UNIT-1

ES605C

6/9/22

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Environmental Microbiology

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It is the study of orgn. that are too small to be seen with naked eyes for eg: bacterias, virus, protozoas etc.

Env. microbio. is defined as the study of microbial interaction with each other (biotic & abiotic) and the associated implications for human health & environment.

Biotic ⇌ Abiotic.

Imp. of Env. microbiology:

1. It Enable us to understand the benefit of microorganism to the society.
2. To understand fate of microorganism in the env. ecosystem.
3. To study the interaction of microorg. with the env.
4. To study the possible effects (beneficial or detrimental) on human activities.

Types of microorganism:

⇒ On the basis of origin:

1. Autochthonous
2. Allochthonous

1. Autochthonous: They r the tiny org. native to the environment - they inhabit. They live in and around existing life forms. For eg: human skin, digestive tract etc.

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② Allocthonous : They are the foreign micro-organisms normally found in particular matrix (food, water). They are foreigener microorganisms. for eg. Lactobacillus bacteria is always found in dairy products.

Microbiology of extreme environment :

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① It is a habitat characterised by harsh condition or unfavourable condⁿ including higher or lower temperature, high/low pressure, acidic/basic environment.

② Examples of Ext. Env. include polar regions, deserts, volcanic region, deep sea event, outer space region etc.

⊙ Extremophiles : They are living organism with the ability to survive in extreme environmental conditions as a result of variant temperature.

They can be categorized into two type :

① Extremophilic
They require one or more extreme condⁿ to survive.

② Extremotolerant
They can tolerate extreme condⁿ but can grow at optimum conditions also.

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Classification of Extremophiles:

① Thermophiles: They are the microorganisms that grow at temp. around 45-50°C. They are generally found in areas like thermal vent, hot springs, boiling springs etc.

They are of two types:

Facultative Thermophiles	Obligate Thermophiles
↓	↓
They can survive at both high as well as moderate temperature.	They require higher temp. for their survival.

Example: Methanosarcina sp.
Methanobacterium sp.

Mode of Adaptation of thermophile:

① Their enzymes and proteins are much more stable to heat.
② Their cell membrane have lipid rich in saturated fatty acid.
③ They contain special DNA binding proteins that arrange the DNA into globular form which are resistant to heat.

② Psychrophile: They are microorg. survive or function at cold temperature. They are found in deep sea, polar region and mountain region. For eg:
Pseudomonas sp.
Sphingomonas sp.

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Mode of Adaptation of Psychophile :

① They produce proteins and enzymes that are stable at low temperature.

② Their membrane contain higher amount of unsaturated fatty acids which help to maintain a semi-fluid state of membrane.

③ In their structure they have high G+C regions (Guanine , Cytosine) which mainly codes for tRNA (transport RNA) which helps in the biosynthesis of all amino acids.

④ Hyperthermophiles : They are the microorganisms which are can survive and grow at extremely high temperature i.e above 80°C They are found in areas like volcanic site, Hydrothermal vents , Hot springs etc.
Eg : Pyrococcus furiousus , Hypothermus sp.

Mode of Adaptation :

1) Their protein molecules exhibit hyperthermo stability i.e they can maintain structural stability at higher temperature (such proteins are homologous) to their functional analogus in organism which grows at lower temp. but evolved to exhibit their function at higher temperature).

2) They don't have any fatty acid membrane instead they have lipids branched with hydro-carbon.

3) Horizontal DNA transfer (a particular type of gene) takes place b/w species in the presence of high heat and UV radiation.

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Microorganism in extreme pressure :

Barophiles : They grows at pressure greater than atmosphere pressure. They are found in deep sea vent.

Eg. Sporosarcina sp.
Photobacterium

Mode of Adaptation :

1) They have gel like membrane which use the nutrient uptake at higher pressure.
2) They have lower concentration of prolin protein and higher concentration of glycin protein which helps in structural stability of cell membrane.

Microorganism in extreme pH :

a) Acidophiles : Organisms are survived at higher acidic conditions. They are found in acidic lake, acid-sulphate soil, metal and coal mines.

For eg : Lacto-bacillus and Thiobacillus.

Mode of Adaptation :

1) Their cell membrane are impermeable which restrict the entry of protons.
2) Their membrane pore size are two small which again prevent the entry of protons in to the cytoplasm.
3) They have mechanism to remove excess protons from the cytoplasm like in bacillus bacteria living in acidic envir., the protons pumped out of cytoplasm to maintain pH homeostasis during respiration proc

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4) Their cell have buffer molecule that have basic amino acid which help in proton removal.

b) Alkaliphiles : They are the organisms that can live and grow in environment in extremely high pH around 9-13, It is of two types :

- ⊙ Facultative : They can grow both in neutral as well as alkaline condition.
- ⊙ Obligate : They require high pH for their functioning.

eg. alkaliphile Clostridium sp. and Halobacterium.

Mode of Adaptation :

1) They adapt mechanism like acquiring proton (H^+) from outer environment, reducing leakage of protons, production of organic acid and prevent diffusion of $OH^\ominus$ ion from the environment.
2) They also perform fermentation process which produce acid inside their body.
3) Their cell membrane consist of 90% branched fatty acids which helps in maintaining the pH stability by reduction of proton leakage.

Microorganism in extreme sugar concentration :

Osmophiles : They are group of micro-organism survive at higher Osmotic pressure areas like soil or water rich in sugar.

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They are found in areas having higher sugar conc. like food industries.
eg. Torula sp. , Zygosaccharomyces sp.

Mode of Adaptation :
1) They provide different osmoprotectants like alcohols that prevent change in osmotic pressure.
2) Their protein and enzymes have more charges and hydrophobicity that protect them against the change in solute composition

Microorganism in extreme salinity :
These organism require higher salt conc. for their survival. They are found in saline lakes, saline pond, sea water etc eg. Holomonas sp., Erwinia sp..
Mode of Adaptation :
1) They accumulate larger salt conc. in their cytoplasm to reach equilibrium state.
2) They accumulate inorganic ions inside their body to maintain salt concentration.
3) Their proteins and enzymes have large proportions of glutamates and aspertate which Ise the hydrophobacity.

Association of two or more different species.

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↑ Microbial Interaction

Symbiosis : It can be positive and negative.

Ectosymbiosis : One organism can be located on the surface of another.
The Ectosymbiont (smaller animals) is always located on the larger organism.

Endosymbiosis : One organism can be located within another organism.

Ecto/Endosymbiosis :
Microorganism lives on both the inside and outside of another organism.
Eg : of Endosymbiosis :
Thiothrix sp. - sulphur using bacteria which lived on the surface of may-fly larva. and which itself contain a parasitic bacteria.

Mycorrhizal fungi : associated with plant roots contain endosymbiotic bacteria as well as bacterias living on their surfaces.

- ⊙ Symbiotic Relationship can be intermitent and cyclic or permanent.
- ⊙ Symbiotic interaction don't occur independently. Each time a microorganism interact with other org. and their environment, a series of feedback responses occurs in the larger biotic community that will impact other part of the ecosystem.

Types of Microbial Interaction :

Positive Interactions :

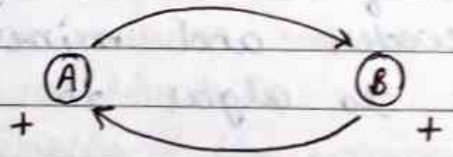
1) Mutualism
2) Protocooperation
3) Commensalism

Negative Interactions :

1) Predation
2) Parasitism
3) Ammensalism
4) Competition

1) Mutualism : Mutuus (reciprocal interaction) define as the relationship in which some reciprocal benefits occurs to both the partners.

- This relationship have some degree of obligation.
- The partners can't live seperately.
- Mutualist and host are dependent on each other.



Eg : ① The Protozoan termite relationship

→ The flagellated protozoa live in the gut of termite and wood roaches.

→ These flagellates exist on a diet of carbohyd acquired as cellulose ingested by their host.

→ The flagellates engulf wood particles, digest the cellulose and metabolise it to acetate and other product.

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→ Termites oxidise the acetates released by their flagellates.

→ Because the termite (host) is almost always incapable of synthesizing cellulases enzyme (it catalyse the hydrolysis of cellulose), dependent on the mutualistic protozoa for its existence.

② Lichens: It is the association b/w fungus & green algae. Fungus is called mycobiont and algae is the phycobiont.

→ The characteristic morphology of Lichen is a property of the mutualistic association and is not exhibited by either symbiont individual.

→ The phycobiont is a photoautotroph dependent on light, CO_2 and minerals, the fungus gets it organic carbon directly from the algae and in return the fungus protect the algae from excess light & it also provide water and minerals and firm substrate to algae to grow independently.

⊙ Syntrophism: It is an association in which the growth of one organism either depends on or is improved by growth factors, nutrients or substrates provided by another organisms growing near by.

In this sometimes both organisms benefitted.

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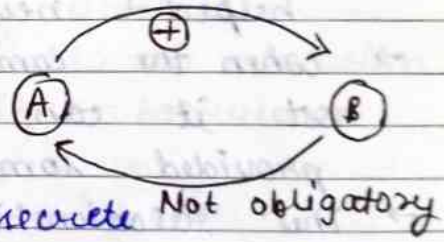
It is also called cross feeding or satellite phenomenon
Eg: Association b/w hydrogen consuming microbes and fermenting bacteria in ruminant.
In this the degradation of organic compounds done by fermenting bacterias which release fatty acids and hydrogen. The production of Hydrogen inhibit the ability of fermenting bacterias to further degrade the organic compounds.
The Hydrogen consuming microbes like methanogens consume Hydrogen thus enable fermenting bacteria to continue degradation process.

2) Photocooperation: It is a +ve symbiosis which involve syntrophic relationship in which one organism lives on the by-product of other.
In this both the organisms are benefitted but it is not obligatory.



Example: a marine worm-bacterial relationship
: Alvinella - the worm secrete mucus from the tiny glands on their back which feed bacteria & in return the bacteria provide insulation to the worm.

Quorum sensing (Auto-induction): A phenomenon where the accumulation of signalling molecules enables a single cells to sense the no. of bacterias (cell-density)

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- The microorganism produces specific auto-inducer compounds and as the population ↑se the conc. of these compounds reaches critical level by which specific genes are expressed. (sporulation, anti-biotic production, competition etc).

- Many bacteria rely on QS to control their gene expression responsible for disease.
- For eg: the infection by pathogenic bacterias by in human body.

- The gram -ve bacteria produces acyl homoserine lactones.

3) Commensalism: It is a relationship in which one organism is benefitted (commensal) and other org^n is neither harmed nor helped (neutral).

→ When the commensal is separated from the host it can survive without being provided some factor or host origin.

→ This relationship btw microorganism includes situation in which the waste product of one microorganism is the substrate for another species (eg. cow dung).

Eg: In the intestine of human (colon) the anaerobic e-coli bacteria shows commensalism as it depends on human waste for their food requirement.

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→ The microorganism is found on the skin and on the surfaces of plants and animals shows commensalism by feeding on their waste produce by their surface.

Negative Interactions :

Predation :

- When one organism called the predator engulf and digest another organism called the prey.
- The prey can be larger or smaller than the predator and this normally results in the death of the prey.
- For eg : Bdellovivrio sp., a peri-plasmic predator that penetrates the cell wall and grows outside the plasma membrane of the prey.
  Vampiro coccus, with its unique epi-biotic mode of attacking a prey bacterium.
  Daptovacter showing its cytoplasmic location as it attacks a susceptible bacterium.

- Beneficial effect of predation :
  1. It helps in maintaining the microbial loop by digestion process — phytoplanktons ← Protozoans ↓ Top consumers (carnivore) ← Zooplanktons ← Bacteria
  2. It helps in protection and Tred fitness of the organism.

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③ Helps in survival and ↑ed pathogenicity. The intracellular survival of legionella ingested by ciliates.

Parasitism: In this negative interaction, the parasites lives or grow on or inside the body of host organisms for their nutritional requirements. In this relationship the host is always harmed.

⊙ It can involve physical maintenance in or on the host body.
⊙ It is characterised by relatively long period of contact.

Diff. b/w Parasitism and predation:
Depending upon the eqm b/w the two organisms this may shift and a parasitic relationship become a pathogenic one. which can be defined as predation.

★ Ammensalism: It is the relationship in which the product of one organism have -ve effect on other organism.
For eg: the production of anti-biotics that kill or inhibit the pathogen micro-organism.
⊙ Bacteriocines produced a proteinaceous toxin which target the other strain of the same species.

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Competition: It arises when different microorg. within a population or community try to acquire the same resource or a particular nutrient.
This principle of competition was studied by E.F Gauss in 1934 and named this principle as competitive exclusion principle. (when competition b/w species results in the elimination of one species from a given habitat).

Importance of microbial Interaction:
- Analysing the impact of human host microbiota composition and activity.
- Understanding the underline governing principle that shape a microbial community which is key for microbial ecology, enginering for various bio-technological applications.

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Major groups of microorganisms

Classification

- Artificial
- Natural — Natural characters
- Phylogenic — evolutionary histories

Five Kingdom Classification: R.H Whitaker - 1969

It is based on

1. Cell type (Prokaryotes and Eukaryotes)
2. Presence of cell wall
3. Body organisation (unicellular / multicellular)
4. Mode of Nutrition
5. Mode of Reproduction
6. Phylogenetic relationship

Based on these characteristics living organisms are divided into 5 Kingdoms:

1. Monera → Prokaryotes
2. Protista → Eukaryotes
3. Fungi → Eukaryotes
4. Plantae → Eukaryotes
5. Animalia → Eukaryotes

Classification of micro-organisms:

- Bacteria → Prokaryotes, Unicellular
- Fungi → Eukaryotes, multicellular
- Algae → Eukaryotes, multicellular
- Rotifers / worms → Eukaryotes, multicellular
- Virus / Viroids → Eukaryotes, multicellular

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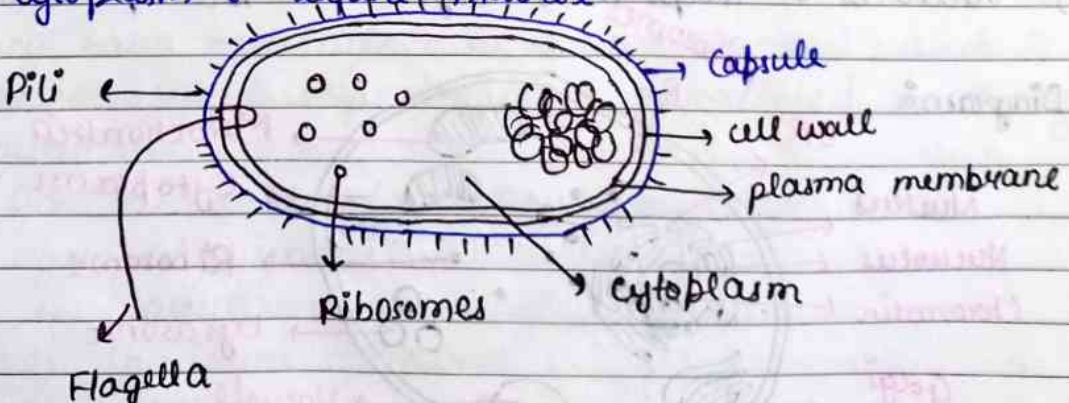
Prokaryotes : Organisms do not contain nuclei or membrane bound organelles. All Prokaryotes are unicellular organisms.

Prokaryotic cell features :

1) Nucleoid : Central region of the cell that contain DNA.
2) Ribosomes : Responsible for protein synthesis
3) Cell wall : Provides structural stability and protection from the outside environment.
4) Plasma membrane / cell memb. : protects cell from outside environment.
5) Capsules : some bacteria have layers of carbs. that surround the cell wall & help the organisms to attach to the outer surface.
6) Flagella : A thin tale like structure which helps in movement.
7) Pilli Pili : multiple rod-like structure involved in different functions like dNA transfer and attachment.
8) Cytoplasm : liquid-matrix



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Eukaryotes : Organisms whose cells have true nucleus and other cell organelles enclosed in a plasma membrane.

Cell features :

1) Nucleus : Consist of chromatin fibre, nucleolus and a liquid matrix called Nucleoplasm surrounded by the nuclear membrane.

2) Plasma membrane : It is the phospholipid-bilayer i.e present on each organelle and on the entire cell.

3) Cell wall : Some of groups have cell wall (outer protective covering) which helps in cell movement as well as cell division.

4) Ribosomes : protein - synthesis

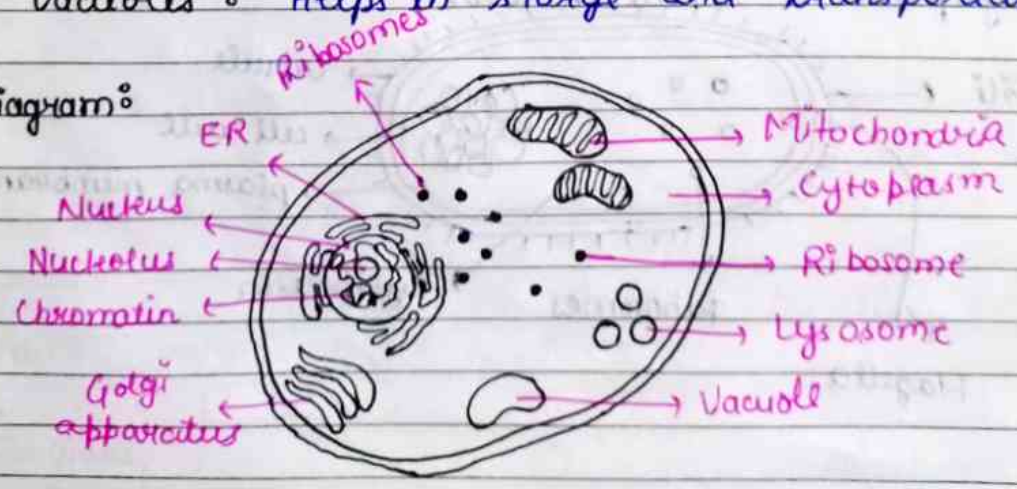
5) Mitochondria : Power-House of the cell - energy production as ATP.

6) Cytoplasm : Liquid matrix surrounding all organelles.

7) Endoplasmic Reticulum : Helps in transfer of proteins

8) Vacuoles : Helps in storge and transportation.

Diagram :



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Kingdom Monera : In 1990, Carl Woose changed Five Kingdom classification into six Kingdom. He divided Kingdom Monera into two domains - Archaebacteria and Eubacteria

Features :

1) It includes all the prokaryotes mainly bacteria, cynobacteria (-blue-green algae) and mycoplasma.
2) They are the most primitive life form of the earth.
3) Their cell wall is made up of peptido glycan and murein.
4) They have no cell bound organelles.
5) They have naked DNA called nucleoid.
6) Flagella may or may not present.
7) They have asexual mode of nutrition reproductio

Bacteria

1) Most abundant microorganism, the sole member of Kingdom Monera.
2) They live in extreme habitat like hotsprings, deserts, snow etc.
3) They have autotrophic (photo + chemo) and Heterotrophic (saprotrophic + parasitic) mode of nutrition.
4) On the basis of shapes bacterias were groped under 4 categories :
   1) Cocus - spherical shape
   2) Spirillum - spiral
   3) Bacillus - rod
   4) Vibria - Comma

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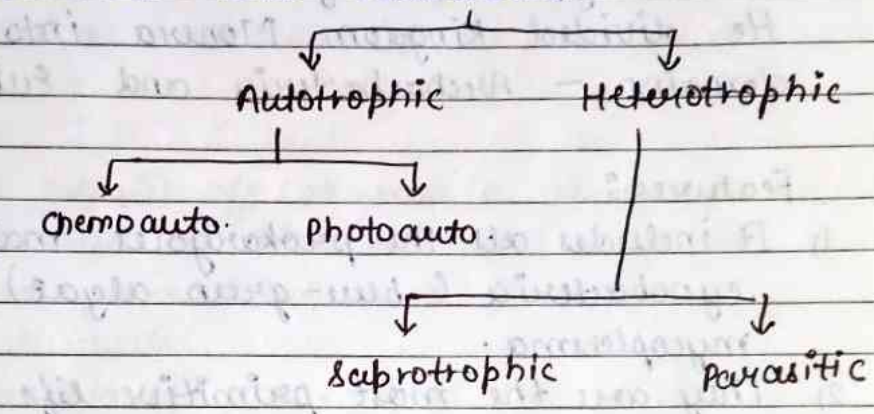
5) Bacterias were classified under two categories — Archaebacteria and Eubacteria.



Archaebacteria :

1) They are the most primitive prokaryotes lives under extreme conditions and in marshy areas.
2) They differ from other bacteria in having cell wall without peptidoglycan.
3) Methanogens are eg. of archaebacteria which are found in the gut of ruminants and help in the production of biogas.

Eubacteria :

1) They are the true bacteria.
2) They have rigid cell wall as well as motile flagellum.
a) Autotrophic eubacteria :
   1) They contain chlorophyll and carotenoids pigments which help in
   2) preparation of food.
   2) They may be unicellular, colonial and filamentous.

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3) They habitat freshwater, marine, terrestrial.
4) Some of them contain Heterocyst which is used during nitrogen fixation.
5) Eg. Blue-green algae, Nostoc, Anabaena

Chemosynthetic eubacteria:
1) They oxidise inorganic substances like nitrates, nitrites, ammonia, sulphide etc to produce energy and also helps in the recycling of N, P, S.
For eg: Calyptogena beggiatoa (H_2S)

b) Heterotrophic eubacteria:
1) Most abundant and act as decomposers. They are helpful in the production of curd, antibiotics, nitrogen fixing in leguminous plants.
2) Some of them are pathogenic and cause diseases like cholera, typhoid, tetanus.

- saprotrophic Eubacteria:
They take nutrition from the dead decaying matter and produce their energy.
For eg: Lactobacillus.
- Parasitic eubacteria:
Take nutrition from the host body and are pathogenic to them.
eg: Vibrio choterae

Mycoplasma: (PPLO)
Pleuro pneumonia like organism
smallest living cell.

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1) They lack cell wall and are the smallest living cell.
2) They can live under extremely hostile conditions and can survive without oxygen.
3) They causes diseases in plants and animals.

Kingdom Protista:
It includes Chrysophytes, Dinoflagellates, Euglenoids and Protozoans.

Characteristics of Protista:
1) They are unicellular eukaryotes.
2) Most of them are aquatic and have photosynthetic, saprophytic, parasitic or holozoic mode of nutrition.
3) Their genetic material consist of two or more no. of DNA.

Chrysophytes:
1) They are called diatoms / Golden algae / desmids.
2) They are fresh water, marine and have photosynthetic mode of nutrition.
3) Their cell wall have silica which is indestructible and they fit together like a soap box.
4) They accumulate to form diagomaceous earth (Gritty soil)

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Dinoflagellates :

1) They are marine, photosynthetic and have cellulose in their cell wall.
They contain two flagella - one long and one short.
2) They multiply and cause the formation of Red Tides.

Euglenoids :

1) They found in stagnant water.
2) They are photosynthetic, flagellated and without cell wall.
3) They r the link b/w animal and plants.
4) They have a protein rich layer called pellicle.

Protozoans :

1) They are unicellular, eukaryotic heterotrophs which are parasitic or predators.
2) They are further classified into 4 categories:
→ Ameboid : Pseudopodia for movement
Eg. Amoeba

→ Flagellated : Flagella for movement
eg : Trypanosoma

→ Ciliated : Cilia for movement
eg. Paramecia

→ Sporozoans : spores to create infection.
eg. Plasmodium.

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Kingdom Fungi :

1) They are achlorophyllus, heterotrophic, spore forming eukaryotes.
2) Their cell wall consist of chitin.
3) The reserved food of fungus is glycogen.
4) They have saprophytic, parasitic or symbiotic mode of nutrition.
5) They have both asexual as well as sexual mode of nutrition.
6) Sexual Reproduction involves 3 steps :
   ① Plasmogamy : Fusion of protoplasm
   ② Karyogamy : Fusion of two nuclei
   ③ Meiosis : The zygote forms haploid spores.

Characterized Into four categories :

1) Phycomycetes : Rhizopus
   Mucor
   → Found in aquatic habitat and on decaying wood in the moist places.
   → Their mycelium is aseptate and coenocytic.
   → Their spores are produced inside the sporangium.

2) Ascomycetes : Neurospora, Aspergillus
   → Found on the dead & decaying organisms as well on the dung.
   → Their mycelium are branched as well as septate.

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→ They are also called sac fungi.
→ Their sexual spores are called Ascospores.

3) Basidiomycetes : Eg. Agaricus
→ Their mycelium are branched and septate.
→ Club Fungi
→ sexual reproducing spores are called basidium.

4) Deuteromycetes : Trichoderma
→ They are called "The Fungi Imperfect".
→ Mycelium is septate and Branched.
→ They have saprophytic as well as parasitic mode of nutrition.
→ Reproduce only by asexual mode of nutrition.

UNIT- 2

AQUATIC MICROBIOLOGY Date...4.Oct.2022

Aquatic Microbiology :

1) It is the science that deals with the living organisms in fresh water or salt water ecosystem.

2) Aquatic microbial habitat are :
   1) Freshwater
   2) salty / Marine
   3) Estuarine

◉ Freshwater Habitat : It is a natural or man/ made habitat that are permanently or periodically under fresh or unsalty water.

→ Further of two type :
   ① Lentic freshwater — still water — eg. Pond
   ② Lotic freshwater — Flaoing water — eg. River

→ Both Lentic and lotic habitat can be divided into 3 zones :
   i) Fringing zone -
   ii) It exist along the edges of the river and pond within 50 m from both the banks.
   ii) Pelagic zone :
This the region of water body which is not associated with the bottom or shore.

iii) Benthic zone : Constitute bottom of the water body.

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Lentic Ecosystem :

1, Divided into different zones based on light penetration as well as water density.

2, It can be divided into 3 zones based on light penetration which is as follow :

→ Littoral zone :

- This zone is the shore area of lake of or pond and consist of area from dry land that slopes into the open water and can be narrow or wider.
- Oligotropic lakes or new lakes have narrow littoral zones whereas Eutropic / older lakes have wider littoral zone.
- This zone is shallow and gets a lot of nutrients from the run off therefore it contains abundance of plants & algal growth along with microorganism population.

→ Limnetic zone :

- It is the open area of the lake or ponds that occupies a much larger section of water.
- This zone further has 2 separate sections :

(1) Euphotic zone :/ epilimnion :

It is Upper portion of limnetic zone near the surface that receives sunlight.

- The zone ends where the sunlight fails to penetrate the water.

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⊙ This zone thrive algal and other living population along with other photosynthetic microorganism. because oxygen level is usually higher in zone.

② Profundal zone / Hypolimnion :

⊙ It is the colder water zone that can be found below euphotic zone.

⊙ This zone is located under the region where the sunlight doesn't penetrate the water.

⊙ The profundal zone typically has low population of fishes and other photosynthetic plants & microorganism because of lack of oxygen.

→ Benthic zone :

⊙ The bottom of the pond or lake which consist of organic sediments and soil. As the water bodies ages this zone will rise.

⊙ It is considered as the ponds or lakes digestive system (due to decomposing microorganism).

⊙ This zone occupied by the bacterias that decomposes organic dead matter either aerobically or anaerobically.

Neuston layer : ⊙ Uppermost layer / surface of the Hydrosphere.

⊙ It is the interface b/w the atmosphere and the hydrosphere.

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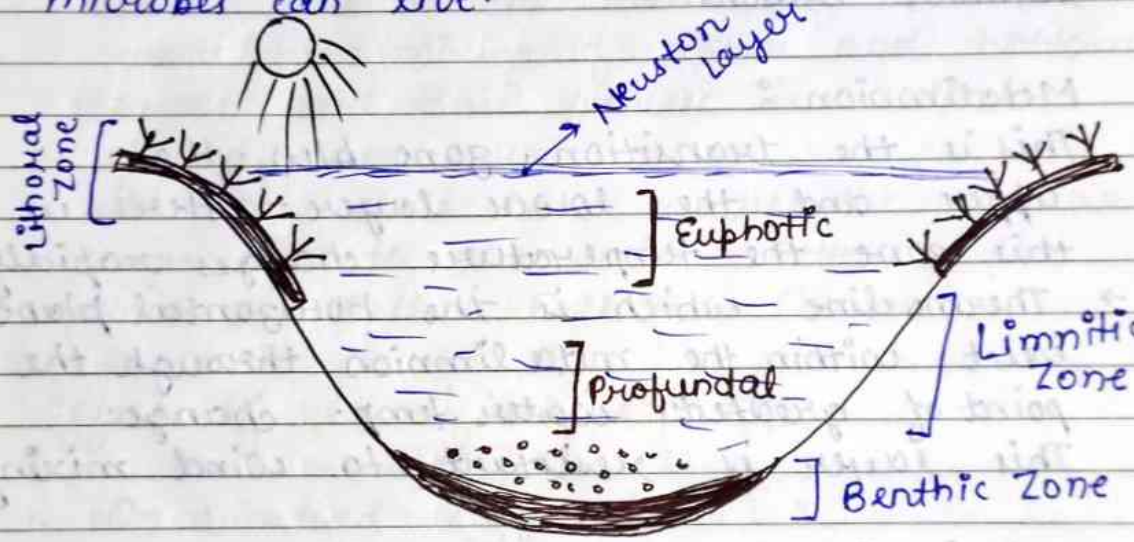
- It is adversely affected by extreme cond^n like sun radiation and temp^e fluctuations.
- Insoluble and less dense organic matter accumulate in this layer therfore constitute a thin gel like structure where microbes can live.



On the basis of water density due to change in temperature the water ecosystem can be divided into 3 zones.
It is also called Lake stratification

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Many lakes are deep enough to form layers of water at different temp. such thermal stratification occurs because of the large difference in density b/w warm and cold water.
The lakes can be stratified in three layers:

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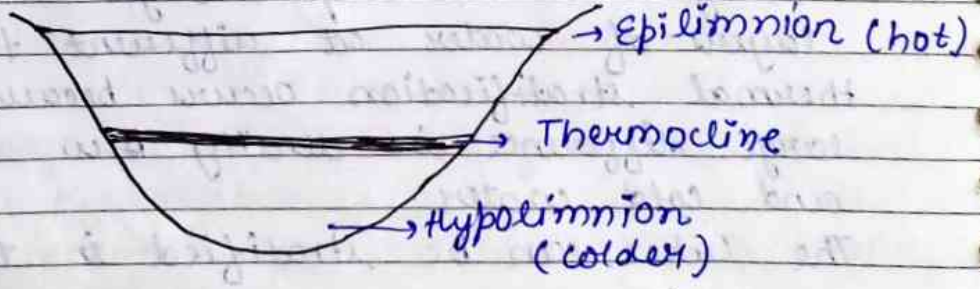
1) Epilimnion : The upper warm, lighter and well mixed zone is called Epilimnion.
This zone has varied temperature depending upon the environment.
This zone is occupied by larger no of aerobic microorganisms specially during summer seasons.

2) Metalimnion :
This is the transition zone b/w the upper and the lower layer. Here in this zone the temperature changes rapidly.
→ Thermocline which is the horizontal plane exist within the metalimnion through the point of greatest water temp. change.
This layer is resistant to wind mixing.

3) Hypolimnion :
The lowest layer found beneath the metalimnion, colder, darker and relatively undisturbed layer.
It is occupied by larger community of anaerobic microorganisms.

In summer



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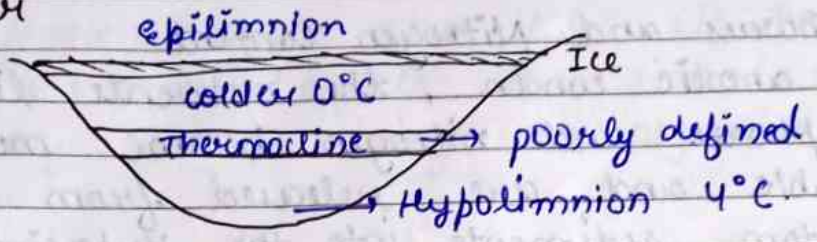
In Winter



Spring Turn Over: The rising density of warm water due to the action of wind cause the mixing of upper and bottom layer and this process is called spring turn over.
When the whole lake reaches the similar temp. due to the wind action, the process is called Fall Turn Over.

Effects of stratification:

1, On Dissolved Oxygen (DO):
Just after summer stratification the Hypolimnion is rich in dissolved oxygen. However bcz of metalimnion which is act as a barrier b/w epi and Hypolimnion, The Hypolimnion is essentially cut off the O_2 exchange with atm and therefore it is too dark for plants like algae to grow for photosynthesis.

Without photosynthesis the hypolimnion can become anoxic as the summer progresses which cause serious consequences to living biota.

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2) Phosphorus and Nitrogen Content:
In anoxic condn, the nutrients like phosphorus and nitrogen become more soluble and are released from the bottom sediments into the Hypolimnion. During summer and due to the action of wind the bottom water partially mixed with the upper water which allow some of nutrient to escape into epilimnion which cause the growth of algal bloom.

3) Temperature:
In summer stratified lakes, water temp. vary from surface to the bottom.
Diff. fish species as well as microorganism prefers diff. water temp. hence the temp. change influence their growth & reproduction.

In colder stratified lakes more species of organisms found in Hypolimnion as compare to epilimnion.

4) Metals & other compounds:
Some metals & other elements like Fe, Mg, Sulphide become soluble in anoxic environment & released from bottom to upper layer.
These comp. ~~com~~ cause the change in taste & odour.

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Some of these comps like H_2S, if Pre is conc. above 1 mg/lt are lethal to many water living microorganisms.

Imp° Biofilms:
They are the aggregate of microbial cell associated with the surface in almost and irreversible manner i.e can not be removed by gently rinsing.
They are attached with the biotic or abiotic surface integrated with the matrix they have produced.

Formation: 5 steps -
1) Attachment: conditioning layer is formed which have a loose collection of carbohydrate and proteins which gets write with mineral and water. It attract the microbial cells to get attached with the surface.

2) Irreversible Attachment: As soon as conditioning layer is formed electric charge accumulate on the surface which attract the bacteria having opposite charge that result in IA of microbial cells.
The charges are sufficiently weak that the microorganisms could be easily removed by mild cleanser and sanitiser.

3) Proliferation: In this phase bacteria get attached to the surface as well as

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with each other by secreting EPS (extra cellular polymeric substance) that entrap the cells within a gel like matrix.

4) Maturation : The biofilm env. consist of nutrient rich layer which supports the rapid growth of microorg. complex diffusion channels are present in a mature biofilms to transport nutrients and other components required for bacterial growth as well as for removal of waste product.

5) Dispersion : It is the process of dispersal of biofilms in which actively growing cells gradually shed off the ~~growing~~ (new) daughter cells. Because as long as fresh nutrients are kept providing, biofilm continues to grow and when they get nutrient deprived they return to their planktonic mode by detaching themselves from the surface. This process probably happens to allow bacterial cells to get sufficient nutrients.

Eg : Pseudomonas : 5 hrs – spores
Vibrio : 2 hrs

Composition of Biofilm : It is composed of bacterial colony which is distributed in a matrix. These colonies are rod-like or mushroom shaped having

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diff. types of bacterias.
BASED ON THE BACTERIAL TYPE - the comp. of microbial colony contain 10 - 25 % of microbial cells and 79- 90% of matrix.
Extensive bacterial growth assist in the rapid formn of layers of microbes accompanied by excretion of EPS. in abundant amount.
At bottom of the biofilm, a dense layer of micro org. is bound together in polysaccharide matrix with other organic & inorganic components.

Water Channels: These are present in b/w the micro colonies which act as simple circulatory system for the distribution of nutrients & other harmful metabolites.

EPS: / Exo-polysaccharides - which is produced by the bacteria and are the major component of biofilms it constitute about 50-90% total organic matter in a biofilm. It is mainly composed of polysaccharides which may be neutral or anionic in case of gram negative bacteria or cationic in case of gram +ve bacteria.
The anionic property of polysaccharide is confirmed by presence of Euronic acids.
EPS also contain proteins, DNA, RNA

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as well as some lipids and humic substances.

Factor affecting biofilm production:

1) Substratum effects:
As the surface roughness ↑es microbial colonization ↑es bz of the ↑e in surface area and diminizing shear forces. And considering extent and rate of attachment, it has been seen thad micro. attached more rapidly to hydrophobic and non-polar surfaces like Teflon and other plastic rather than hydrophilic surfaces.

2) Conditioning films forming on the substratum:
When a material surface expose to any aqueous medium it gets immediately coated with polymer or become conditioned. The coating or film is found to be organic in nature. The nature of these films is quite diff. from the surfaces exposed in the human host.
For eg: Acquired pellicle - a proteinaceous conditioning film develops on tooth enamel surface and as composed of glycoprotein, lysozyme, phosphoproteins, albumin, lipids and gingival crevices fluid.
Oral cavity bacterias get adhered within hour of exposure to this pellicle conditioned surface.

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3) Characteristics of the aqueous medium :
— such as temp°, pH, nutrient strength and ionic strength possibly play an imp. role in the attachment of microorganism.
For eg: _____ of pseudomonas fluorescens to glass surface is affected by Tse in the conc. of several cations.

4) Environmental Factors :
a) Availability of Nutrients :
It has been shown by studies that the species of bacteria Monocytogenes biofilm is affected by the level of phosphate conc. and it also get stimulated by the presence of carbohydrates.

b) Presence of oxygen : It regulates biofilm formation in e-coli bacteria. In the absence of oxygen supply, biofilms doesn't form as bacteria couldn't adhere to the substrate surface.

c) pH :
pH affects several micro. during biofilm formn for eg. the optimum pH required for the formation of biofilm - by V. chloral is 8.2. and in acidic environment the bacteria loose their ability to form biofilm. On the other hand the e. coli bacteria don't need alkaline environment but they easily grow in acidic medium like urethral catheters.

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d) Temperature:

When temp. was kept high, most of the organisms did not form biofilm as the bacteria was not able to adhere itself to the substrate surface.

Diseases caused due to biofilm formation:

Gram +ve microorganisms	site of infections & diseases
⊙ Acidogenic gram +ve cocci eg. streptococcus	Dental caries
⊙ Staphylococci	Musculoskeletal infections
⊙ S. epidermidis, E. faecalis	Urinary catheter cystitis
⊙ S. epidermidis, S. aureus, Micrococcus species, Candida, Streptococci	IUDs
⊙ Viridans streptococci	Mechanical heart valve

Gram -ve microorganisms	site of infection and diseases
⊙ P. aeruginosa	Cystic fibrosis pneumonia
⊙ Proteus mirabilis, Bacteroides, E. coli	Orthopedic devices.

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Applications of Biofilms:

① Plant Protection Agent:

Biofilm formation triggers a no. of beneficial effects such as biocontrol & symbiosis. These biofilms forms on the surface of leaves, roots & stems and can act as bio control agent.

For eg: the rhizobacteria colonized on the plant surfaces and act as a barrier b/w the plant and the pathogens. They can also act as biofertilizers to promote the plant growth through N_2 fixation and mineral uptake.

② Bioremediation:

It is the process that employies living organism or their derivative for the treatment of Hazardous subs. from the env. into harmless compound.

The biofilm mediated remediation exhibit higher efficiency in converting toxic waste into the non-toxic compound & enhanced adaptibility of several degrading micro. to diff. toxic chemicals. It can be in-situ (on the site) or ex-situ (off the site).

It can be achieved through incorporation of limiting nutrients and $e^{\ominus}$ (Biostimulation). or by addition of microbes at the polluted site (Bio-augmentation) to promote the bio-remediation process.

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③ Wastewater Treatment :
The presence of organic inorganic contaminants present in various water resources can be neutralised and degrade by using biofilm based treatment technology.
For eg: Biologically active carbon (BAC) process is one of the water treatment technology which used activated carbon mediated biofilms to physically removed microorganisms that cause water borne diseases.

④ Prevention & control of Corrosion :
Both chemical and biological factor can accelerate the rate of corrosion. The use of bacterial biofims is now used in diff. industries to prevent corrosion bcz of their nature friendly behaviour and cost effectiveness.
The potential strategy used by biofilms to control corrosion is
a) Removal of corrosive substance like O_2 by aerobic bacteria.
b) Inactivation of corrosion inducing bacteria like sulphate reducing bacteria.
c) Production of protective q. coating such as γ- polyglutamate by biofilms.
d) Biofilm formn serving as barrier to hinder dissolution of ~~matter~~ metals.

Environmental Microbiology

Date.....

UNIT-3

Biodegradation Microbiology

Interaction of biological, chemical and environmental factors in biodegradation process. (2020)

Bioremediation process : Defⁿ & classifⁿ (ex-situ & in-situ) (Factors) & Principle) (2020)

Biodegradation

↓

Environmental parameters (Abiotic)

Nutrients, Temp., pH, moisture, Salinity

Biological

Light, water, Oxygen, Temp.

Chemical & Biological (Biotic)

↓

Temp.
Oxygen
Nutrients
pH
Soil Moisture
Bioavailability
Salinity
Pressure

Microbial degradation
Fungi
Algae
Plants.

Date.....

Biodegradation :

- ⊙ Breakdown of organic matter by microorganisms, such as bacteria and fungi.
- ⊙ The process whereby organic wastes are biologically degraded under controlled condn.
- ⊙ It uses naturally occuring microorganisms like bacteria and fungi or plants to degrade or detoxify subs. hazardous to human health or environment.

Types of Biodegradation

Natural Attenuation

(1) Processes that naturally transform contaminants to less harmful forms or immobilize contaminants so that they are less of a threat to environment.

(2) These processes include aerobic & anaerobic biodegradation, sorption, volatilization & chemical or biological stabilization.

(3) For eg : Pseudomonas, Marinomonas.

Aerobic degradation

(1) Breakdown of organic pollutant by microorganisms when oxygen is present.

(2) Oxygen is used in chemical response that breakdown the pollutant molecule into water & CO_2.

(3) For eg : Pseudomonas putida (gram-ve bacteria) (bioremediation of toluene).

Anaerobic degradation

(1) It is a series of processes in which microorganisms break down biodegradable material in the absence of oxygen.

(2) Used to treat waste water sludge & biodegradable waste

(3) For eg : Dechloromonas aromatica (Rod-shaped bacterium) which oxidizes aromatics. Only orgn able to oxidize benzene anaerobically.

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Environmental factors in Biodegradation Process:

Environmental (Abiotic factors)

Temperature
- It regulates the growth and activity of microorganisms.
- Temp. is different at diff elevations.
- The species diversity & microorganism count is affected by environmental changes.

Moisture
- water present in soil is responsible for various physiological processes of microorgn present in soil.
- Growth of microgn. is thus governed by presence of moisture in soil.

pH
- The presence of cations and anions governs the pH of soil, which in turn affects microbial growth.

UV radiation → UV radiation reduces all fraction of total organic carbon which indicates dominance of mineralization.

Pollutant Quality
- Rate of decompos^n depends on structural and chemical properties of litter.
- For eg: the litter of Bryophytes are decomposed at a slower rate due to presence of lignin called complex chemicals.

Aeration
- The oxygen present in pores of soil helps in growth of microorganisms.
- In the water logged soils, the aerobic microgr^n are absent.
- Only anaerobic microorg^n grow & decomposed.

Inorganic chemicals
- Elements like Potasium, sodium, calcium, magnesium are released into soil.
- Some of these used by microorg^n for their growth.
- It affects rate of decomposition.

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Biological factors (Biotic)

- **Microbial degradation (Bacteria)**
  - Microbial organisms transform the subs. through metabolic or enzymatic process.
  - Growth & cometabolism.
- **Fungi**
  - Major Role as decomposers & symbionts
  - Diverse metabolic capacity due to which they are suited for purpose of bioremediation / biodegradation.
- **Algae**
  - Algae characterized by secretion of various enzymes involved in bioremediation / biodegradation of several organic pollutants into less hazardous metabolites.
- **Plants**
  - Used to degrade the pollutant (Phyto remediation)
  - Most plant associate with different bacteria & association can use the biodegradation rate of organic comps.

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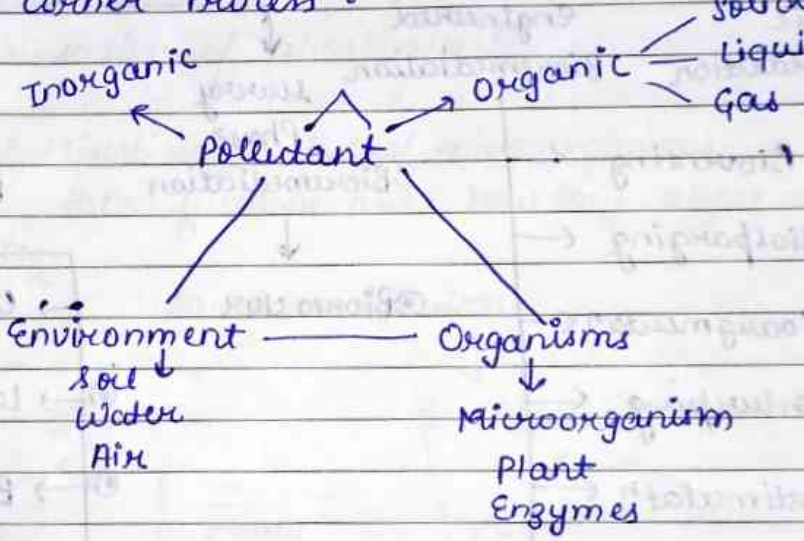
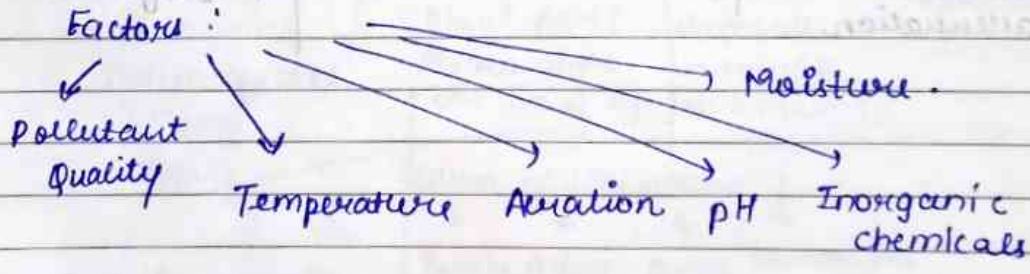
Bioremediation :

⊙ Bioremediation is any process that uses organisms (microorganism, plant & algae) or their enzymes to remove the desired pollutant and to return the polluted env. to its original condn.

⊙ Triple Corner Process :



Factors :



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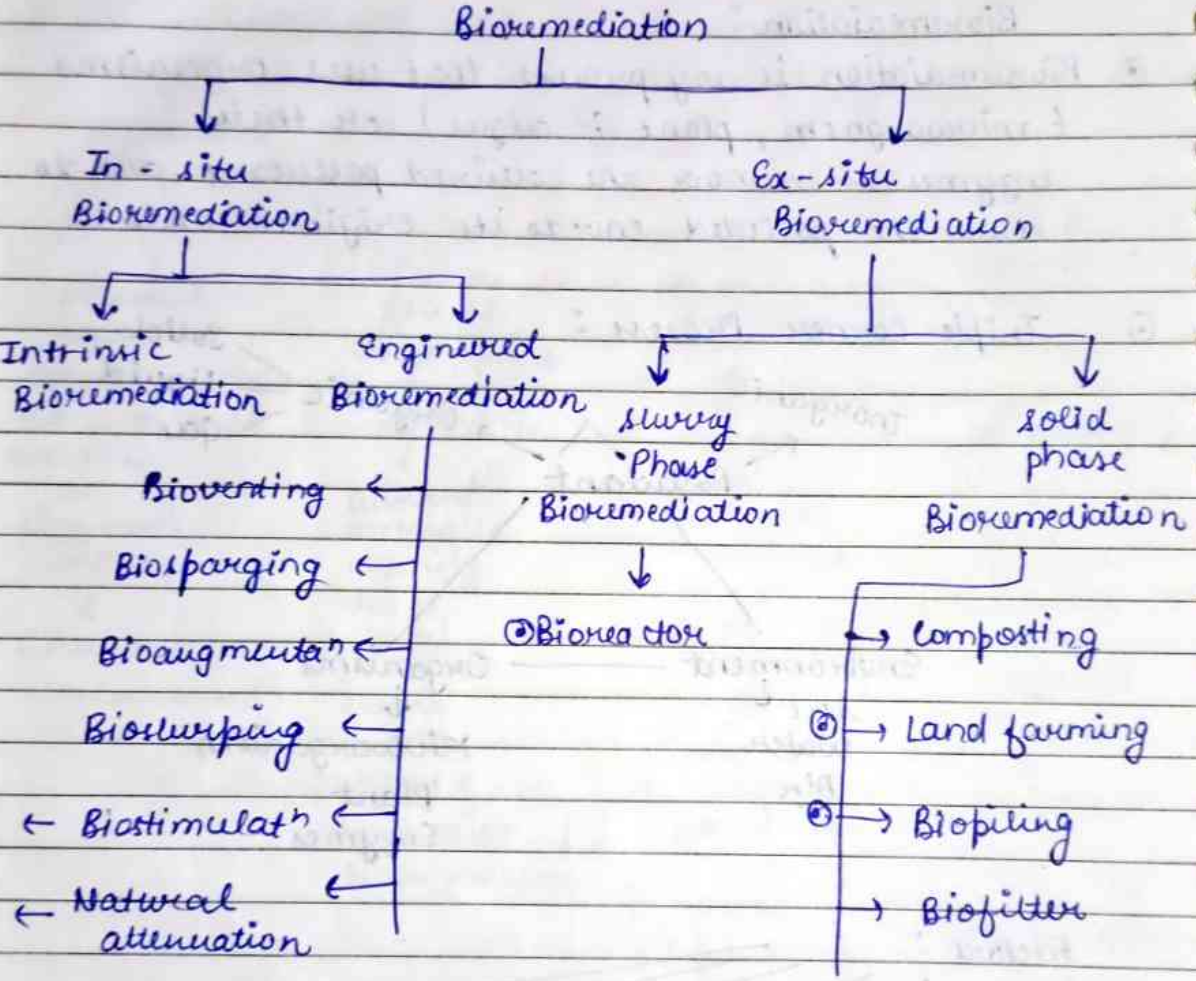
Bioremediation
In - situ Bioremediation
Ex - situ Bioremediation
Intrinsic Bioremediation
Engineered Bioremediation
Slurry Phase Bioremediation
solid phase Bioremediation
Bioventing
Biosparging
Bioaugmentation
Biosurfping
Biostimulation
Natural attenuation
Bioreactor
Composting
Land farming
Biopiling
Biofilter

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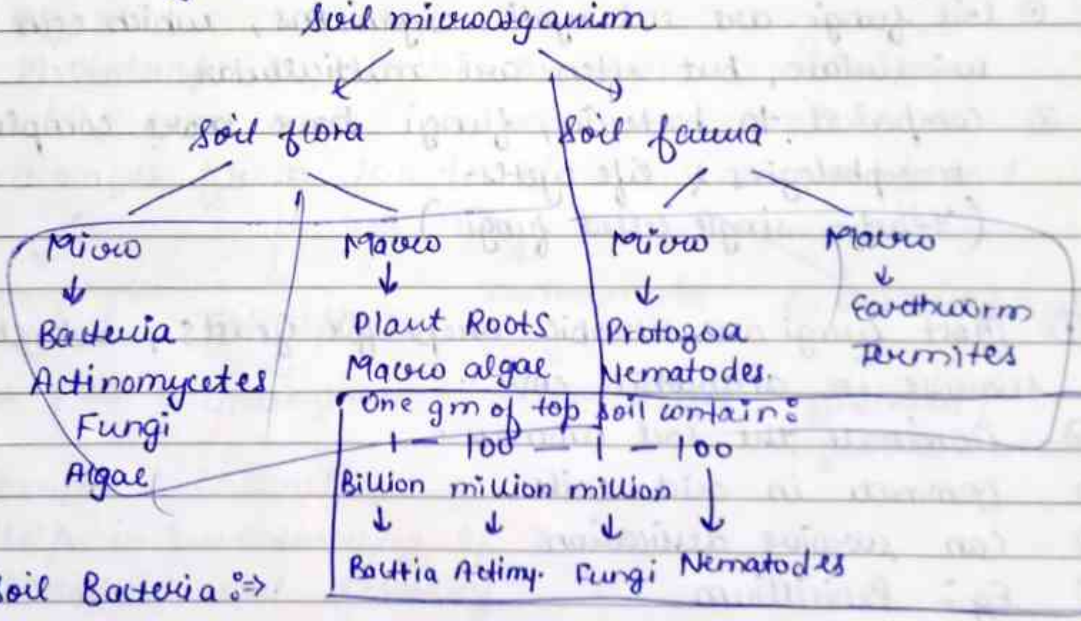
Unit-4 Soil Microbiology

- Distribution of different types of soil microorg.
- Factors influencing microbial population
- Rhizosphere effect
- Microbes in decomposition
- Mineralisation and recycling process (20 ✗)
- Bioremediation
- Solubilisation of phosphates ⊙

Soil Microbiology: study of microorganisms in soil, their functions and how they affect soil microbiology.



① Soil Bacteria :⇒

- ⊙ These are very small, one-celled organisms that can be seen with electron microscope.
- ⊙ Constitute the highest biomass of soil organisms. More abundant near roots, one of their food resources.
- ⊙ For agriculture, rhizobium & Actinomycetes are important.

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- Bacteria are imp. in soil because they contribute to the carbon cycle by fixation (photosynthesis) & decomposition.
- Prokaryotic org. - usually 0.5 - 1mm wide and 1 to 2 mm long.
- A variety of cell shapes exists for bacteria, including rod, spherical, spiral and filamentous.
- Example: Bacillus, Arthrobacter, Pseudomonas, Micrococcus

(2) Fungi:

- Soil fungi are eukaryotic organisms, which can be unicellular, but often are multicellular.
- Compared to bacteria, fungi have more complex morphologies & life cycles.
  (Yeast - single celled fungi).

- Most fungi are aerobic except for yeasts, which can survive in anaerobic env.
- Dominate the soil biomass.
- Dominate in acid soils
- Can survive desiccation
- Eg: Penicillium
  Trichoderma in soil.
  Mycorrhizal fungi — plant roots.

(3) Actinomycetes:

- Actinomycetes are filamentous bacteria, most of which are gram +ve bacteria & more abundant in neutral to alkaline soil.

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- Anaerobic, that form colonies or extensive mycelia.
- ∴ Mycelia break down, resulting in rod-/cocoid-shaped form.
- Size - 0.5 to 1.5 μm.
- Population largest in surface layer of soil and decreases with depth.
- Eg: Streptomyces
  Streptomyces scabies — potato scab
- Ability to produce antibiotics
  Streptomycin — TB.

(4) Algae:

- Phototrophic, aerobic organism, obtain CO_2 from atm, unicellular, colonial
- No. ranges from 100 to 10,000 per gm of soil.

Cyanophyta	Chlorophyta	Xanthophyta	Bacillariophyta
↓	↓	↓	↓
Blue-green	Grass-green	Yellow-green	Diatoms / golden-brown

- Example: Chlorella
- Help in weathering of rocks
- Maintain soil fertility.
- Mucilage secreted by BGA helps in ↑ water retention capacity of soil.

Soil → fauna → Microfauna → Protozoa.

(5) Protozoa:

- Abundant in upper layer of soil (15 cm).
- Unicellular, eukaryotic

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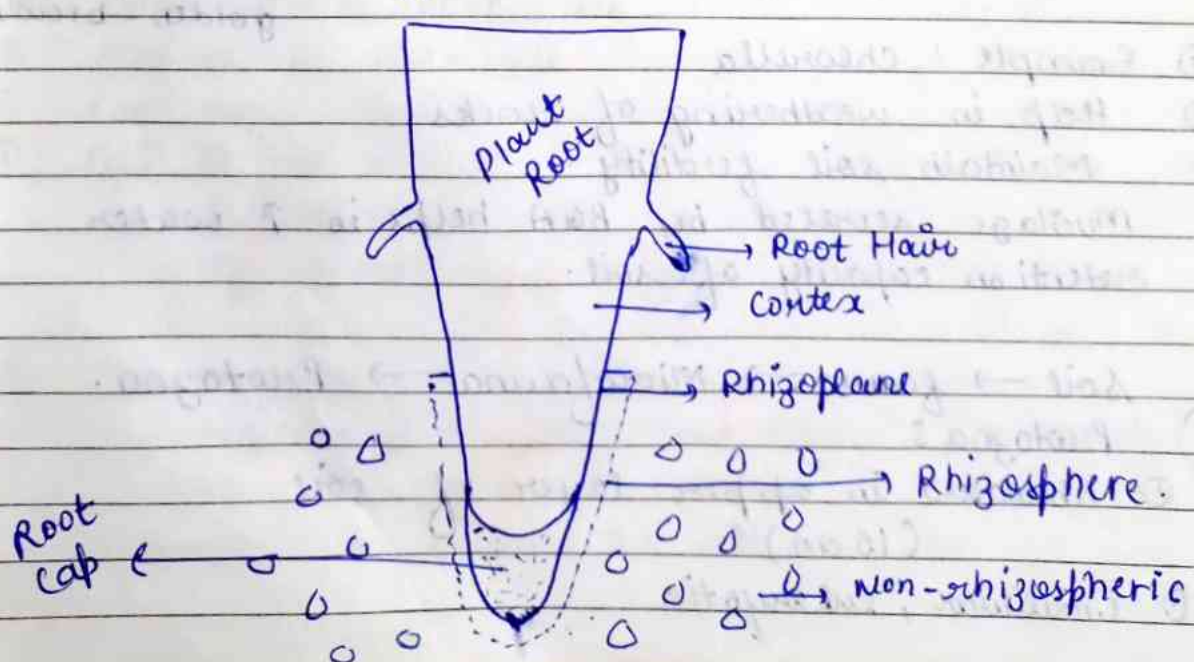
- 3 categories : flagellates, amoeba, ciliates.
- Eg : colpidium
- Cause disease in human beings which are carried through water & other vector.
  eg. Amoebic dysentry by E. coli.

Factors influencing microbial population :

1) pH - Acidic quality in their surrounding.
2) Temp. - Per temp. - More easy mic. grow.
3) Moisture - To exchange materials
4) Diff. seasons
5) Types of vegetation & its growth ~~seasons~~ stages
6) Soil mineralogy
7) Light
8) Soil organic matter
9) Organic & Inorganic chemicals.

Rhizosphere effect :



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Microbiology of rhizosphere :

① The rhizosphere is the narrow region of soil around the plant root that is influenced by several factors like root exudates and the associated soil microorganisms.

② The soil surrounding the plant root where root exudate migrates & microbiologically activity is exceptionally high is called rhizosphere.

③ Surface of root is called rhizoplane.

④ Plant root produce and release various exudates containing sugar, aminoacids, organic acids, fatty acid, vitamin, nucleotides & other organic matter that promotes growth of microorganisms.

⑤ Therefore rhizospheric soil is characterized by greater no. of microorganisms than soil away from plant roots.

⑥ The no. of microorganisms ↓ses continuously as the distance from the plant root ↑ses.

⑦ R : S = $\frac{\text{No. of microbe in rhizospheric soil}}{\text{No. of microbe in soil free of plant root}}$

R : S > bacteria (20 : 1)
less for fungi & actinomycetes.

⑧ Effect of rhizosphere is almost negligible for algae & protozoa.

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(2) Most bacteria depends on easily available decomposable matter of root exudates.
∴ No. of bacteria is exceptionally high in rhizosphere.

Example of rhizospheric microorganisms :⇒
(1) The microbial population in the rhizosphere is known as rhizosphere microbiome & the microbial population in such an area are much higher than the bulk soil.
(2) Large no. of bacteria, fungi & actinomycetes are found in rhizosphere.

i) Bacteria : Bacillus, Pseudomonas, Azotobacter, Arthrobacter, Rhizobium.
→ many nitrogen fixing bacteria and phosphate solubilizing.

ii) Fungi : Trichoderma, penicillium
→ some fungi are found associated with root forming mycorrhiza & other occurs freely in soil.

iii) Actinomycetes :
Streptomyces, Frankia etc.

Factors Affecting Rhizospheric Microorganisms

① **Proximity of soil to Root:**
→ No of rhizospheric orgn is more near the root & their no. continuously ↓ses with ↑se in distance from root.
→ More nutrient availability near to root surface.

② **Temperature & Light Intensity:**
→ Low Temp. & low light intensity ↓ses the rate of exudate secretion from root – rhizospheric org. ↓ses.
→ No. of microbes in rhizosphere ↑ses when temp. & light intensity ↑ses as multiplication rate is high.

③ **Type of soil:**
R:S > sandy soil (Due to organic matter present near roots).
low in clay soil.

④ **Age of plant:**
With age – rate of exudates secretion is altered so that no. of rhizospheric microbes change.

⑤ **Type of plant & location of root:**
Root cap & region of root from where lateral root arises are primary sites of exudate secretion.

⑥ **Depth of root:**
Rhizospheric microorganism ↓se with ↑se in depth of root, which is mainly due to anaerobic condition.

⑦ **Root Respiration:**
→ Plant root release CO_2 during respiration that makes the soil acidic.
→ Acidity of soil ↓se no. of rhizospheric bacteria.

⑧ **pH of soil:**
pH of rhizosphere become acidic due to root respiration & by oxidation of sulphur.
→ Acidification of rhizospheric soil ↓se no of microorganisms.

⑨ **Pesticides and Antibiotics:**
→ Spray of pesticides and antibiotics on agriculture crops ↓se the no. of rhizospheric organisms.

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Positive Role of Rhizospheric Microbes:

– Promote plant growth

① Rhizobium, Azotobacter, Clostridium – fix atm. Nitrogen & make it available for plant growth.

② Bacillus – phosphate solubilizing microbe
Release free phosphate from inorganic salt of phosphate
↓
plant growth

③ Several rhizospheric microbes (Azotobacter, Arthrob. & Pseudomonas) produce growth hormone such as Gibberellin, IAA – plant growth.

④ Mycorrhiza – Mycorrhizal fungi promote plant growth.

⑤ Rhizospheric microbes induce development of lateral root, root hair development & mucilage secretion from plant root.

⑥ Microbes also Use rate of exudate secretion.

Negative Effect of plant root on rhizospheric microbes:

① Some plant root produces antimicrobial chemicals such as glycosides, and antifungal agents – inhibits growth of rhizospheric microorg.

② ~~Some~~ plant root release CO_2 during respiration that make habitat acidic & anaerobic.

③ Some plant root produce chemicals that bring fungistasis.
↓
Inability of spore to germinate.

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Solubilisation of phosphates :

① Phosphorus is one of major plant growth limiting macronutrients required for proper plant growth.

② Essential in plant activities → root growth, cell division and enlargement
N fixation in legumes
Transporting of genetic traits
Transform^{n} of sugar to starch.

③ Phosphorus in soil solution exists as insoluble inorganic phosphate and insoluble organic phosphates.

④ Phosphate solubilizer :
PSB capable of solubilizing inorganic phosphorus from insoluble compound
→ have capability of dissolving soil Phosphorus, can mineralize organic phosphates to become Inorganic Phosphates.
→ Plant absorb phosphate in $H_2PO_4^-$ & HPO_4^{2-}
→ absorb 10-15 % & rest in insoluble form of phosphate.

⑤ Mechanism of phosphorus solubilization.
→ Lowering soil pH :
⊙ lowering of soil pH by microbial prod^{n} of organic acid.
⊙ PSM uses phosphate availability by producing organic acids that lowers the soil pH.
⊙ PSM - create acidity by evolution of CO_2.
⊙ Prod^{n} of OA coupled with use of pH by action of Microorg. resulted in P solubilization.

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→ **Chelation:**

- Organic and inorganic acid produced PSM dissolve the insoluble soil phosphate by chelation.
- Hydroxyl & carboxyl grp of acid chelate the cations bound to phosphate — converting it into soluble forms.

→ **Mineralisation:**

- Other mechanism of solubilizing soil P is mineralisation.
- Organic phosphate is transformed into utilizable form by PSM through proces of mineralisation.
- Mineralisation & immobilization of soil organic P plays a vital role in phosphorus cycling of agricultural land.

Microbes in decomposition:

→ Microbiology of decomps^{n} is study of all M/O involved in decomps^{n}, the chemical & physical processes during which organic matter is broken down & reduced to its original elements.

→ Bacteria, fungi, protozoa, actinomycetes & other saprophytic orgn feed upon decaying O.M initially, while in later stage of decomps^{n} mites, millipedes & centripedes enrich the composting materials.

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→ Decompos^n of organic matter by M/O.

i) Cellulose decompos^n
↓
Most abundant OM in plants.
Fungi decompose cellulose
↓
Penicillium, Trichoderma, Verticillium

→ Bacteria: Bacillus, Pseudomonas

ii) Hemicellulose decomp^{sn}
↓
polymer of simple sugar such as pentose, uronic acid
Fungi → Aspergillus
Bacteria → Bacillus, Vibrio.

iii) lignin Decomp^n.
↓
Highly resistant to microbial degradation.
F → Polyporus
B → Micrococcus

Significance of Microbial decomp^n of OM.
→ Retain Nitrogen & other nutrients
→ Glue soil particles together for best str.
→ Protect roots from diseases & parasites.
→ Produce hormones that help plants grow.
→ Retain water
→ Make retained nutrients available to plant.

Ques: Role of M/O in soil.
Explain nitrogen mineralization & its imp. in (pH), Organic waste recycling?

Ques: What does a soil Microbiologist study?
Types of soil orgnsms?
Role of soil microorg. in plant mineral nutrition. → fav. page.

Ques: Compare the microbial flora of soil in region of rhizosphere & that in an area at a dist. from rhizosphere.