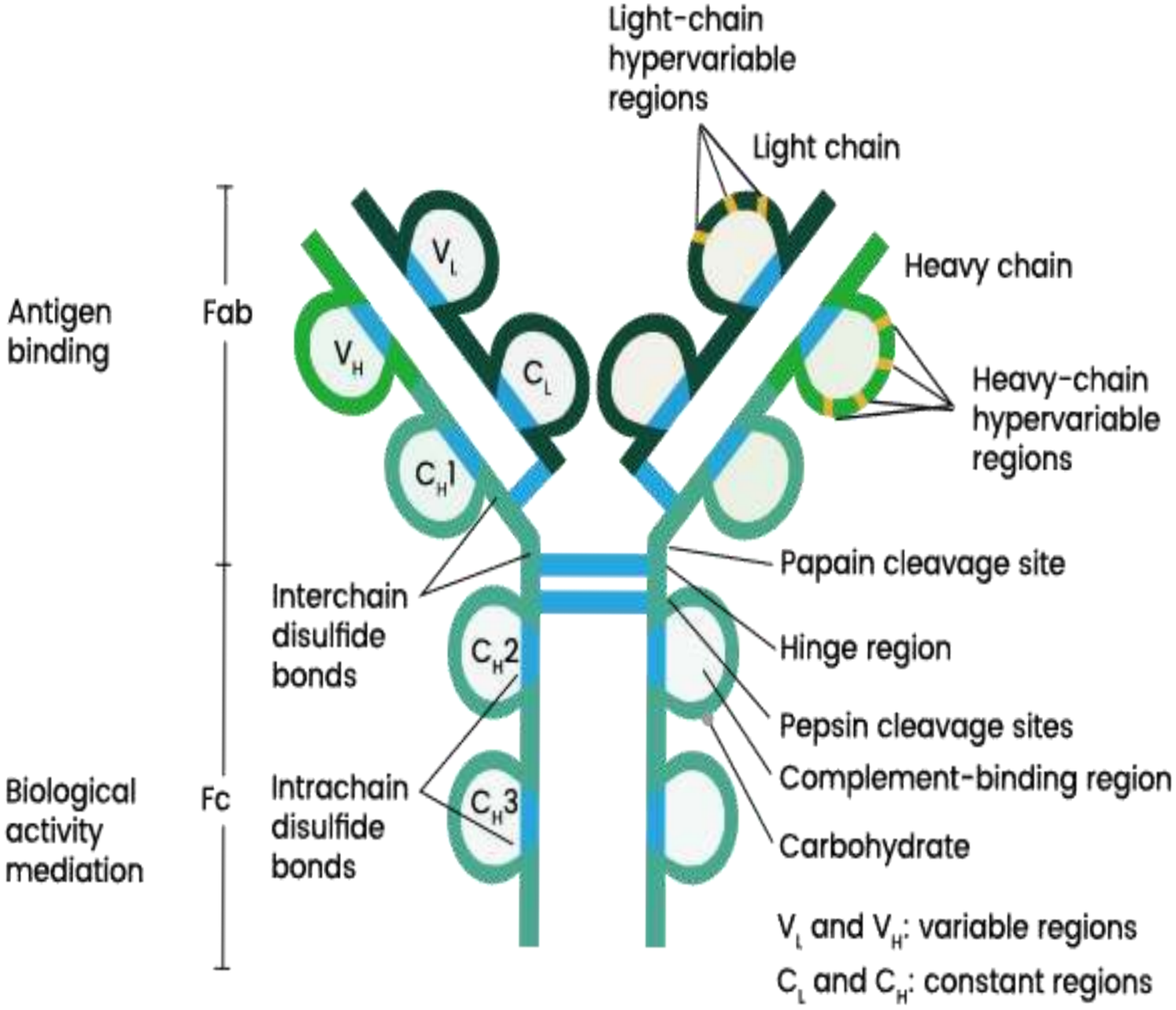
Light-chain hypervariable regions
Light chain
Heavy chain
Heavy-chain hypervariable regions
Antigen binding
Fab
V_L
V_H
C_L
C_H1
Papain cleavage site
Interchain disulfide bonds
Hinge region
C_H2
Pepsin cleavage sites
Complement-binding region
Biological activity mediation
Fc
Intrachain disulfide bonds
C_H3
Carbohydrate
V_L and V_H: variable regions
C_L and C_H: constant regions

Purification of Enzymes

Presented by
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Purification:-Process of obtaining enzyme with maximum retention of activity

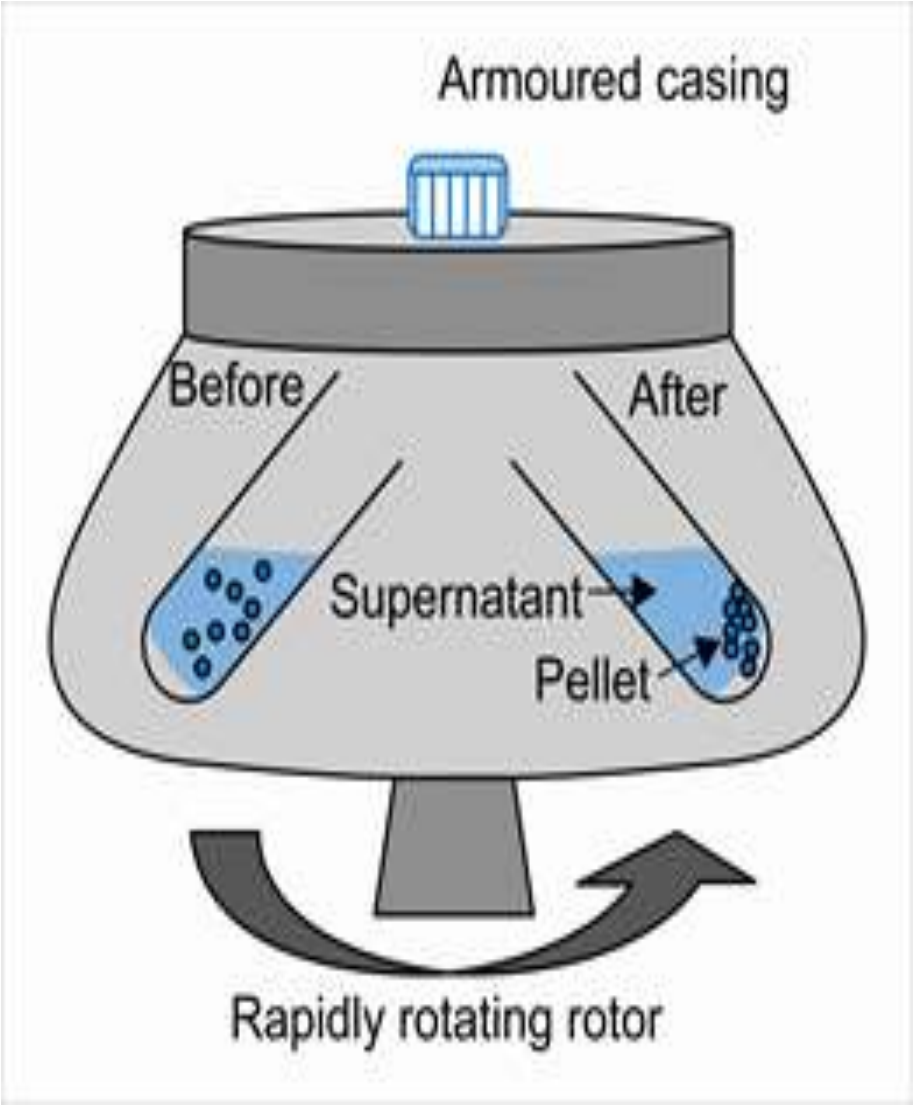
- Crude enzymes fraction separated from other components are further subjected to isolation & purification process.
- Purification-obtain pure form of enzymes with maximum retention of activity.
- To obtain maximum activity-process repeated over and over again.
- Repetition may cause denaturation of enzymes.
- So repetition avoided & instead combination of different processes are used for purification
- Purification carried out based on :-
    a) Size
    b) Change in solubility
    c) Possession of specific binding sites
    d) Molecular charge

Purification based on Molecular size or mass

- Physical methods are used for separation of large proteins i.e enzymes from small protein molecules.
- Enzymes-large molecules
- So, easy to separate based on size or mass.
- Separation methods are based on molecular size, molecular weight, sedimentation properties etc.

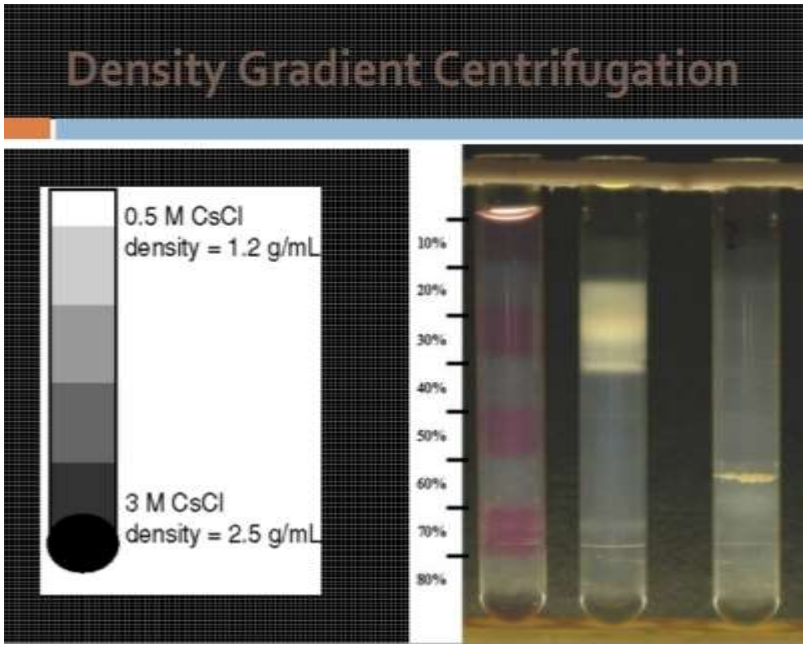
Centrifugation

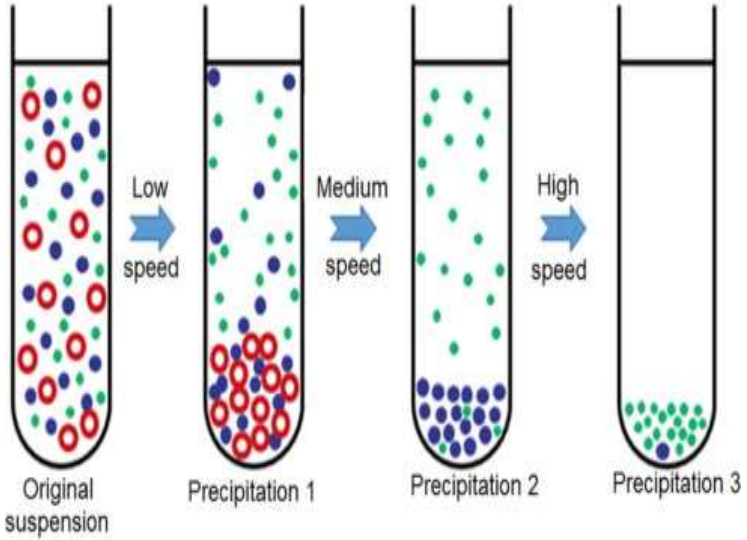
- Use centrifugal force
- Separates particles based on size & density.
- Proteins in solution tends to sediment at high centrifugal forces & thus opposing to diffuse, allowing proteins to separate from mixture.
- Rate of sedimentation depends upon size,shape,molecular weight,mass,density along with some other properties like viscosity of solution.
- Higher molecular weight,higher sedimentation rate.

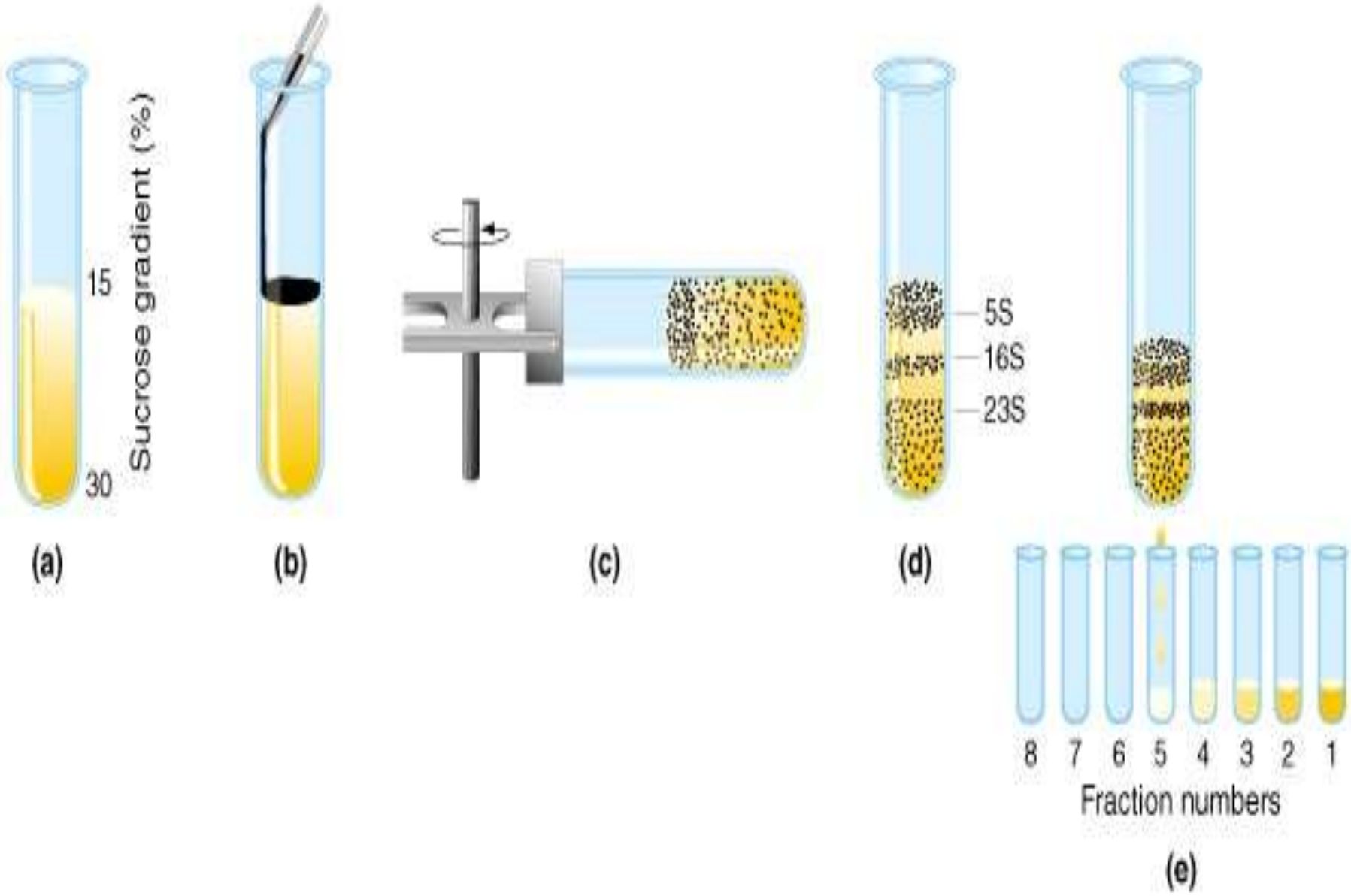


Density gradient centrifugation

- Widely used for separation of proteins,other macromolecules and viruses
- Here, prepare gradient of sucrose and water with high density at bottom.
- Size of gradient is 1cm.
- Cesium chloride also can be used.
- To achieve stable gradient,whole plastic tube with already formed gradient is kept in refrigerator at 4 ^{o}c for 12 hrs.
- Mixture to be separated is layered on top of gradient in horizontal position.
- Place tubes in rotor and centrifuge it at high speed.
- Duration - 30 min to 3 hrs.
- Sample can be separated from layer by puncturing at bottom of tube,by thawing out the fluid by syringe,by punchring tube at respective position.





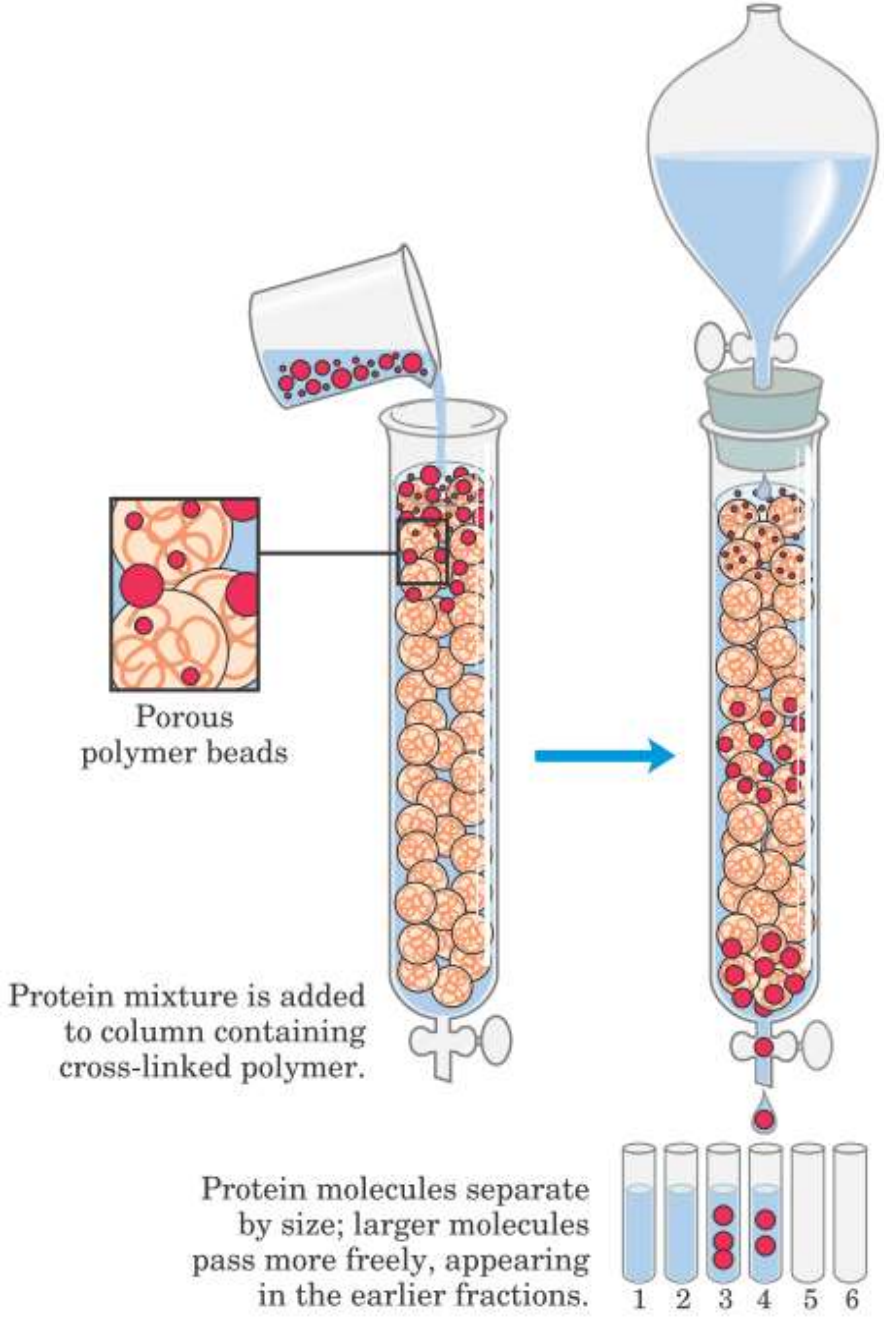

Sucrose gradient (%)
15
30
(a)
(b)
(c)
5S
16S
23S
(d)
8 7 6 5 4 3 2 1
Fraction numbers
(e)

Molecular Exclusion Chromatography

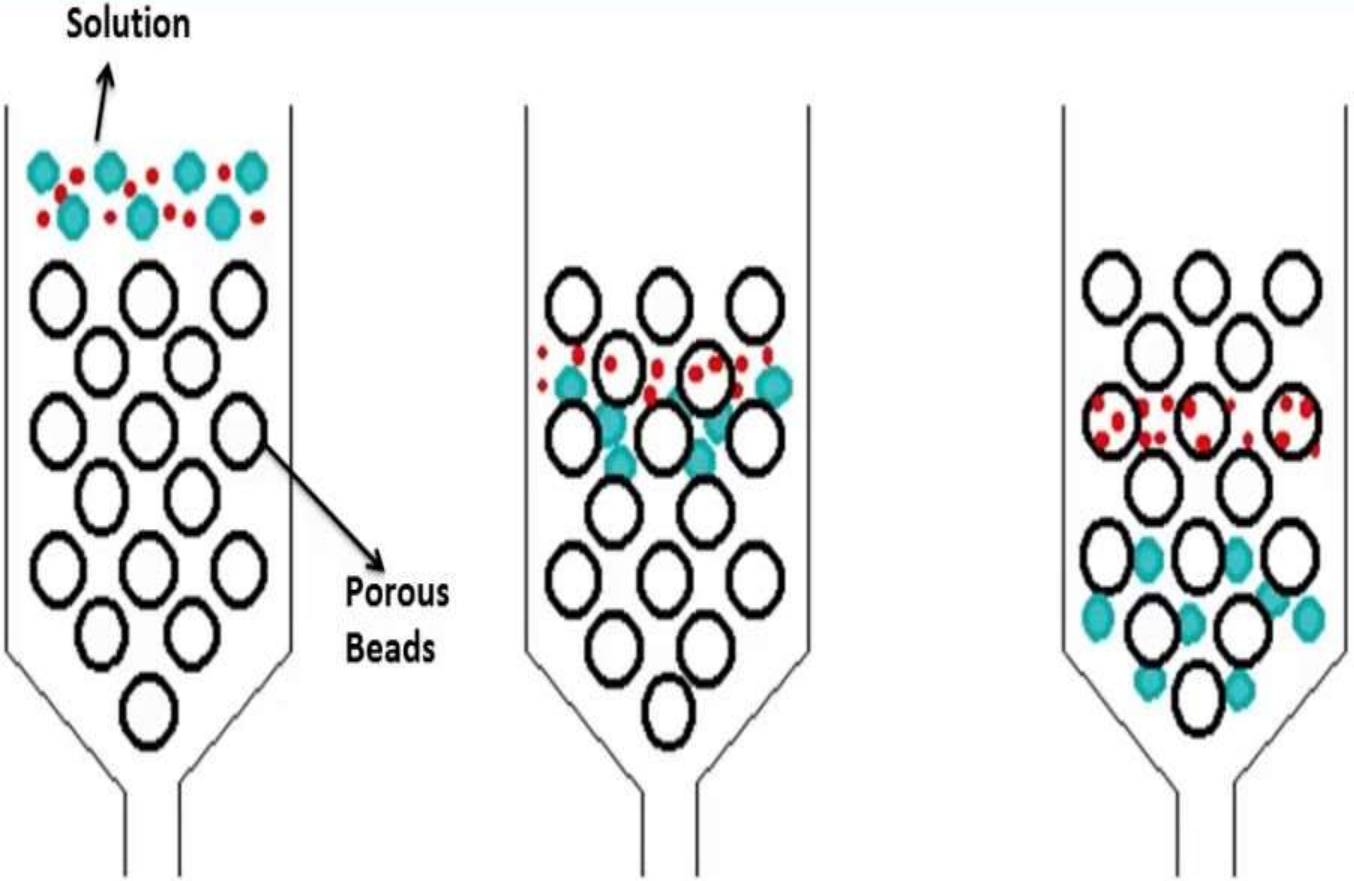
- Also known as molecular sieve chromatography.
- Type of chromatography where separation is based on size & molecular weight
- Type of column chromatography
- Process performed with column which consists of a hallow tube tightly packed with extremely small,highly hydrated porous beads of diifferent sizes.
- Polymeric beads - previously washed & equilibrated with suitable buffer.
- Common gel used- sephadex (polysaccharide dextran carefully cross linked to give small beads of hydrophilic insoluble nature which are placed in water to swell and form gel)
- Biogel P – cross linked polymer of acrylamide

Process :-

- Gel –packed in glass cylinder of diameter 2 inch & 3 feet in length
- Mixture of enzymes dissolved in suitable buffer allowed to flow by gravity down the packed column of inert beads.
- Smaller proteins can penetrate into pores in beads & so retarded in their flow down the column.
- Large protein can't penetrate into beads & pass down the column more rapidly.
- Proteins with intermediate size will pass down the column at intermediate rate depending upon degree to which they can penetrate into the beads.
- The molecules are said to be excluded and remain in the excludes volume of aqueous phase outside the beads
- Large gel columns are expensive
- This method is generally used in the later stages of purification



How it Works???



Purification based on change in solubility

- Solubility is the property of solid , liquid or gaseous chemical substances called solute to dissolve in a solvent.
- The solubility of any components in a given solvent depends upon a balance of force between solute & solvent.
- The solute dissolves in solvent based on force of attraction or repulsion between them.
- During purification of enzymes it is possible to alter these forces & hence the enzyme of interest can be precipitated . There are three important ways of changing the solubility of enzymes as -
    a) By changing the pH
    b) By changing ionic strength
    c) By decreasing dielectric constant
- These methods can be applied on large scale & are often used in the initial stages of purification of enzymes.

1.Isoelectric Precipitation

- This methods purifies enzyme by changing the pH
- Isoelectric pH is that pH where protein molecule has net zero charge,such molecules with no charge are called ‘ Zwitter ions ‘.
- Each enzyme has it’s isoelectric pH (pI).

  e.g Glucose 6 phosphate isomerase pI = 9.8

  catalase pI = 5.4

  lactate dehydrogenase = 6.3
- In general when Protein is having net charge + ve or – ve, it’s force of interaction with water increases & it gets solubilized.
- But protein becomes least soluble when the pH of solution is at it’s isoelectric point,because at pI proteins looses charge.
- Thus by making proper adjustment of pH ,desired enzyme can be precipitated out from the solution .But it is importnant to check that enzyme of interest is not inactivated by exposure to these changes in pH.

2.Salt precipitation

- Based on changes in ionic strength.
- Commonly used method of purification especially in initial separation of enzymes.
- Principle involves the properties of ionic strength & affinity of enzyme & salt towards water. Here ,proteins can be separated by altering their solubility in presence of high salt concentration.
- This technique makes use of neutral divalent salts like Ammonium sulfate , magnesium chloride,K2HPO4.CaCl2, etc.
- Sometimes monovalent salts like Nacl or sodium acetate may also be used,
- Though Ammonium sulfate is most widely used.

Salting in -

- Based on principle that ,at low concentration of salt (solute) solubility of protein increases.
- When proteins are present in aqueous environment(in water) then protein tends to fold in such way that charged hydrophilic amino acid surrounds proteins & hydrophobic amino acids are folded in & protect from water.
- So hydrophilic charged amino acids interact with water molecule by solute-solvent interaction & get solubilizes in water. Water forms solvation layer around protein molecules.
- When low concentration of salt was added in a solution containing proteins then it interact with proteins & stabilizes their structure by increasing the charge. This results in increase in solubility of proteins & the phenomenon is known as " Salting In "
- It was explained by Dubye-huckel"s theory.

Salting out -

- Increase in salt concentration decreases solubility of proteins in water & hence proteins get precipitated.
- When high concentration of salt was added then salt ions gets dissociated. These dissociated salt ions interact with oppositely charged ions of water. As a result interaction between hydrophilic amino acids of proteins & water becomes weak. So water starts interacting with proteins.
- Here, salts forms shell around protein molecules.
- As there is no water available to interact with , hence proteins interact with other by hydrophobic interaction & forms aggregates which gets precipitated in solution.
- Since proteuins consists of different variations of amino acids the salt concentration at which proteins gets precipitated varies from proteins to proteins.
- E,g Fibrinogen – 0.8 M ammonium sulfate
- serum albumin -2.4 m ammonium sulfate

Factors affecting salting out-

- Temperature
- pH
- Presence of other impurities.

Methods of addition of salt –

a.Solid addition

b.liquid addition.

3.Solvent Precipitation

- Addition of water miscible solvents like ethanol,

methanol or acetone to solution causes precipitation of proteins.

- Miscible organic solvent decreases the dielectric constant of solution (water) which in turn causes proteins to come together.
- Dielectric constant –

It is the ability of solvent to retain ions in solution in their ionic state & also preventing oppositely charged ions from reacting with each other.

- In absence of organic solvent water forms a layer around proteins called 'solvation layer'.
- Addition of solvent causes displacement of water from proteins.
- It causes increase in affinity between positive & negative charges on proteins by attractive electrostatic force & thus proteins get precipitated.
- This method can be used on large scale as a initial procedure for purification.
- Addition of organic solvent can lead to inactivation of enzymes & hence it is important to work at low temperature.

- # Miscellaneous Method

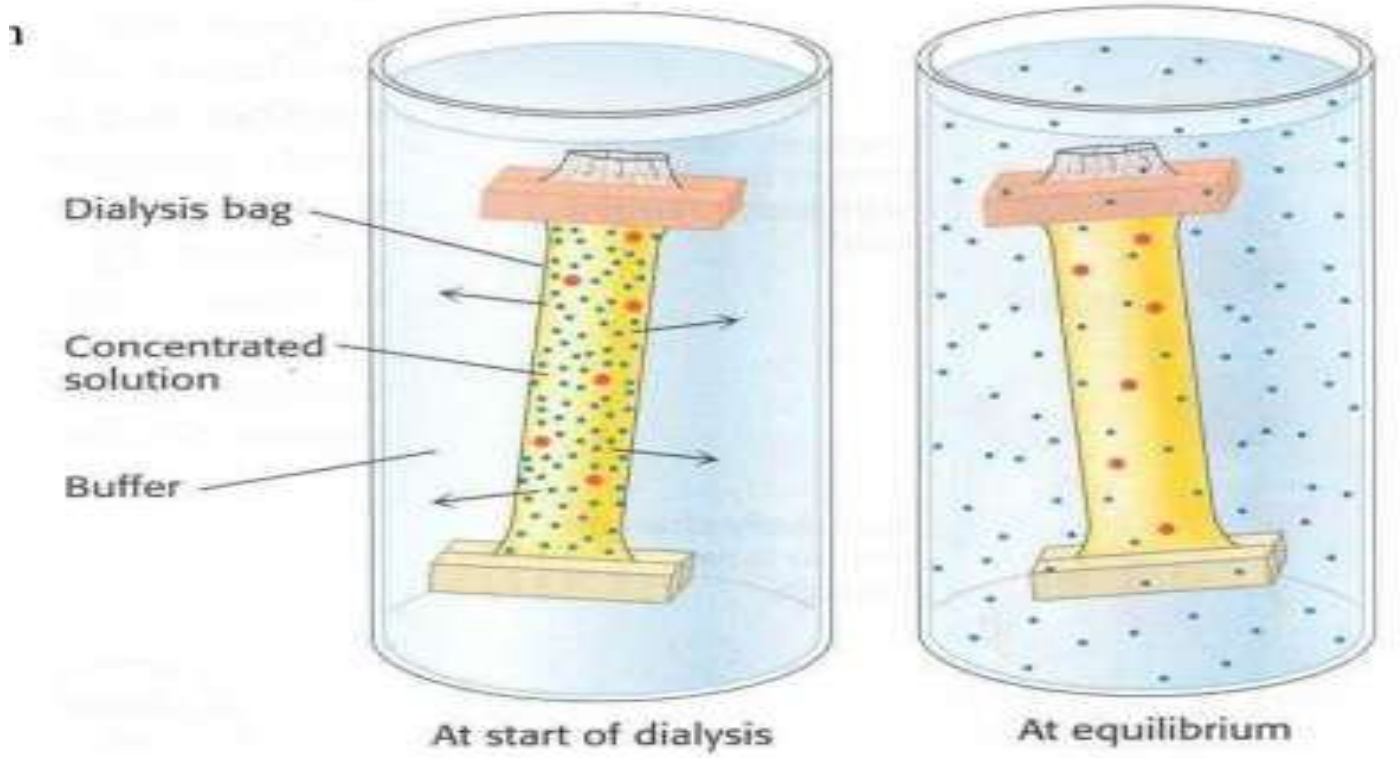
- Some water soluble, non-ionic polymers such as polyethelene glycol, pectate , polyacrylic , polymetacrylic acid etc. can also cause enzyme precipitation.
- Polyethylene amine used as protein precipitant at large scale,
- This substances primilarly act through the removal of solvent sphere of the enzyme proteins.

Dialysis & Ultrafilteration

- It is the process of separating molecules on solution by the difference in their rates of diffusion.
- Defination –

  It is diffusion of solute from solution of high concentration to low through semipermeable membrane until equilibrium is reached.
- Oldest technique ,which is not used for separation of enzyme from each other but widely used during purification procedure to remove salt or organic solvent.
- A dialysis membrane such as cellophane act as a sieve with holes large enough to permit the passage of globular substances upto 20,000d molecular weight, but not all ranges of molecule.
- It doesn't allow passage of proteins through semipermeable membrane.
- It is possible to alter pore size by various mechanical, chemical processes.
- Thus enzyme solution is packed in cellophane bag.
- It is then suspended in water.
- The smaller molecules like salt, solvent will pass out through the pores until equilibrium is reached and enzymes will be retained.

- The membrane enclosing the proteins solution allows water & small solute to pass through freely but don’t allow passage of large solute.
- Thus, by replacing the outer aqueous phase wit distilled water several times, the concentration of solute molecule in the crude mixture can be decreased.
- In ultrafiltration ,small molecules of ions pass through the dialysis membrane under pressure.



Purification Based on Possesion of Specific Binding Site

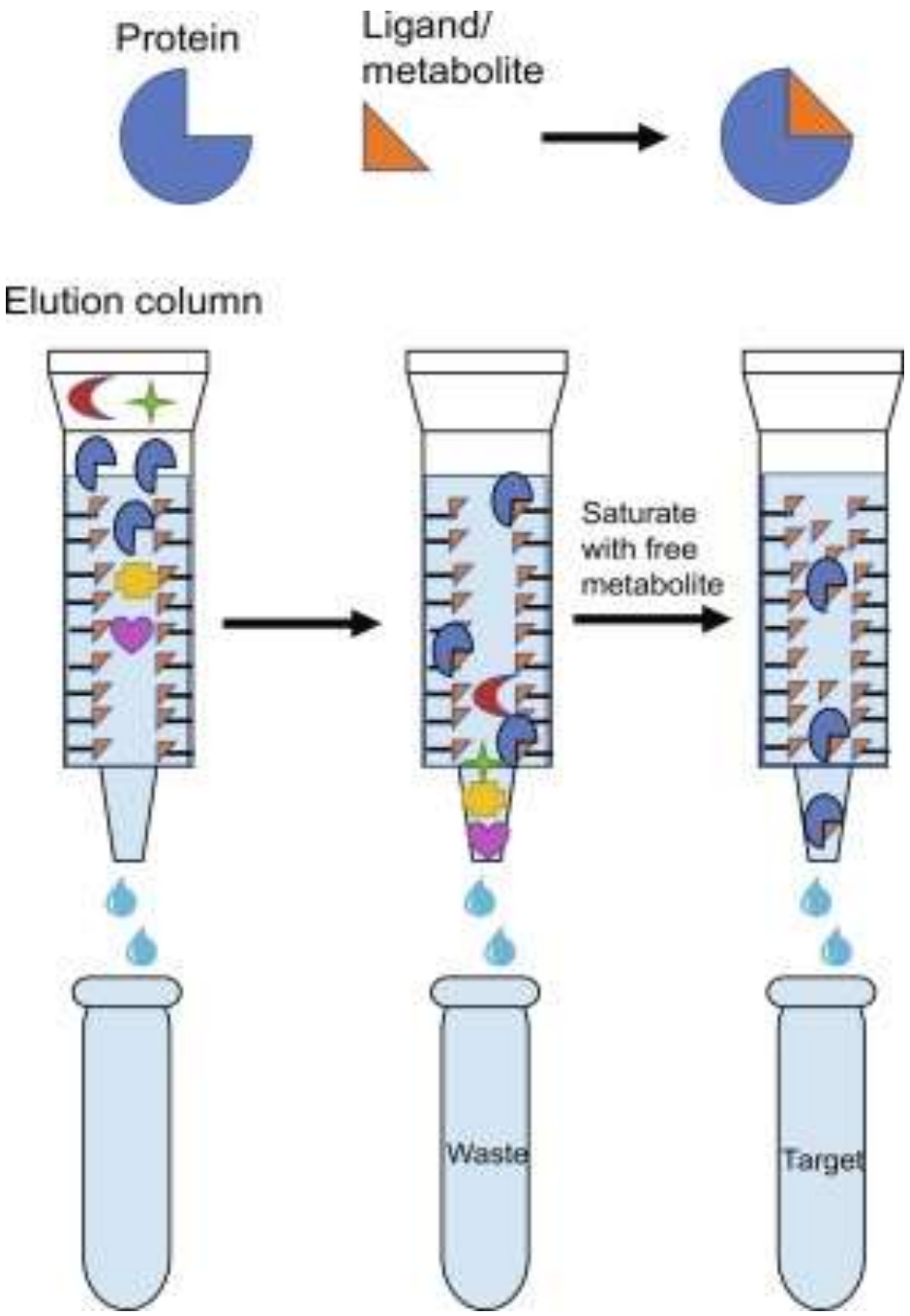
- Enzyme normally display highly specific interaction with their substrates.
- By considering the specificity (affinity), enzymes can be separated from each other , by using methods like affinity chromatography & affinity elution.

1.Affinity Chromatography -

- In affinity chromatography , biological specificity of enzyme is implanted.
- This techniques separates proteins on basis of reversible interaction between a protein & specific ligand coupled to a chromatography matrix.
- This technique offers high selectivity & high resolution of enzyme of interest.

Method-

1. Here , a column is prepared by tightly packing inert gel beads in glass column.
2. A substrate or competitive inhibitor which interact specifically with the enzyme of interest is covently linked to an inert matrix (e,g. Agarose or Sephadex) in such a way that groups reacting with enzymes remains free.
3. When mixture of enzyme solution was passed down a column containing affinity matrix,only desired specific enzyme is retained & other proteins & enzymes are washed away.
4. In Desorption,the bound enzyme can be desorbed or eluted with either an excess of ligand or by changing the pH or ionic strength of solution in a such way as to weaken the binding of enzyme from column.
5. Comparatively simple & highly efficient methods for purifying enzyme in single step from mixture containing very small amount of enzyme



- Certain problems are associated with these affinity chromatography, because attaching a suitable substrate analogue or inhibitor (ligand) to matrix can be a difficult task.
- Linking of ligand to the matrix may interfere with the binding to enzymes & may lead to loss of enzyme specificity.
- Affinity chromatography to work successfully , the strength of enzyme interaction must be in a correct range.
- If it is too weak , the enzyme will not be retarded by column & if interaction is too strong, removal of bound may only be possible under harsh condition which may lead to inactivation of enzyme.
- Problem are possesses by enzyme having more that one substrate .E.g NAD dependent dehydogenases . In such case many enzymes would be retained by NAD+ bound column.
- In spite of all these problems , affinity chromatography has made very significant contribution to purification of enzymes.

2.Affinity Elution -

- It is complementary method to affinity chromatography.
- In affinity elution , first suitable substrate or ligand or fixed to a inert carrier matrix in a column.
- Enzyme mixture is then adsorbed on column having substrate or ligand to it.
- The bound enzyme is then eluted (desorpted) by another appropriate highly specific substrate.
- This process has many advantages over affinity chromatography.
- The column of high capacity are more readily available since ion exchangers are much cheaper than affinity matrix.

Purification Based on charge present on Enzyme

Ion Exchange Chromatography :-

- Method depends upon electrostatic reaction between ions of opposite charge
- Proteins are seperated based on charge present on them at given pH.
- Components of samples-

  anioinic –bind to possitively charged ion exchanger

  cationic- binds to negatively charged ion exchnager
- Net charge on enzymes depends on it's isoelectric point (pI)

  At isoelectric pH -proteins have zero(null) charge

  Below isoelectric pH -proteins have positive charge

  Above isoelectric pH -proteins have negative charge
- Ion exchangers are usually consist of modified derivative of support matrix material such as cellulose,sephadex etc.
- Two types of ion exchangers-

  Cation exchangers -posses negatively charged groups & so bound cations(+) charge.E.g=Carboxylate (coo-),phosphate (PO3--),sulfonate

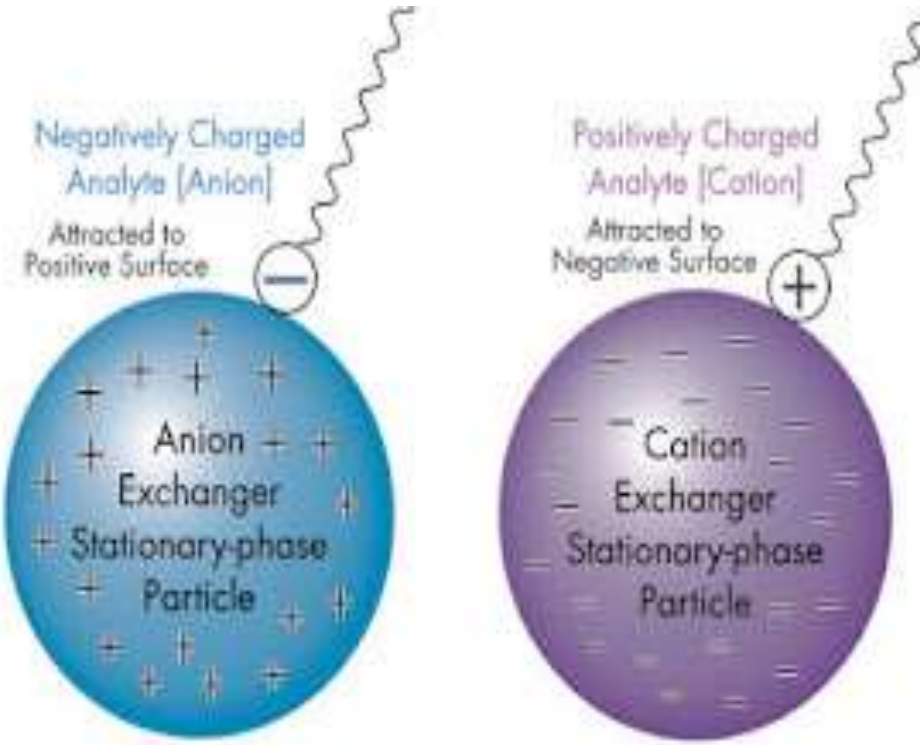
  Anion exchangers–posses positively charged groups& so bound anion(-) charge. E.g- DEAE sephadex,DEAE cellulose, Guanidoethyl cellulose.

Method:-

1. Column of ion exchangers is prepared
2. Mixture of enzymes having charged groups is eluted down the mixture the column
3. Oppositely charged enzymes binds to ion exchanger by attraction (ionic bonds) with help of charged groups on them.
4. Condition like pH is set in such a way that will favor interaction between charged proteins & ion exchangers using suitable buffer.
5. Desorption done by 2 ways:-

   a) by increasing ionic strength of solution leading competition between enzymes and increased conc. of cation/anion.

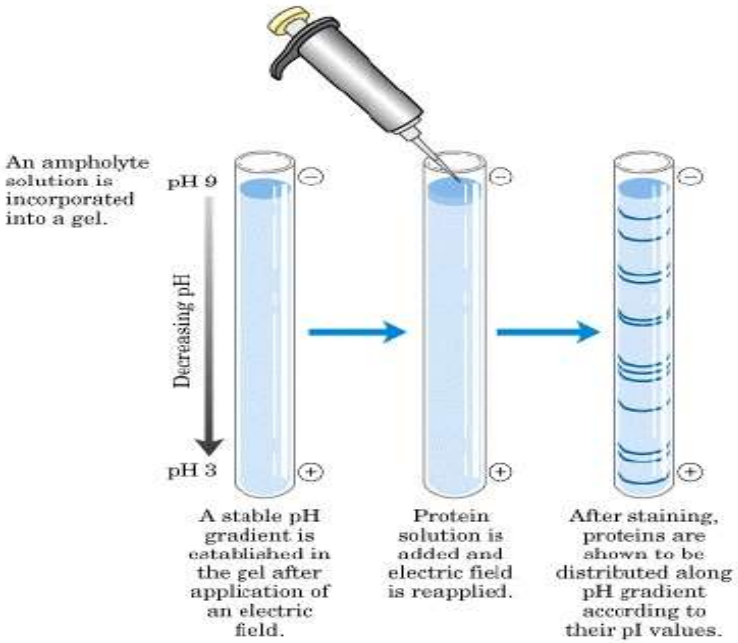
   b)By adjusting pH such that,when enzyme mixture passed through the ion exchanger, desired enzymes doesn't bind to resins & passes straight through column,Iseaving other enzyme bound column.



2.Isoelectric Focusing

- Based on equilibrium position of charged ions in a pH gradient.
- If electrophoresis performed in gel with pH gradient,protein molecules will migrate towards oppositely charged electrode & get Precipitate where the pH of solution is same as it's isoelectric pH & remain there as where gradient potential differences are maintained.
- this method is used in later stages of purification.
- At isoelectric pH ,proteins looses it's charge stop migrating .if neutral enzyme tries to diffuse in neighbouring region then it will aquire charge & its brought back to isoelectric pH region.
- Artificial pH gradient can not be prepared as conc. Gradient but can be preapared only electrophoretically.
- At the anode,acid is used as electrolyte fragil &to be stabilize with suitable buffering agent.
- Use of polymine or poly carboxylic acids enables the formation of stable pH gradient.

Isoelectric Focusing



- pI of a protein: net charge=0
- A pH gradient is established by allowing a mixture of organic acids and bases (ampholytes). Protein migrates until it reaches the pH that matches its pI

- Example- protein with isoelectric pH 6.0 in position A (at acidic six) will migrate towards cathode & will settle at pH 6.0 . If it moves to position B, it will aquire –ve charge & again will be brought to pI.

Immobilization of Enzymes

Presented by
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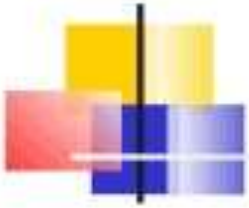
What Is Enzyme Immobilization ?



Enzyme immobilization may be defined as a process of confining the enzyme molecules to a solid support over which a substrate is passed and converted to products.

What Is An Immobilized Enzyme?

An immobilized enzyme is one whose movement in space has been restricted either completely or to a small limited region.

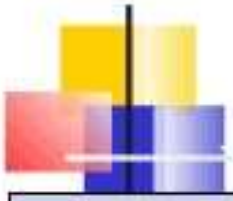
Why Immobilize Enzymes?

- Protection from degradation and deactivation.
- Re-use of enzymes for many reaction cycles, lowering the total production cost of enzyme mediated reactions.
- Ability to stop the reaction rapidly by removing the enzyme from the reaction solution.
- Enhanced stability.
- Easy separation of the enzyme from the product.
- Product is not contaminated with the enzyme.

An Ideal Carrier Matrices For Enzyme Immobilization

- Inert.
- Physically strong and stable.
- Cost effective.
- Regenerable.
- Reduction in product inhibition.

CLASSIFICATION OF CARRIERS



Inorganic Carriers

- High pressure stability.
- May undergo abrasion

Examples:

1.Commercialy SiO_2 available materials-
- Porous glass.
- Silica.

2. Mineral materials - (clays)
   Celite ,Centonite

Organic Natural Carriers

- Favourable compatibility with proteins.

Examples:

1.cellulose derivatives-
- DEAE-cellulose
- CM-cellulose.

2. Dextran.

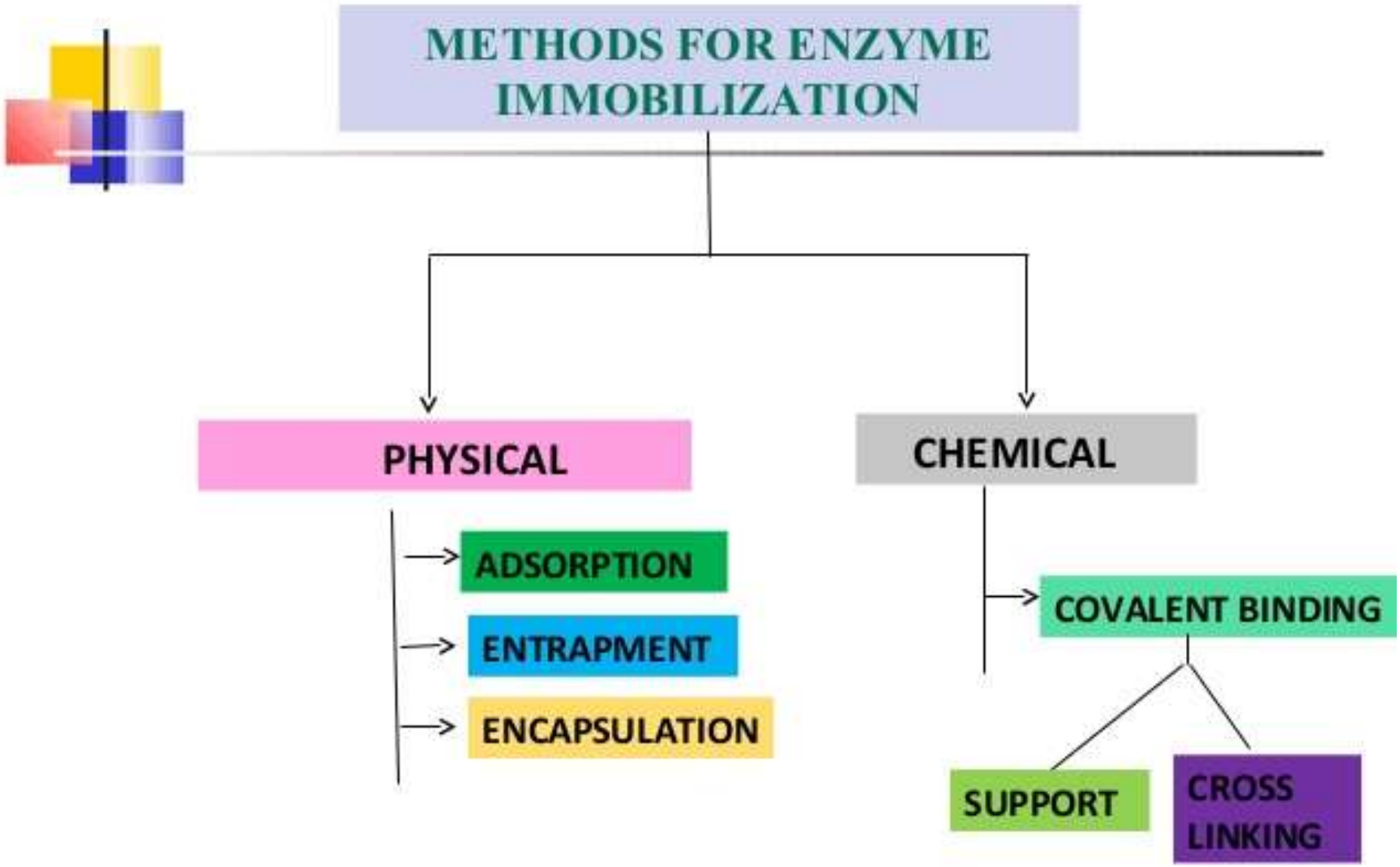
3.Polysacharides
Agarose, Starch
Pectine ,Chitosan

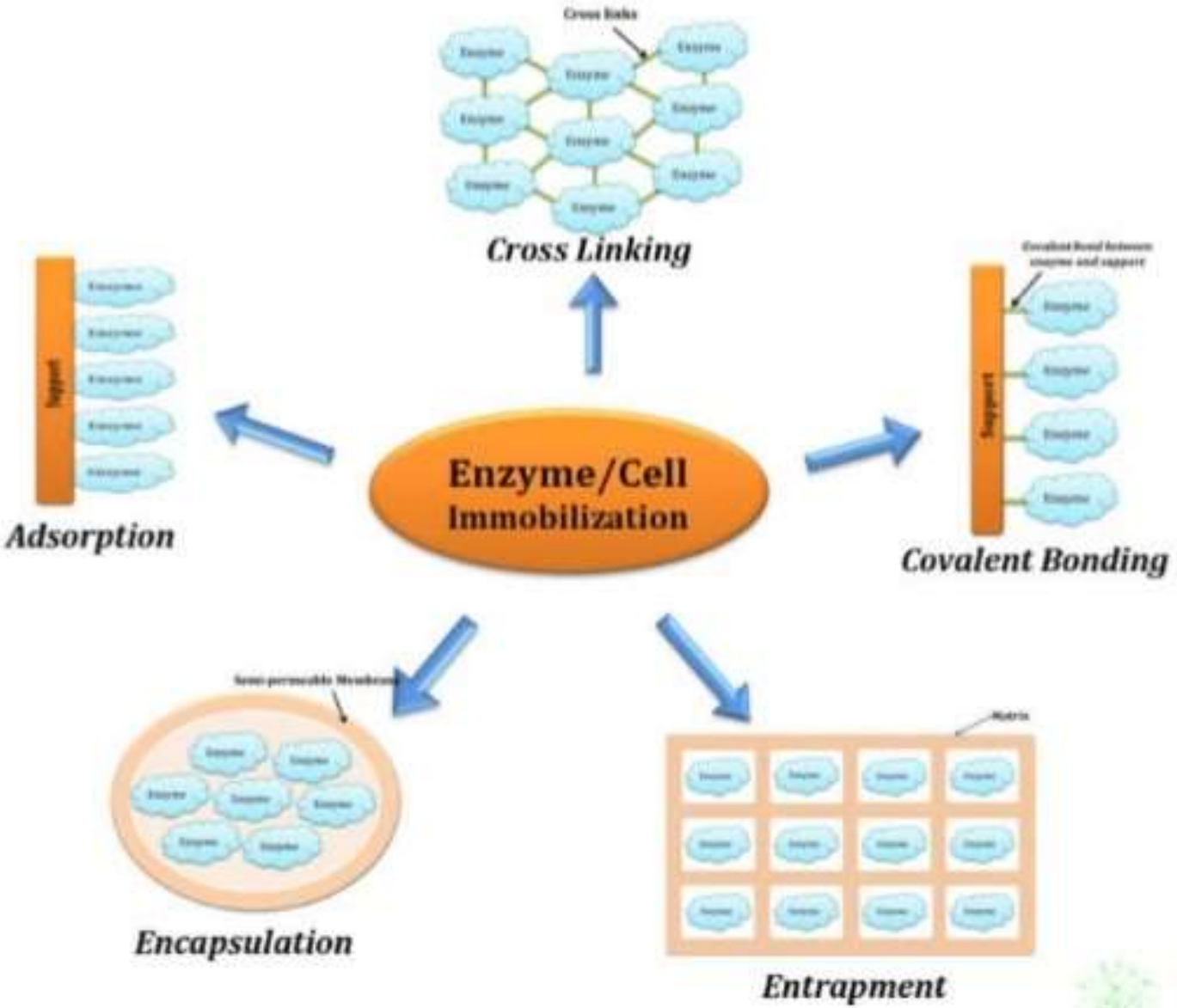
Organic Synthetic Carriers

- High chemical and mechanical stability.

Examples:

1.Polystyrene
2.Polyvinylacetate
3. Acrylic polymers


METHODS FOR ENZYME IMMOBILIZATION
PHYSICAL
CHEMICAL
ADSORPTION
ENTRAPMENT
ENCAPSULATION
COVALENT BINDING
SUPPORT
CROSS LINKING



Enzyme/Cell Immobilization Methods

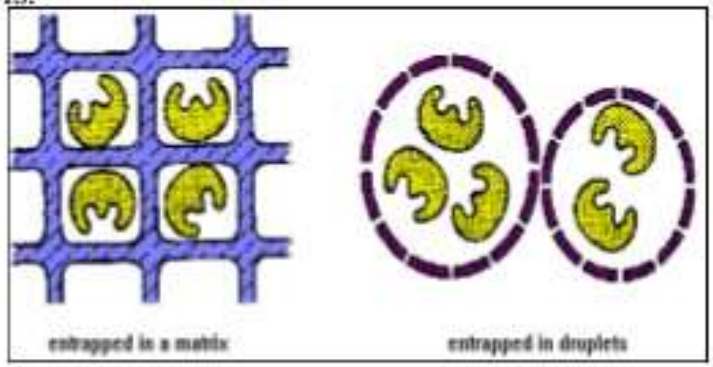
Entrapment

- In entrapment, the enzymes or cells are not directly attached to the support surface, but simply trapped inside the polymer matrix.
- Enzymes are held or entrapped within the suitable gels or fibres.
- It is done in such a way as to retain protein while allowing penetration of substrate. It can be classified into lattice and micro capsule types.

Inclusion in gels: Poly acrylamide gel, Poly vinyl alcohol gels.

Inclusion in fibers: Cellulose and Poly -acryl amide gels.

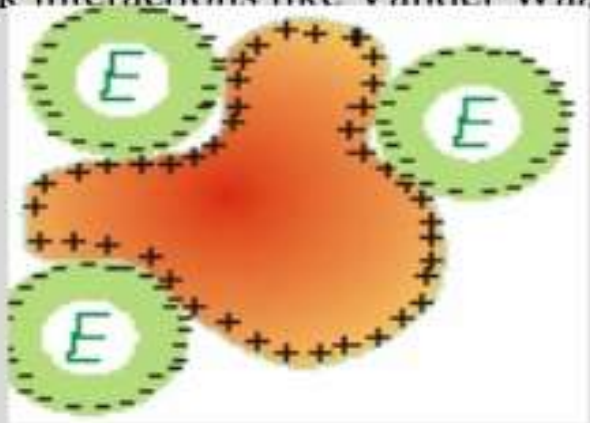
Inclusion in micro capsules: Polyamine, Polybasic - acid chloride monomers.



Physical Methods For Immobilization

ADSORPTION

- Involves the physical binding of the enzyme on the surface of carrier matrix.
- Carrier may be organic or inorganic.
- The process of adsorption involves the weak interactions like Vander Waal or hydrogen bonds.



- Carriers: - silica, bentonite, cellulose, etc.
- e.g. catalase & invertase

CARRIER BINDING -> PHYSICAL ADSORPTION

This method is based on the physical adsorption of enzyme protein on the surface of water-insoluble carriers. Examples of suitable adsorbents are ion-exchange matrices, porous carbon, clay, hydrous metal oxides, glasses and polymeric aromatic resins.

The bond between the enzyme and carrier molecule may be ionic, covalent, hydrogen, coordinated covalent or even combination of any of these.

Immobilization can be brought about by coupling an enzyme either to external or internal surface of the carrier.

The external surface binding method is advantageous as it does not involve conditions like pore diffusion. The disadvantages, however, include exposure of enzymes to microbial attack, physical abrasion of enzyme due to turbulence associated with the bulk solution.

The major disadvantage of the internal immobilization method is the pore diffusion.

CARRIER BINDING -> PHYSICAL ADSORPTION

Methods of immobilization by adsorption:-

he absorptive immobilization of enzymes can be done by following methods:

1) **Static Process:-** This is most efficient technique but requires maximum time. In this technique, enzyme is immobilized by allowing it to be in contact with the carrier without agitation.

2) **Dynamic Process:-** This process typically involves the admixing of enzyme with the carrier under constant agitation using mechanical shaker.

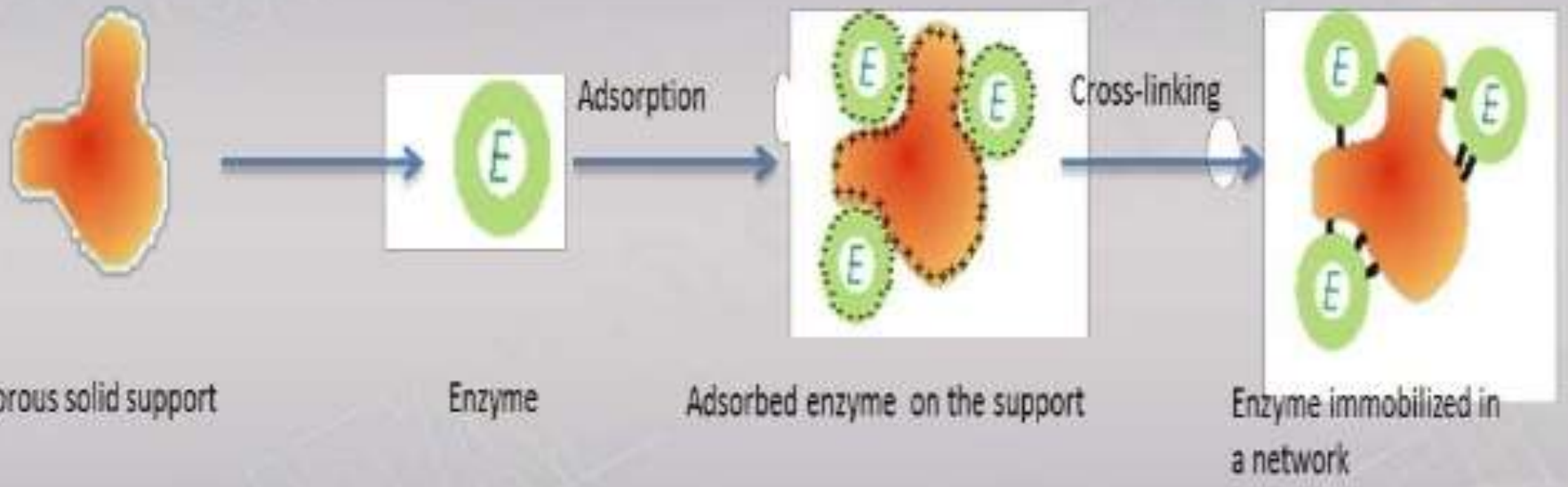
3) **Reactor loading:-** This process is employed for the commercial production of immobilized enzymes. The carrier is placed into the reactor and enzyme solution is transferred to the reactor with agitation of the whole content in the reactor.

4) **Electro-Deposition:-** In this technique, carrier is placed in the vicinity of one of the electrode in an enzyme bath and electric current is applied leading to migration of enzyme towards the carrier. This results in deposition of enzyme on the surface of the carrier.

CARRIER BINDING -> PHYSICAL ADSORPTION

Adsorption

Adsorbtion + and cross-linking



COVALENT BONDING

- Covalent binding is the most widely used method for immobilizing enzymes. The covalent bond between enzyme and a support matrix forms a stable complex. The functional group present on enzyme, through which a covalent bond with support could be established, should be non essential for enzymatic activity.
- The most common technique is to activate a cellulose-based support with cyanogen bromide, which is then mixed with the enzyme.
- The protein functional groups which could be utilized in covalent coupling include:
- Amino group
- Carboxylic group
- Phenol ring
- Indole group

Advantages of covalent coupling:-

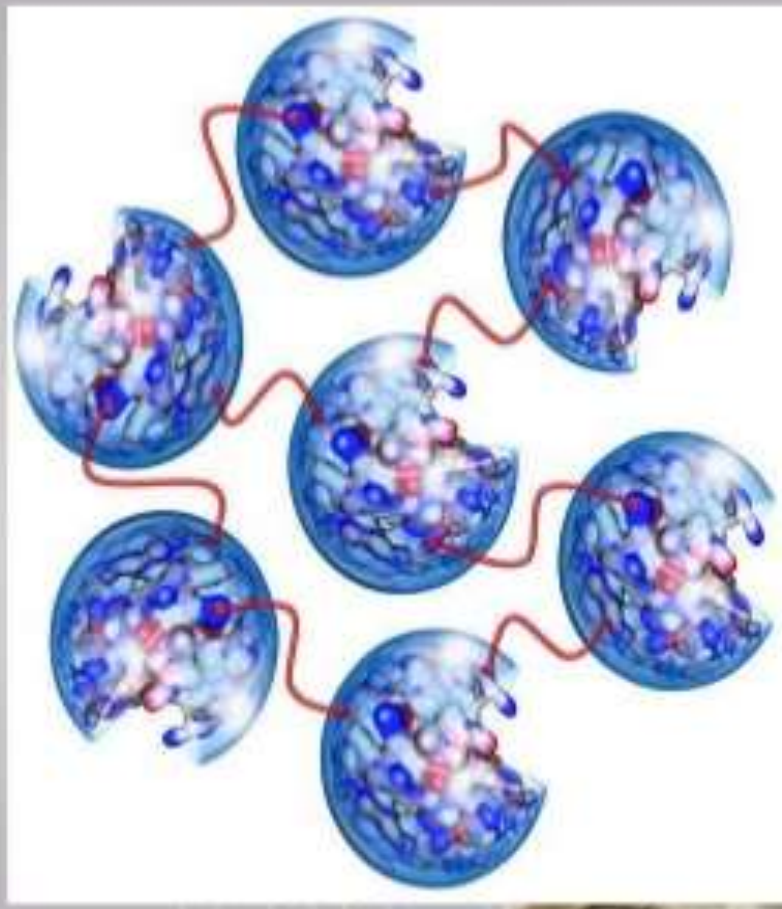
- The strength of binding is very strong, so, leakage of enzyme from the support is absent or very little.
- This is a simple, mild and often successful method of wide applicability

Disadvantages of covalent coupling:-

- · Enzymes are chemically modified and so many are denatured during immobilization.
- · Only small amounts of enzymes may be immobilized (about 0.02 grams per gram of matrix).

CROSS LINKING

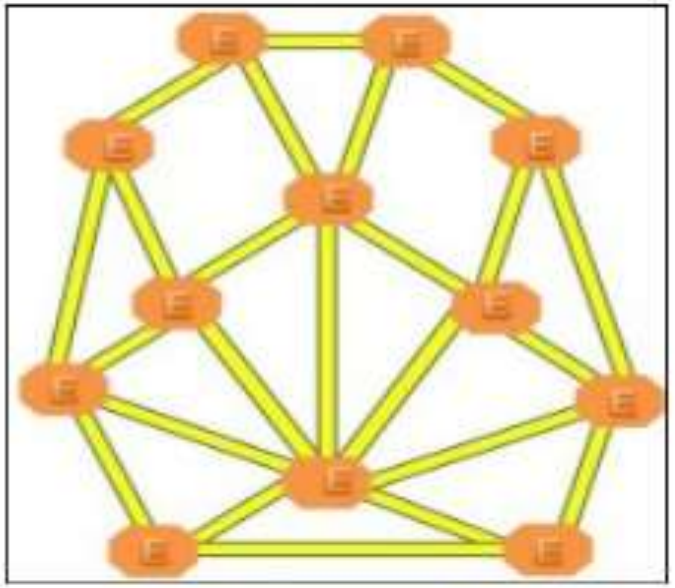
- This method is based on the formation of covalent bonds between the enzyme molecules, by means of multifunctional reagents, leading to three dimensional cross linked aggregates.



- It is used mostly as a means of stabilizing adsorbed enzymes and also for preventing leakage from polyacrylamide gels.

Cross Linking

- Cross linking involves intermolecular cross linking of enzyme molecules in the presence/absence of solid support.
- The method produces a 3 dimensional cross linked enzyme aggregate (insoluble in water) by means of a multifunctional reagent that links covalently to the enzyme molecules.



CROSS LINKING:

The most common reagent used for cross-linking is glutaraldehyde.

Advantages of cross linking:-

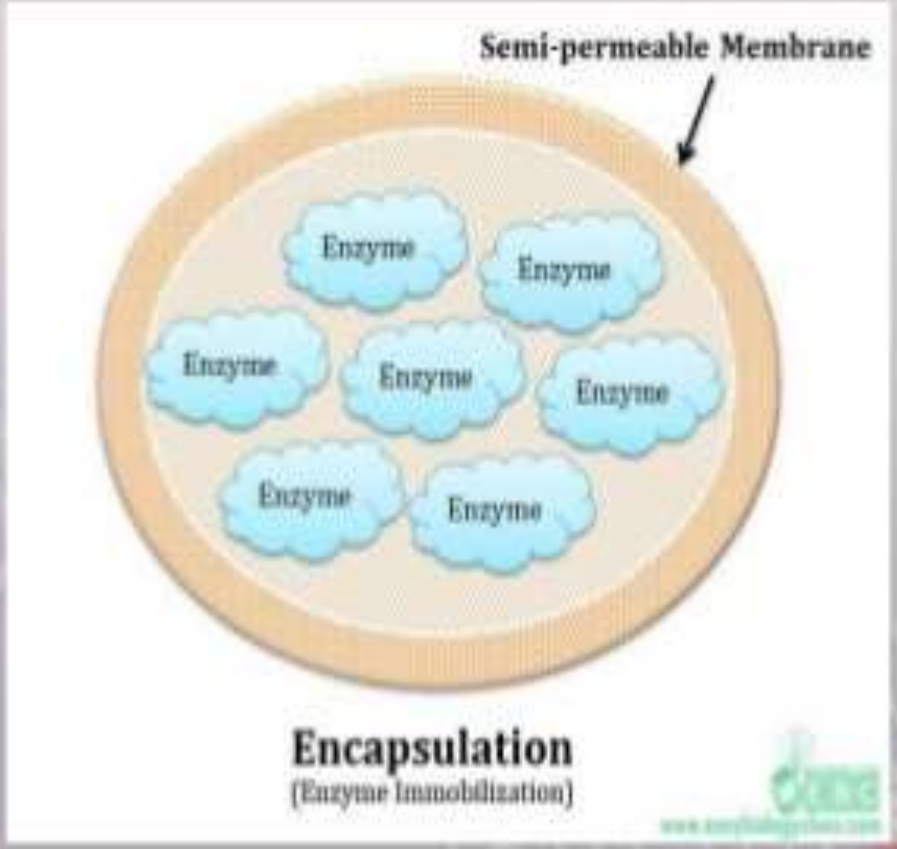
- Very little desorption(enzyme strongly bound)
- Best used in conjunction with other methods.

Disadvantages of cross linking:-

- Cross linking may cause significant changes in the active site.

2. Microencapsulation:-

This entrapment involves the formation of spherical particle called as "microcapsule" in which a liquid or suspension of biocatalyst is enclosed within a semi permeable polymeric membrane.



- **Advantages of entrapment:-**
  - Loss of enzyme activity upon immobilization is minimized.

- **Disadvantages of entrapment:-**
  - The enzyme can leak into the surrounding medium.
  - Another problem is the mass transfer resistance to substrates and products.
  - Substrate cannot diffuse deep into the gel matrix.

Bacterial blight of pomegranate

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History -

- In 1952, bacterial blight of pomegranate was first reported in India (Dehli) by Hingorani and Mehta.
- Pomegranate (Punica granatum) is an important fruit crop native to Iran.
- India is one of the largest pomegranate growing country in the world.
- Bacterial blight is one of the most devastating diseases of pomegranate occurring in major pomegranate growing states of India.
- The states include Maharashtra, Karnataka and Andhra Pradesh.
- In Maharashtra major districts affected by the disease are Sangli, Solapur, Osmanabad, Pune, Nasik, Latur, Aurangabad, Jalna and Ahmednagar.
- In karnataka Bagalkot, Gadag, Koppal, Bellary, Chitradurga, Bijapur are main blight affected districts.

Distribution.

- *Disease was prevalent only in india .*
- *But it was also reported from South Africa in 2010.*

CAUSATIVE AGENT -

Xanthomonas axonopodis pv. pumicae is a causative agent of blight disease of pomegranate. This is a gram negative rod shaped organism. Pomegranate is being claimed as the only host for the organism.

Symptoms -

- The pathogen attacks all the above ground plant parts including fruit splitting that results in huge yield and market losses.
- The initial symptoms appear as water-soaked translucent irregular to circular small black spots (2-5mm) on leaves.
- Gradually the centre of the spots become necrotic and turn dark brown with prominent water-soaked margins.
- In severe cases, spots coalesce and produce a large patch that may result in shedding of infected leaves.
- On stem brown to black spots develop around stem nodes, which results in breaking of affected branches.
- Fruits also infected with water soaked spots in the earlier stages, later became dark brown, slightly raised from the surface with oily appearance.

Mode of spread -

- *Xanthomonas axonopodis pv. Punicae* causing leaf spot of pomegranate infects through wounds and stomatal openings and causes water soaked lesions, which later develops into irregular spots.
- The organism spreads by air borne rout,could survive in soil for four months and cause fresh infections on new flush.

Favourable condition -

- The increase in day temperature (38.6°C) and low relative humidity (30.4%) along with cloudy weather and irregular rainfall favores the disease initiation and further spread of the disease.
- The pathogen survives in infected plant leaves, stems and fruits.
- Wind splashed rain, insects and contaminated pruning tools help in spreading the disease.

Preventing method of disease

Satisfactory control measures are not available for this disease. However, following measures may help in reducing the disease.

1. Use of disease free plant material.

2. Removal and burning of infected plant parts.

3. Phytosanitary cultivation techniques.

4. Bacteriocidal sprays containing antibiotics and copper. Streptocycline at (500 ppm) and Copper oxychloride at (2,000 ppm) is effective in controlling the disease.

PHOSPHOROUS CYCLE

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- Phosphorus is an essential element for living organisms.
- Phosphorus is only second to nitrogen as a mineral nutrient required for plants, animals and microorganisms. Phosphorus is one of the 16 essential nutrients for plant growth.
- Phosphorus moves slowly from deposits on land and in sediments, to living organisms, and then much more slowly back into the soil and water sediment. Because the quantities of phosphorus in soil are generally small, it can be the limiting factor for plant growth.
- The phosphorus cycle differs from carbon and nitrogen cycle is that it does not have an atmospheric component to the cycle.
- The phosphorus cycle involves the uptake of phosphorus by organisms.
- Phosphorus in the environment is mainly found in rocksand natural weathering processes can make it available to biological systems.After decomposition of biological waste, it can accumulate in large amounts in soils and sediments.
- Phosphorus is used by humans as a fertilizer in farmlands and in detergents, Overuse of phosphorus can lead to eutrophication.

Steps involved in the phosphorus cycle are as follow :-

1. Phosphorus solubilization.
2. Phosphorus uptake by organisms
3. Phosphorus mineralization and immobilization

2. PHOSPHORUS UPTAKE BY ORGANISMS -

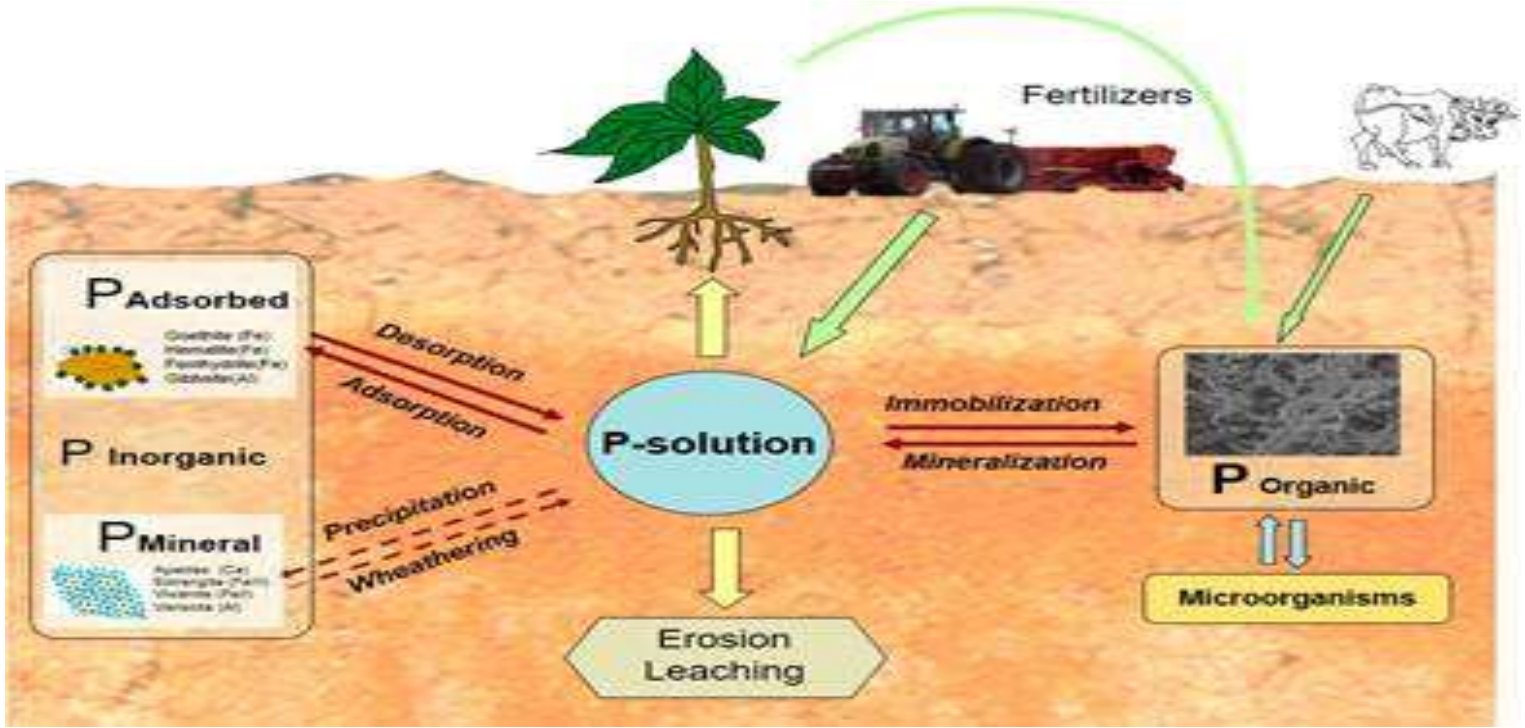
- Orthophosphate, the simplest phosphate, has the chemical formula PO_4^{3-}.
- In water, orthophosphate mostly exists as H2PO4, in acidic conditions or as HPO4 in alkaline conditions.
- Inorganic phosphates (PO4,, HPO4 or H2PO4) are absorbed by plants from the soil and bodies of water and eventually pass into animals through food chains.
- Phosphorus is important in all cells as a component of nucleic acids which form both the genetic material of the cell (DNA and RNA) and energy carrying molecules (ATP and NADP). Phosphorus is also found in phospholipids, cell membranes, bones and teeth. Photosynthesis, metabolism, energy transport, nerve function, and muscle movement are all dependent on phosphates.
- The availability of phosphorus depends on the pH.
- In acidic conditions phosphorus precipitates with iron or aluminum minerals.
- In alkaline conditions phosphorus precipitates with calcium minerals.
- Maximum phosphorus will be available at pH 6-7.
- When phosphorus finds its way into waterways in large amounts, as a result of runoff from the use of fertilizers, it can greatly contribute to eutrophication.

3. PHOSPHORUS IMMOBILIZATION MINERALIZATION AND IMMOBILIZATION -

- Microbial, plants and animals waste products, dead plants and animals are finally added to the soil.
- Some amount of phosphorus is returned to earth in the form of birds excreta - Guano deposits (excreta of marine birds) and dead fish.
- A large number of compounds found in organic matter make up the organic phosphorus in soils.
- Organic phosphorus is held very tightly and is generally not available for plant uptake until the organic materials are decomposed and the phosphorus released via the mineralization process.

■ MINERALIZATION -

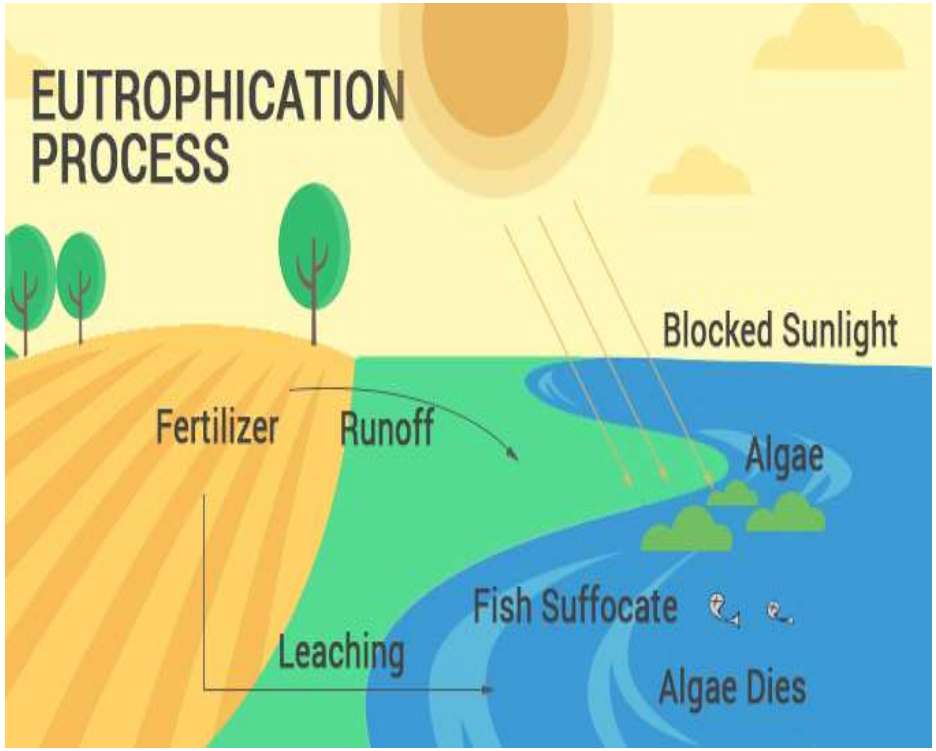
- Mineralization refers to the process where organic phosphorus is converted to inorganic plant accessible phosphorus.
- Microbes carry out mineralization process.
- The rate of mineralization is affected by factors like soil moisture, composition of the organic matter, oxygen concentration and pH.



IMMOBILIZATION :-

- The immobilization refers to the tie-up of plant available phosphorus by soil minerals and microbes that use phosphorus for their own nutritional needs.
- Immobilization is the alternative to mineralization.
- Microbes may compete with plants for phosphorus, if the decomposing organic materials are high in carbon and low in nitrogen and phosphorus (i.e., wheat straw).
- Mineralization and immobilization occur simultaneously in soil. If the phosphorus content of the organic material is high enough to fulfill the requirements of the microbial population, then mineralization will be the dominant process.

- The phosphate salts are not soluble in water, they sink to the bottom and accumulate as sediment in deep ocean floor.
- Phosphorus incorporated in bones and teeth also remain outside the natural cycle for a long time as the bones and teeth are resistant to decay. Therefore, phosphorus cycle is an imperfect cycle.
- High concentration of phosphates in natural water causes 'eutrophication' and pollution.



Soil

Presented by
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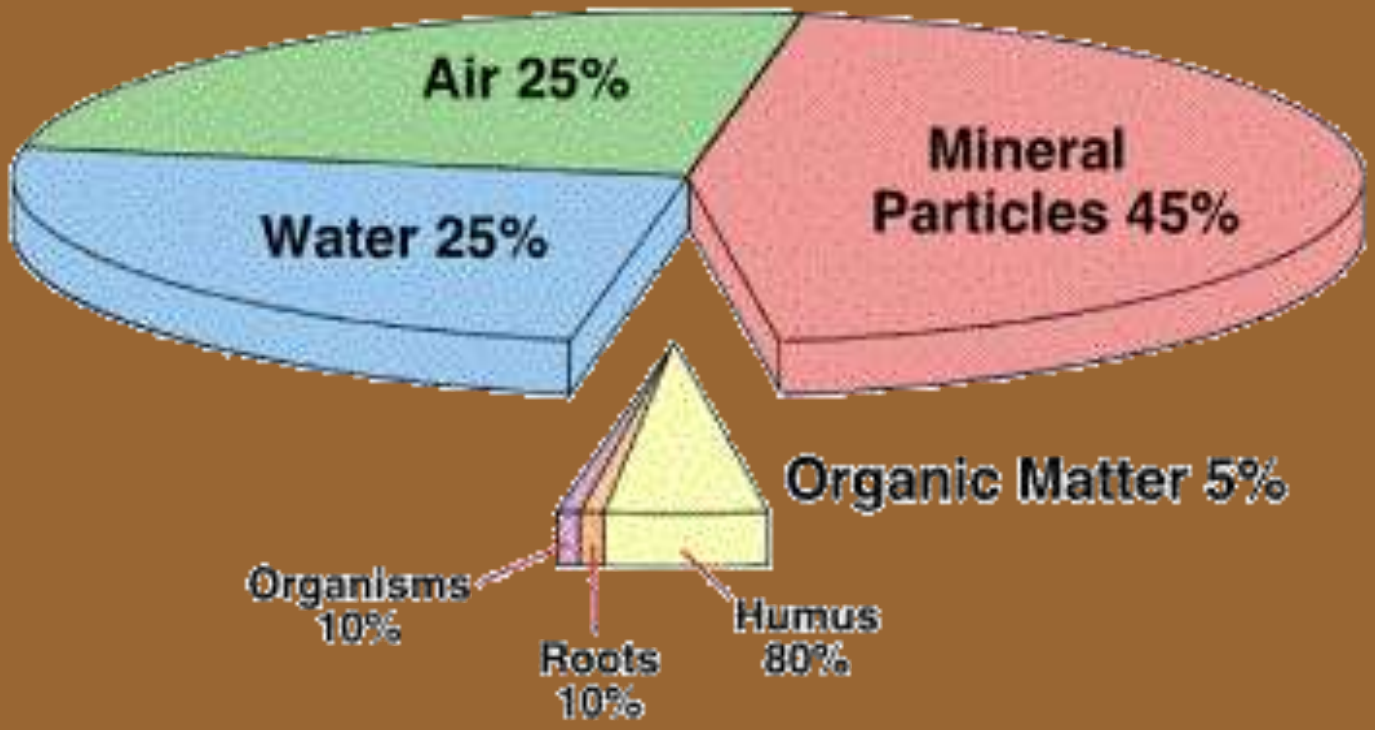
Soil

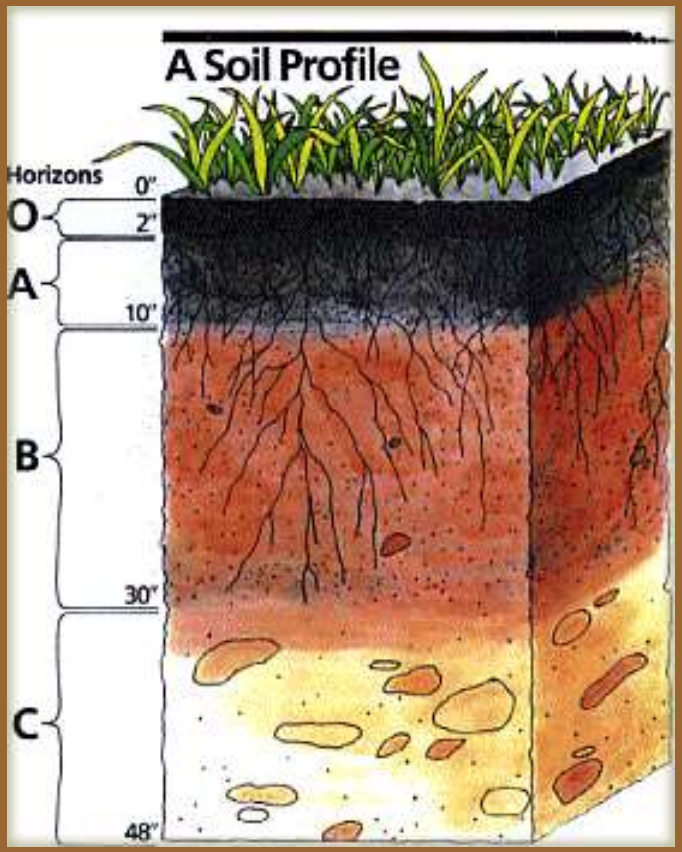
Loose top layer of earth's surface mad e up of rock,mineral particles,humus &divers group of organisms.

General composition of soil



Physical Properties

- Texture
- Structure
- Soil profile
- Soil Water
- Atmosphere
- Bulk Density
- Tilth
- Color

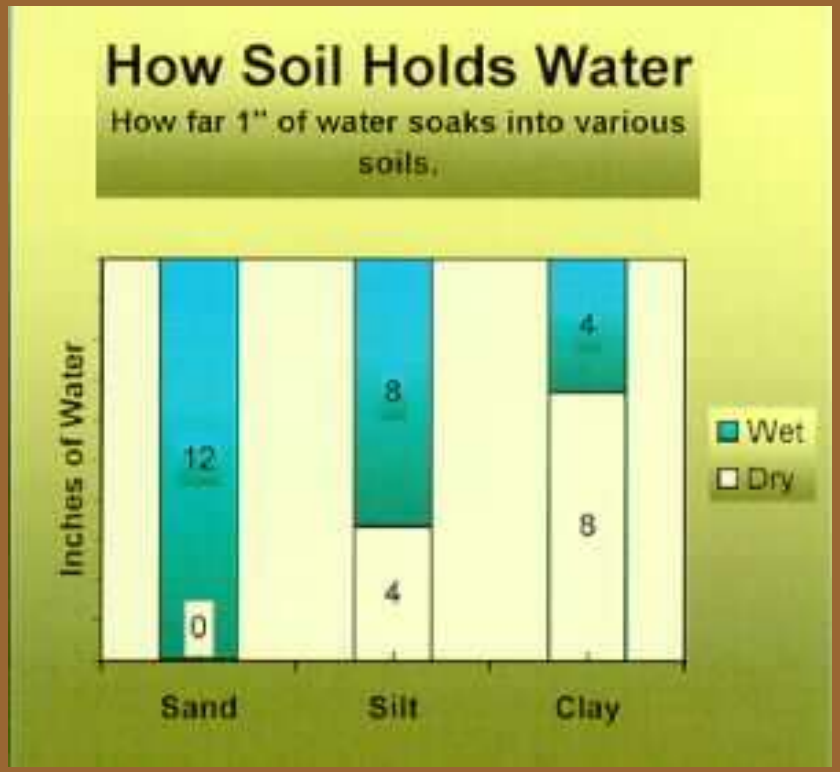


Texture

- Sand
  - Largest particle size: 0
    05 – 2.0 mm
- Silt
  - Middle particle size: 0.
    002 – 0.05mm
- Clay
  - Smallest particle size:
    < 0.002mμm

Texture

- Drives moisture holdin g capacity
  - Clay holds most water
  - Sand holds least water



http://www.dahlias.net/dahwebpg/Soil/Soil_07.htm

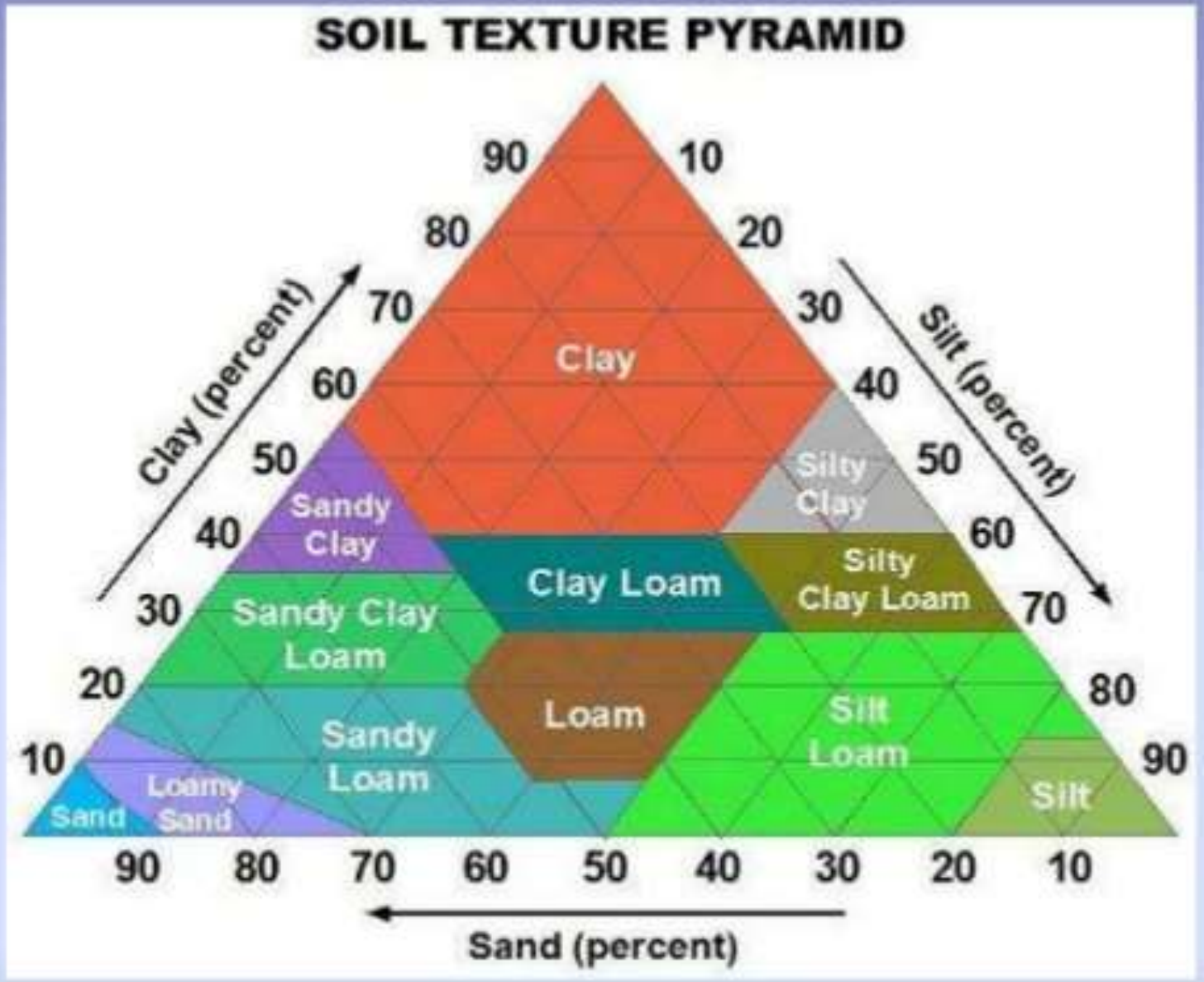
TEXTURAL GROUPS

- On the basis of proportion of different size particle ,soils are classified into different textural groups;

TEXTURAL GROUPS	RELATIVE PROPORTION OF DIFFERENT SIZED MINERAL PARTICLE
Sandy soil	85% sand + 15%clay or slit or both
Loamy sand	70% sand + 30% clay or slit or both
Loam soil	50% sand +50% clay or slit or both
slit	90% slit + 10% sand

TEXTURAL CLASSES OF SOILS

SERIAL NUMBER	SOIL CLASSES OR TEXTURAL NAMES	RANGE IN RELATIVE PERCENTAGE OF SOIL SEPARATES		
		SAND	SLIT	CLAY
1	Sandy soil	85-100	0-15	0-10
2	Loamy sand	70-90	0-30	0-15
3	Sandy loam	43-80	0-50	0-20
4	Loam	23-52	28-50	7-27
5	Slit loam	0-50	50-88	0-27
6	Slit	0-20	8-10	0-12
7	Sandy clay loam	45-80	0-28	20-35
8	Clay loam	20-45	15-53	27-40
9	Slity clay loam	0-20	40-73	27-40
10	Sandy clay	45-65	0-20	35-45
11	Slit clay	0-20	40-60	40-60
12	clay	0-45	0-40	40-100



SOIL TEXTURAL PYRAMID : describing the relative proportion of sand ,slit ,clay in various type of soils.

PROPERTIES

- Loamy soil are a balance between sand , slit and clay particles and considered as the most desirable soils for agricultural.
- Sandy soil have coarse texture .They hold water and mineral poorly . Water and air penetrate easily thats why ,they warm readily in spring and cool quickly in autumn.
- Clayey soil hold large volume of water and retain minerals . They are very slow to warm in spring and cool more slowly in autumn.
- Slity soil are intermediate in characteristics and properties between sandy and clayey soil.

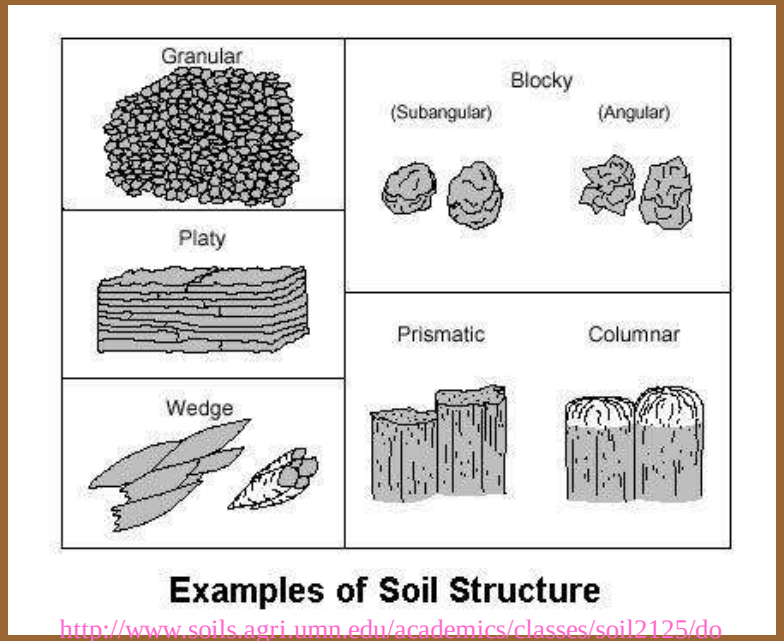
ROLE OF SOIL TEXTURE

- Soil texture is a qualitative classification tool used in both the field and laboratory to determine classes for agricultural soils based on their physical texture.
- It directly influences soil-water relationship ,aeration and root penetration through its relationship with interpartical pore space.

- Soil texture is of ecological interest ,for the dominant particle size present in any area have a effect on the flora and fauna of an area.
- It affects the nutritional status of soil
- The presence of fine textured soil in lower part of soil body may partially compensate for coarser soil in upper layers , through a mixture of fine coarse soil particles can combine many of the advantages provided by either type texture.

Structure

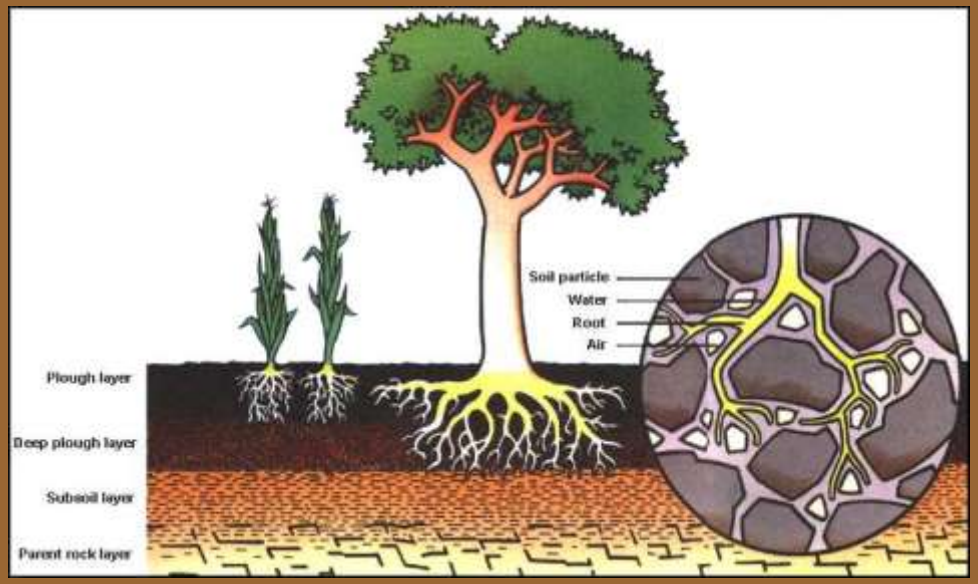
- How closely bound soil particles are
  - Aggregate
- Different shapes allow for different density of compaction
  - Ex. Platy structure packs more particles than blocky



http://www.soils.agri.umn.edu/academics/classes/soil2125/doc/s3chap1.htm

Structure

- Soil structure is important for root p enetration nutrient retention
- Influences erodibility



Soil Structure

Structure – arrangement of individual particles in relation to each other . Soil structure is the arrangement of particles into small groups, or ag gregates.

Aggregates may be bound together with other aggregates in larger mas ses called peds.

Peds come in different shapes that roughly resembel sphere, blocks, co lumns and plates.

If the individual particles are arranged in small aggregates with rounde d edges, we speak granular structure. This is very desirable for plant g rowth because it provides both large and small pores.

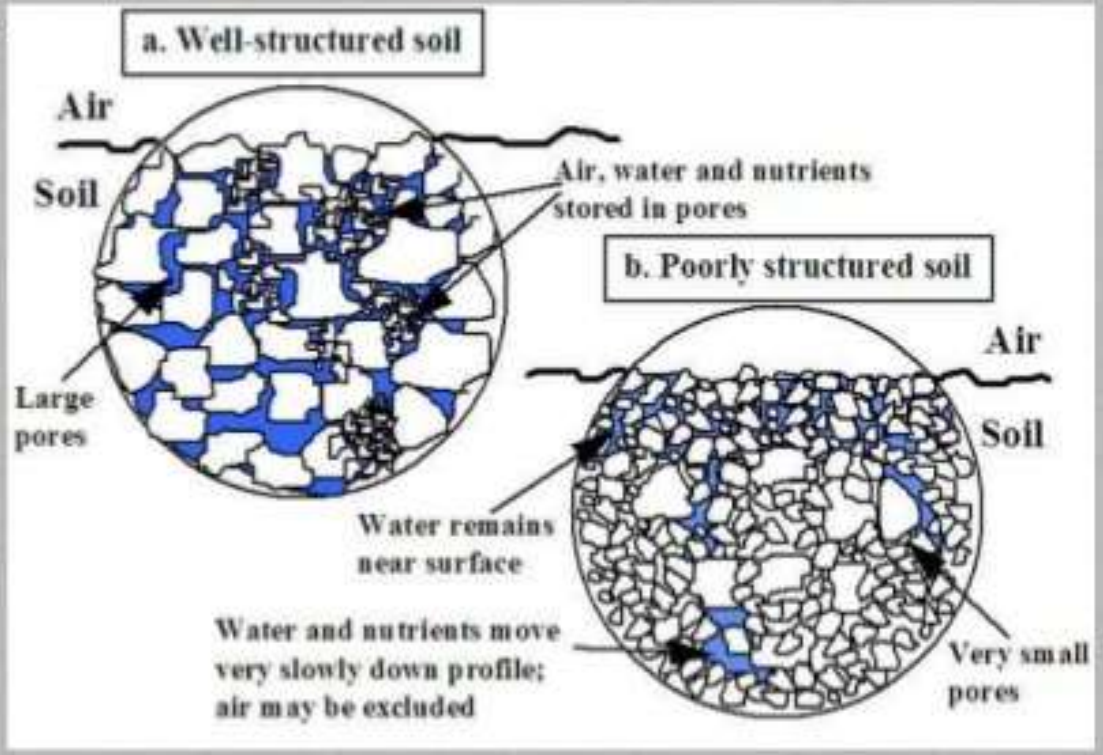
Some soils lack structure. Sandy soils the individual grains act indepe ndently of each other. No binding substances hold the particles togeth er, so the soil has no peds.

SOIL STRUCTURE

The arrangement of primary particles (sand, silt, clay) and their aggregates into a certain definite pattern is called soil structure.

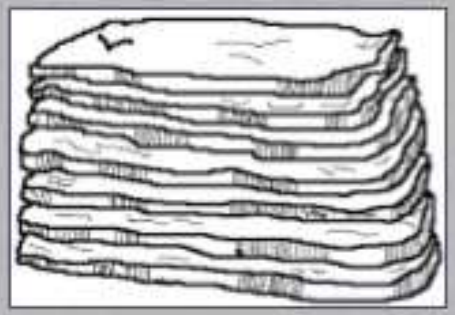
Influence of soil structure on soil physical properties:

- Aeration/ Porosity
- Temperature
- Density
- Consistency
- Colour
- Etc.

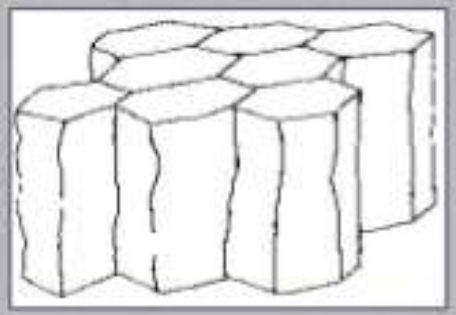


I. TYPES OF SOIL STRUCTURE

1. Platy: Peds are flattened one atop the other; 1–10 mm thick. Found in the A-horizon of forest soils and lake sedimentation.
2. Prismatic and Columnar: Prismlike peds are long in the vertical dimension; 10–100 mm wide. Prismatic peds have flat tops, columnar peds have rounded tops. Tend to form in the B-horizon in high sodium soil where clay has accumulated.



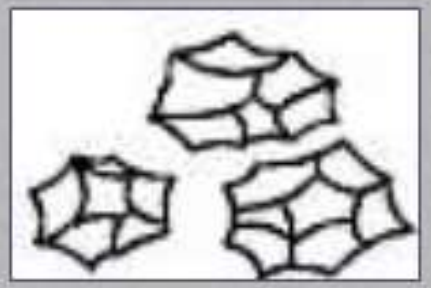
PLATY SOIL



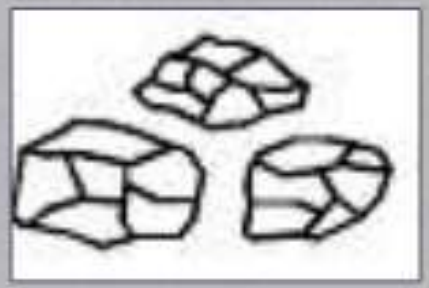
PRISMATIC SOIL

COLUMNAR SOIL

3. Angular and subangular: Blocky peds are imperfect cubes, 5–50 mm, angular have sharp edges, subangular have rounded edges. Tend to form in the B-horizon where clay has accumulated and indicate poor water penetration.
4. Granular and Crumb: Spheroid peds of polyhedrons, 1–10 mm, often found in the A-horizon in the presence of organic material. Crumb peds are more porous and are considered ideal.



ANGULAR BLOCKY SOIL



SUB ANGULAR SOIL

GRANULAR SOIL

Role of soil structure in relation to plant growth

- Soil structure influences the amount and nature of porosity.
- Structure controls the amount of water and air present in the soil.
- It affects tillage practices.
- Structure controls runoff and erosion.
- Platy structure normally hinders free drainage whereas sphere like structure (granular and crumby) helps in drainage.
- Crumby and granular structure provides optimum infiltration, water holding capacity, aeration and drainage.
- It also provides good habitat for microorganisms and supply of nutrients.

SOIL PROFILE

- Soil profile is defined as the vertical section of the soil, exposing various layers or horizons from the surface of the soil to the underlying bedrock. The master horizons are: O, A, B, C and R.

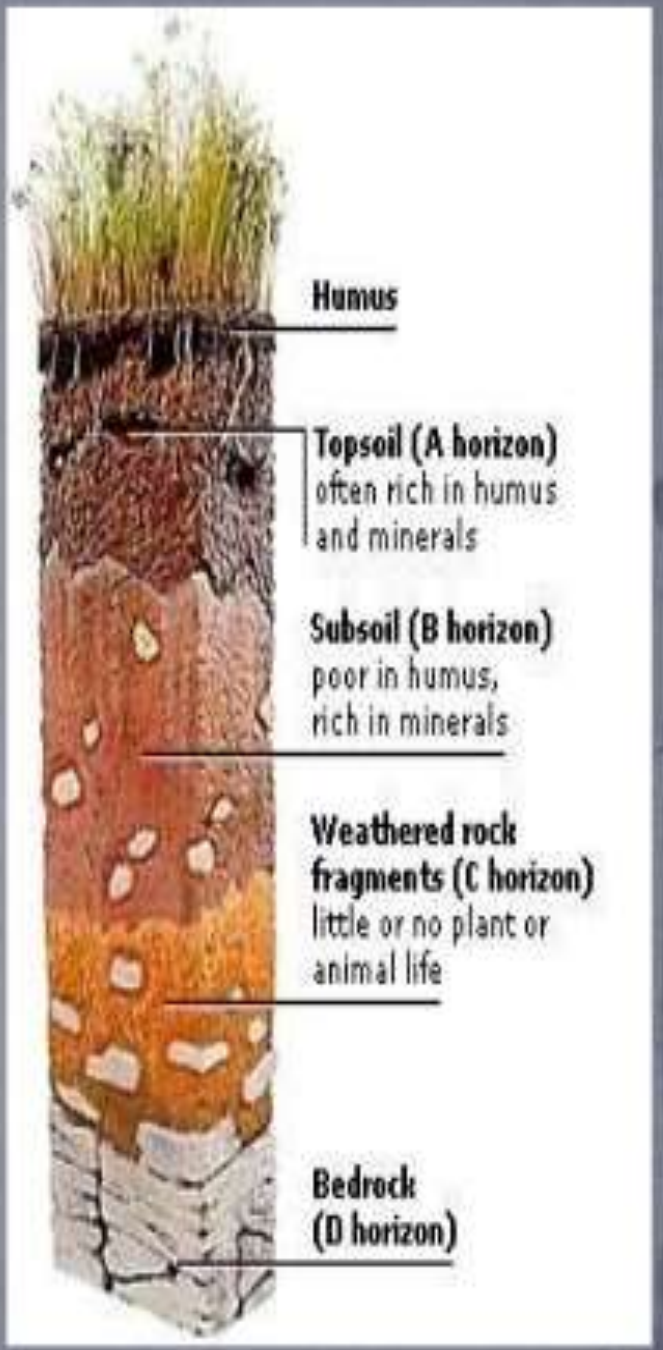
O horizon- uppermost layer and contains most of the organic matter like leaves, animal waste, crop waste, etc.

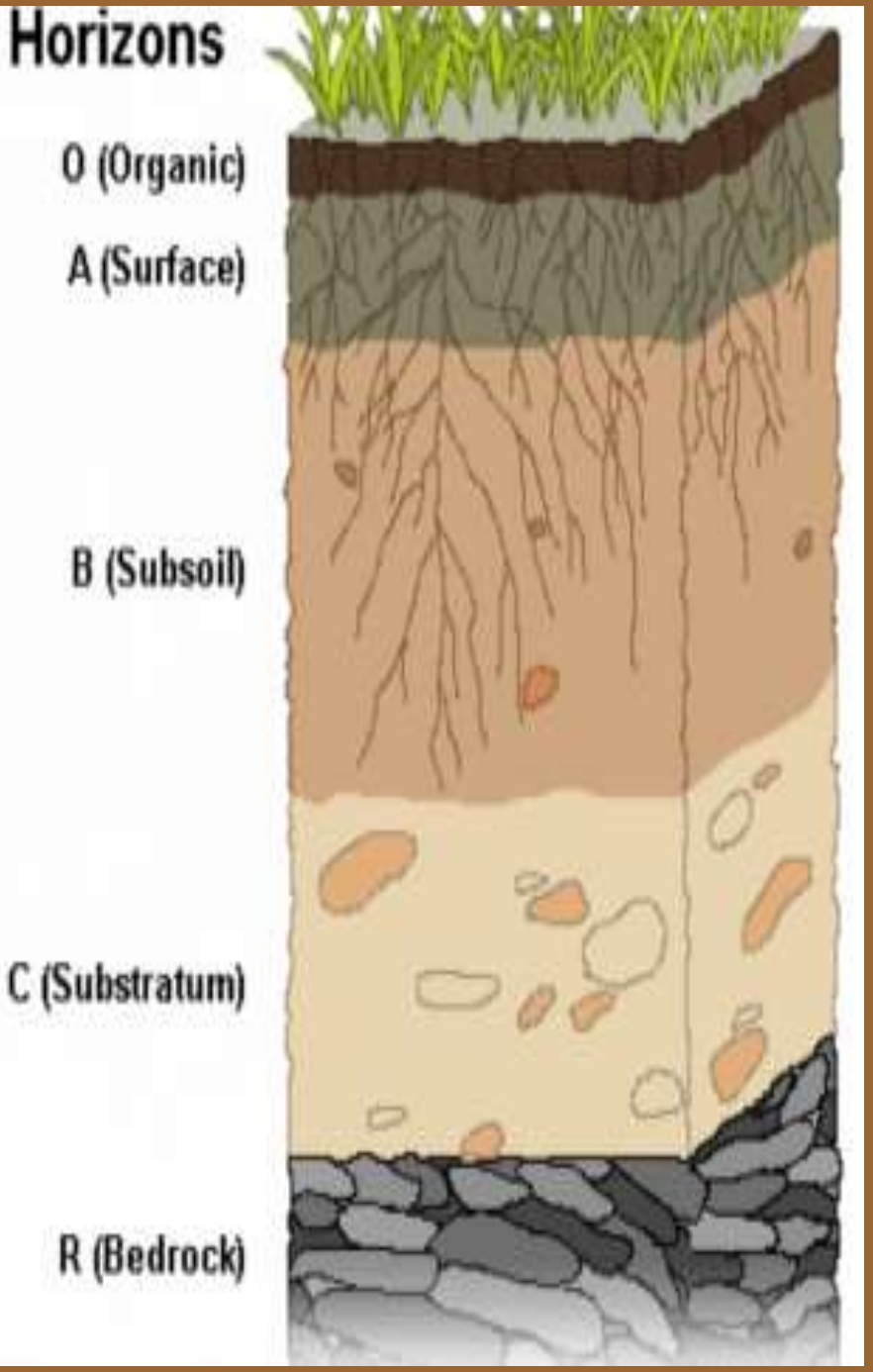
A horizon- topsoil layer, mixture of some humus and inorganic materials, leaching or eluviations zone.

B horizon- subsoil layer, composed of inorganic materials, illuviation or accumulation zone.

C horizon- parent material layer, the layer from which the soil develops, large pieces of rock that have not gone much weathering.

R horizon- Bedrock.




Horizons
O (Organic)
A (Surface)
B (Subsoil)
C (Substratum)
R (Bedrock)

Soil Water

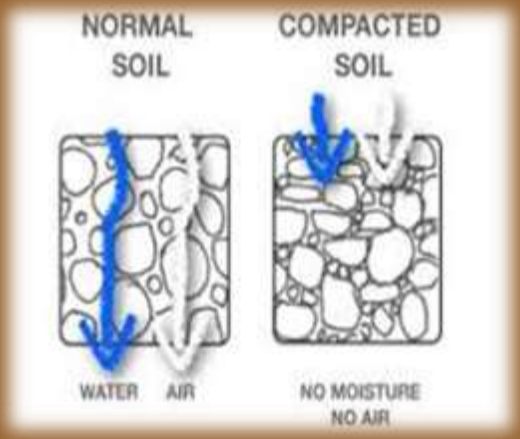
- Water and air together account for approximately 50 perce nt of the total volume of the soil.
- The spaces between mineral particles are called pore space s.
- The pores are filled with water and air.
- The amount of pore space is dependent on texture, structur e and organic matter content of soil.
- The amount of water in soil depends on the amount of prec ipitation and other climatic conditions, drainage, soil comp osition and the living population of the soil.
- Various organic and inorganic components of the soil are d issolved in soil water and thus are made available as nutrie nts for soil inhabitants.

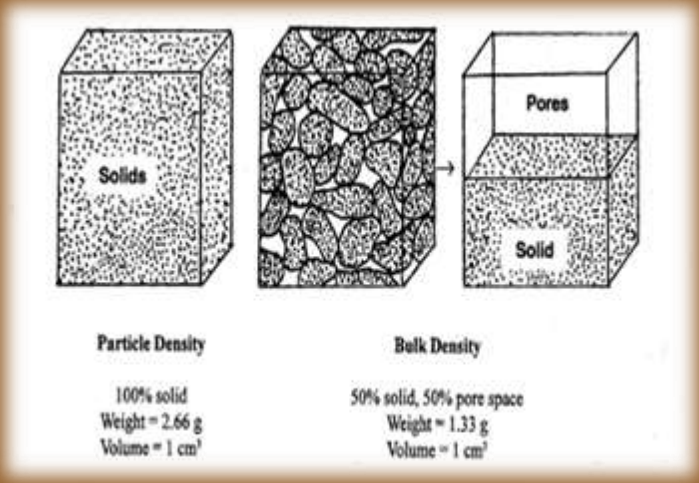
Soil Atmosphere

- The pore space not containing water is filled with air. The air moves in to the pores that are free of water.
- The soil atmosphere is derived from air atmosphere but differ s in composition from it because of biological processes occu rring in soil.
- Soil atmosphere mainly consists of CO2, O2 and N2. The CO , concentration of soil is 10 to 100 times more than air atmos phere but O, is less plentiful.
- Changes in soil atmosphere alter the size and functions of the micro flora as both CO, and O, are necessary for growth.

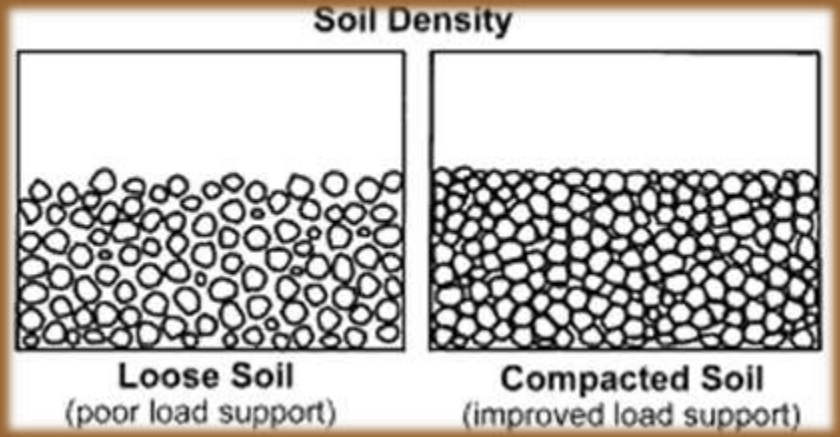
Bulk Density

- Bulk density is the dry weight of soil divided by its volume.
- It is an indicator of soil compaction. This volume includes the volume of soil particles and the volume of pores among soil particles. Bulk density typically expressed in g/cm^3.
- Bulk density is an indicator of the amount of pore space available with in individual soil horizons, as it is inversely proportional to pore space.
- Pore space=1- bulk density/particle density
- Bulk density is always less than the particle density.





- The individual mineral particles in soil have an average density of about 2.7 g/cm^3, and the organic matter has a much lower density in the range of 1.2 to 1.5 g/cm^3.
- The bulk density values for cultivated soils are in the range of 1.0 to 1.25 g/cm^3 .
- Root growth and penetration becomes difficult in a soil having bulk density values ab ove 1.4 g/cm^3. The higher the bulk density,the lower the total pore space filled with air and water.
- Porosity influences both gas diffusion and water movement in soil.
- There are two types of pores in soil: Macropores and micropores.
- Macropores are located between soil aggregates. More macropores in a soil means faster infiltration of water into the profile.
- Micropores are located within aggregates. Water does not move rapidly through these micropores.

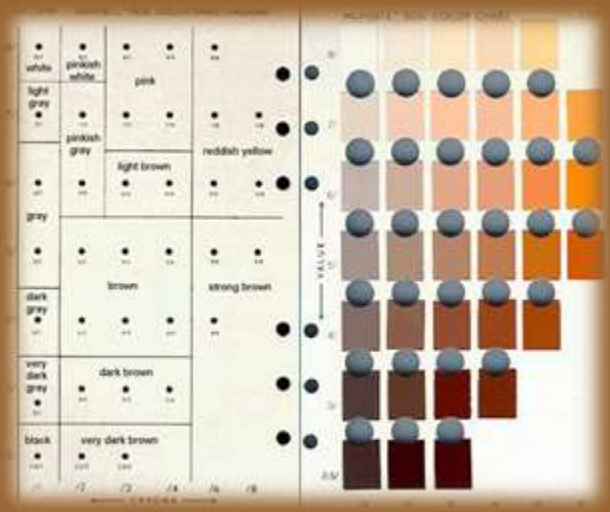


Soil Tilth

- Tilth is a general term for the physical condition of a soil. Soil tilth refers to the state of aggregation of a soil. It is significant in planting or growing a crop
- Factors that determine tilth include the formation and stability of aggregated soil particles, moisture content, degree of aeration, rate of water infiltration, and draina ge.
- A soil with good tilth offers little resistance to penetration by plant roots during the ir growth.
- It also provides ample oxygen and water for plants.
- The presence of both macropores and micropores is important for good tilth.
- Macropores permit infiltration and drainage of water; micropores store water for future plant needs.

Soil Color

- Soil color is one of the easiest physical soil properties to see.
- To measure soil color a standard reference chart such as Munsell color chart is used.
- Color can tell us much about the soil: the amount of organic matter present; the types of minerals and how weathered they are; the current moisture content; how long water is he ld in the soil (soil drainage class); and oxidation states of iron and manganese.
- Many components in the soil influence soil color, among them organic matter; minerals such as manganese oxides, iron oxides, and carbonates and moisture.
- Most common colors of soil are black, brown, red, gray, yellow and white.
- Colors of organic matter range from the brown un-degraded to the rich black of humus.
- Black colors are generally found in the surface layer of soil.



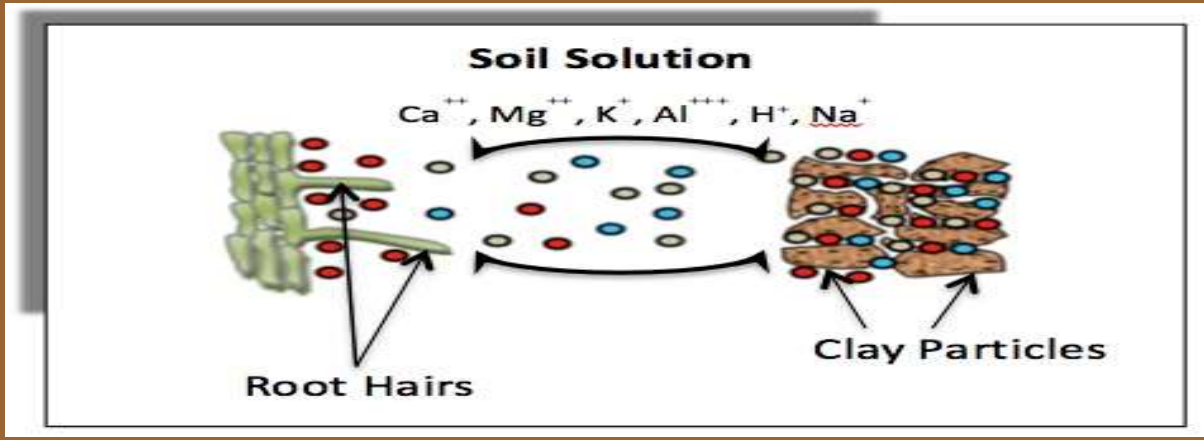
- Red color in soil is caused by the presence of un-hydrated iron oxide
- Yellow color in soil is caused by the presence of hydrated iron oxide.
- White color in soil is caused by the presence of carbonates.
- Black color in soil is caused by the presence of iron sulfides and manganese oxides.
- Grey color in soil is caused by the presence of quartz and reduced iron compounds.
- Light gray-white color is due to calcium or silica
- When the iron is removed, a gray color remains, or the reduced iron color persists in shades of green or blue.
- Gray soils often indicate poor drainage, while red soils can indicate very poor soils.
- Generally speaking, the darker a soil is the more nutrient rich it is. The darker color often in dicates an increase in decomposed organic matter known as humus.
- Decomposed organic matter known as humus.

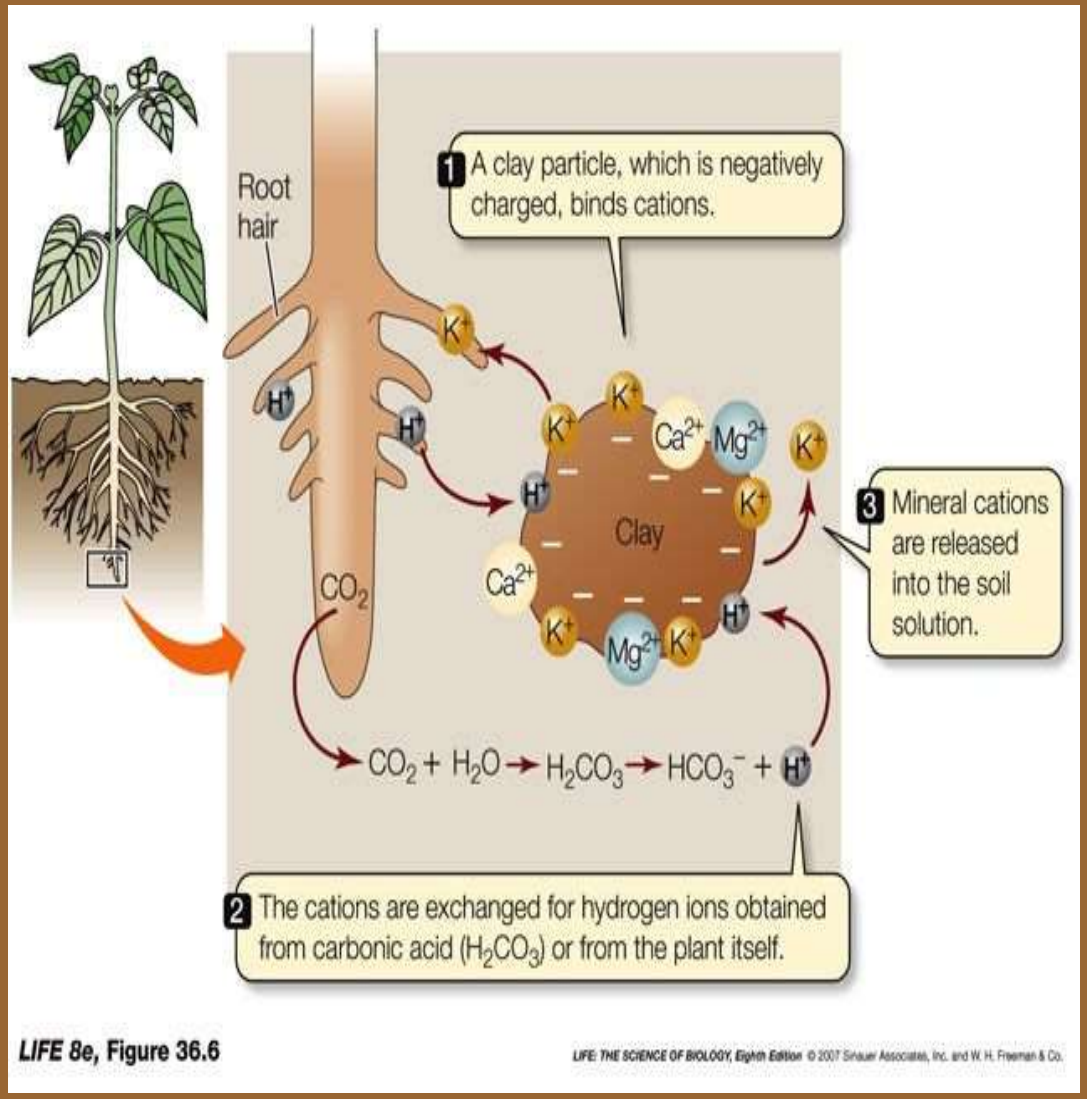
Chemical Properties of Soil

- Cation Exchange Capacity (CEC)
- pH
- Organic Matter

Cation Exchange Capacity

- A cation is a positively charged ion. Most nutrients are cations: Ca2+, Mg2+, K+, NH4, Zn2+, Cu2+ , Fe2+, Al+3 and Mn2+. Many of the heavy metals are also cations.
- Clay and organic matter particles are predominantly negatively charged (anions). Clay and organic m atter particles adsorb cations and prevent them from being "leached" or washed away. The adsorbed cations are subject to replacement by other cations in a rapid, reversible process called "cation exchan ge as;
- The released cations can be taken up by the plants, react with other soil constituents or be carried awa y with drainage water
- The "cation exchange capacity", or CEC, of a soil is a measure of capacity of the clay and organic mat ter particles to remove cations from soil solution, or an estimate of the soils ability to attract, retain, a nd exchange cations. It is reported in milliequivalents per 100 grams of soil(meq/100g).
- CEC is dependent upon the amount of organic matter and clay in soils and on the types of clay.
- In general, the higher organic matter and clay content, the higher the CEC.
- Larger CEC values indicate that a soil has a greater capacity to hold cations. A high CEC soil has a lar ge nutrient reserve: Cation exchange capacity is an important soil characteristic for retaining and supp lying plant nutrients, and for adsorbing contaminants.

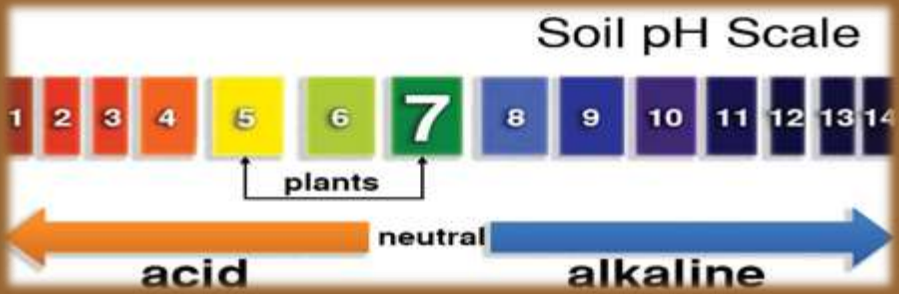




LIFE 8e, Figure 36.6

pH (SOIL REACTION)

- The soil pH is very important because it exerts strong influences on root development, activity of soil bacteria, fungi, symbiotic nitrogen fixation by legumes and the availability of nutrients.
- The pH is a measure of the active hydrogen ion (H) concentration It is an indication of the acidity or alkalinity of a soil, and also known as "soil reaction".
- The pH scale ranges from 0 to 14. Values below the mid-point (pH 7.0) are acidic and those above p H 7.0 are alkaline. A soil pH of 7.0 is considered to be neutral.
- At neutral pH the H' and OH are equal; their concentration is 10 moles/liter.
- A pH of 5.0 is ten times more acidic than a pH of 6.0.Most plants perform best in a soil that is slightl y acid to neutral (pH 5.5 to 7.0).
- Some plants like blueberries require the soil to be more acid (pH 4.5 to 5.5), and others, like alfalfa w ill tolerate a slightly alkaline soil (pH 7.0-7.5).
- The major effect of pH in the soil is on ion solubility, which in turn affects microbial & plant growth
- A pH range of 6.0 to 7 is ideal for most crops.
- Most of the important plant nutrients are soluble in this pH range.Some minor elements (e.g., iron) a nd most heavy metals are more soluble at lower pH.
- In acid soils, hydrogen and aluminum are the dominant exchangeable cations. Aluminum, iron, zinc, copper, manganese and boron become more soluble under acidic conditions and can reach toxic level s. Under acid conditions, aluminum reacts with water and produces hydrogen ions. Hydrogen ions in the soil solution are increased when the salts increase.



- Factors that affect soil pH include parent material, vegetation and climate.
- Some rocks and sediments produce soils that are more acidic than others: quartz-rich sandstone is acidic; limestone is alkaline.
- Some types of vegetation, particularly conifers and microorganisms lower pH by producing organic acids.
- Addition of certain nitrogen fertilizers to soil can also produce hydrogen ions. Sulfur is generally used to lower the pH, whereas, lime is used to raise the pH.

Organic Matter

- The organic matter usually contributes 3 to 6% of the total volume of the soil.
- The plant and animal remains deposited on or in the soil contributes organic matter. The microbial po pulation both live and dead also contributes to organic matter.
- There are 3 main components of organic matter in soils:

1. Dead forms of organic material - mostly dead plant parts
2. Living parts of plants - mostly roots
3. Living microbes and soil animals

- <u>Beneficial impacts of soil organic matter on soil properties:</u>

1. Physical properties: It stabilizes soil structure, improves water holding characteristics, and lowers b ulk density.
2. Chemical properties: It increases CEC, acts as a pH buffer and ties up metals.
3. Biological properties: It provides food for soil microorganisms, increases microbial populations and their activities, it helps store soil moisture, it returns plant nutrients to the soil and it makes soil more tillable.

HUMUS

- When plant and animal remains fall on or are incorporated into the soil, they are subjected to microbial decomposition. From the original material a variety of pro ducts are formed. The original material and the initial products undergo further decomposition by microorganisms and are converted to brown or black material. Such material is referred to as humus. Humus is a product of the synthetic and de composing activities of the microflora.
- Soil humus is a mixture of dark, colloidal organic compounds relatively resistant to decomposition. Humus is formed during the decomposition of organic matter in soils. This decay is mediated by microbes and the enzymes they excrete. Thes e compounds result from the decay of organic litter, and accumulate in the 'O' an d 'A' horizons of the soils.
- The dark organic material in soils, produced by the decomposition of vegetable o r animal matter and essential to the fertility of the soil is called humas. Humus co ntains humic substances. Humic substances are degraded bio-molecules and con sist of heterogeneous mixtures of substances. The humic substances have been d esignated as humic acid. fulvic acid or humin.
- Composition of humus is not definite. It is composed of heterogeneous group of substances.

- In terms of specific elements the humus contains compounds of carbon, hydrogen, oxygen, ni trogen. phosphorus, sulfur and small amounts of other elements.
- In terms of types of compounds, humus contains a number of polymerized substances; alipha tic acids, aromatic molecules, polysaccharides of several kinds, bound amino acids, polymers of uronic acids, phosphorus containing compounds, phospholipids & vitamins.
- The organic constituents of the humus includes amino acids, amino sugars (N-acetylglucosa mine), methyl sugars (rhamnose, fucose), pentose sugars, hexose sugars, sugar alcohols (inos itol, mannitol). aliphatic acids (acetic, formic, lactic, succinic acids), aromatic molecules, uro nic acids (glucoronic and galactoronic acid), purines and pyrimidines.
- The COOH and phenolic OH groups are weakly acidic, which give humus its pH buffering a bility, pH dependent charge and cation chelating ability. In addition, the toxicity of the pheno lic subgroups which make up humus contributes to its resistance to microbial decomposition.
- <u>The humus composition depends upon</u>:

a. The material from which it is made constituents of dead and living plant and animal cells.

b. The types of microorganisms involved and

c. The environmental conditions under which the organisms are working - temperature, moisture, aeration, pH, etc.

- Numerous factors influence the decomposition of organic matter. Among these are the prope rties, amount and stage of decay of the organic matter, and the availability of oxygen, temper ature, soil moisture, nutrients and soil texture.

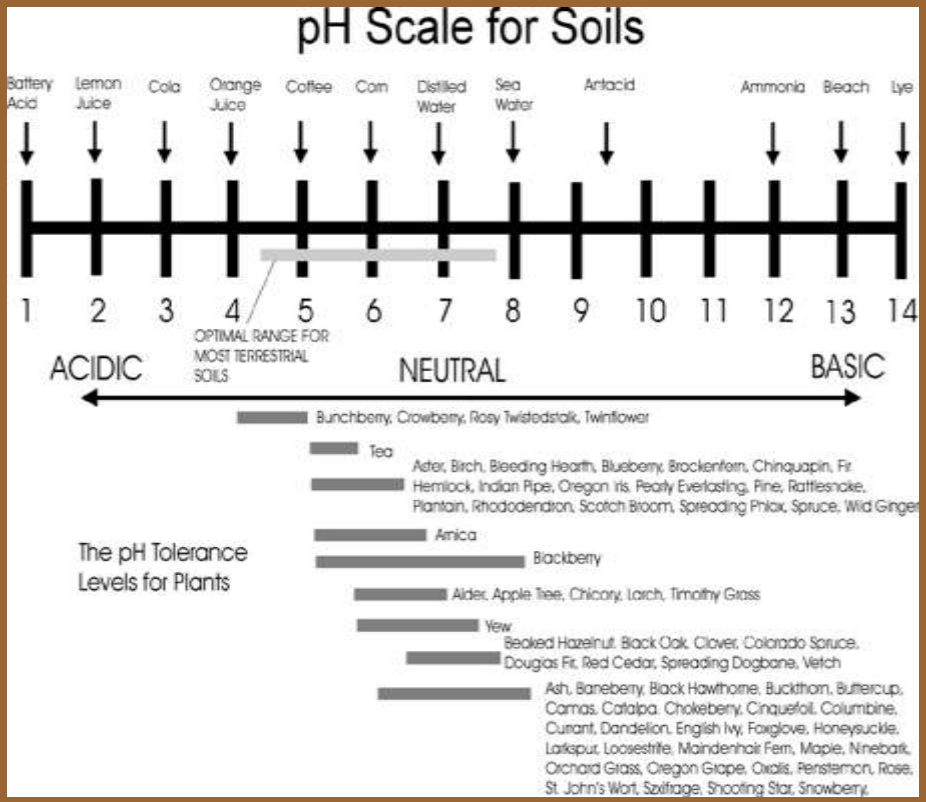
- FUNCTIONS OF HUMUS :-
- 1. Humus improves the physical condition of the soil, making it soft. It increas es the water holding capacity of soil and aerates the soil. Due to this soil beco mes sticky and resist erosion.
- 2. It contributes to buffering capacity of soil.
- 3. It is a great storehouse of food materials for higher plants. It holds and slowl y releases nitrogen, phosphorus, minerals and CO, under the action of microor ganisms.
- 4. Feeds and stimulates beneficial soil microbial populations.

Chemical Properties

- pH
- Fertility
- Soil Water
- Leaching/Weathering
- Soil Organic Matter

Soil pH

- Plants grow best at 6.5 -7.5
- To decrease pH:
  - Lime
  - Fish meal
  - Wood ash



Soil Water

- Water retained within soil pores
- Depends on soil:
  - Texture
  - Porosity
  - Bulk density
- Contains nutrients for plant intake