Microbial Ecology and Biodiversity

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CONTENT

- Definition of ecology and important terms
- Microbial interactions
- Microbial habitats
- Biofilm: growth and control
- Terrestrial habitats: Soil and groundwater
- Water habitats

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General definitions

Ecosystem: Sum together of all organisms and abiotic factors in an environment (Lehninger: Principle's of Microbiology)

A dynamic complex of plant, animal and microbial communities and their abiotic surroundings, all of which, inter-act as a functional unit (Brock Biology of Microorganisms).

Habitat: A portion of the environment in which a particular species or organisms or micro-organisms reside.

Population: A group of micro-organism of same species living in a habitat.

Communities: Different species of microbes/organisms living in the ecosystem.

Microbial diversity: The vast array of micro-organisms that exist in the environment. Microbial diversity is expressed in two ways:

1. Species richness: The total number of different species present.
2. Species abundance: Given by the proportion of each species in a community.

Microbial interactions

- **Symbiosis:** A relationship between two micro-organisms in which both or one might be benefitted
  - Ectosymbiosis: Fungi associated with plant roots or mycorrhizal fungi (plant gives sugar and lipids, Fungus helps in uptake of water and nutrients)
  - Endosymbiosis: bacteria living inside such mycorrhizal fungi help the Fungi with
- **Mutualistic symbiosis:** A relationship in which both organisms are benefitted
- **Commensalism:** A relationship where one of the organisms is benefitted while there is no benefit for the other organism.
- **Parasitism:** A relationship in which one organism, the parasite, is benefitted at the expense of the other organism, the host.

Microbial diversity and interactions

- Microbes which are metabolically connected are known as **guilds**.
- Sets of such guilds form communities. Microbial communities affect the ecosystem by interacting with the abiotic factors and the macro-organisms.
- Interaction with the environment in such cases contributes to the biogeochemical cycles of important nutrients.
- Example: Sulphur in the form of H_2S can be metabolised to form sulphur or sulphate both by phototrophic as well as chemotrophic organisms. This sulphate acts as a key nutrient for plants. Sulphate is also reduced by sulphur reducing bacteria.

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Microbial habitats

- Habitats that sustain life supports microbial growth. Variations in habitat even in small mm scale can produce community diversification.
- Common habitats that we will study: soil, water, waste areas.
- Ideally, a habitat is an environment which provides the essential life conditions for the microbial growth and life. However, each microbial species has certain pre-conditions that is required for its survival. These pre-conditions include the physico-chemical (temperature, pH, redox conditions, alkalinity) conditions of the habitat as well as the quantity of the resources (macromolecules, nutrients, presence of certain trace constituents).
  These pre-conditions define the "**niche**" of the habitat.
- For each microbial species there exists one specific niche. This is known as the **"prime niche"** where the microorganism thrives best. In it's prime niche, the microbe is the dominant species.
- However, the microbes may be found in other situations where it survives less successfully.

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Microbial habitats: continued

- To understand microbial habitats we need to understand the microbial environment.
- Microbial environments are often very small in nature and are rightly known as micro-environments.
- Imagine the size of a typical microbe around 1 µm. A distance even in 10 mm is equivalent to you going to the other side of the city.
- Thus, you would expect to find microbial diversity even in such minuscule scale of measurement.
- Consider the figure given alongside: This figure represent the depletion of oxygen inside a soil particle. Thoughts?

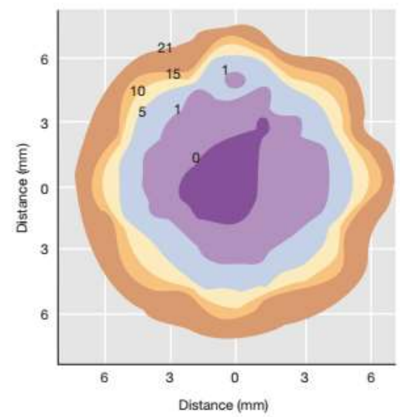

Fig. 1: Contour map of O_2 concentration in % as sensed by micro-sensors. Each zone is a microenvironment

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Microbial habitats: continued

- Microbes exists in environment usually in substrate limited conditions.
- The microbial habitats in nature go through a 'feast-famine' phase.
- Nutrient supply in nature is intermittent in nature which leads to a nutrient spike followed by a famine phase when the microbes are nutrient scarce.
- Microbes survive by storing food in the form of PHAs, polysaccharides etc.
- Such substrate limited conditions can produce growth rates which are quite dissimilar to that seen in Petri-dish growth.
- For example, doubling time of *E. coli* in rumen of a human is 12 h as compared to that in a nutrient abundant Petri dish wherein doubling time can be as low as 20 mins.
- Reasons for such growth rate difference:
    1. Resources or growth conditions are not optimal
    2. Spatial distribution of nutrients are not uniform
    3. Competition with other microbes in the environment might lead to lesser substrate availability.

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Biofilm growth

- Microbial growth over different surfaces occur through biofilms. Biofilms provide certain advantages over planktonic cells
    1. Better access to nutrients as nutrients tend to absorb on surface.
    2. Enhanced antibiotic protection of microbes through such colony formation.
    3. Providing cells a means to anchor to the favourable habitat as compared to the planktonic cells, thus preventing washout
- Biofilm formation can occur on diverse surfaces. Biofilms can be defined as assemblages of bacterial cells attached to a surface and enclosed in an adhesive matrix that is the product of excretion by cells and cell death
- The matrix is typically a mixture of polysaccharides, proteins, and nucleic acids that bind the cells together. Biofilms trap nutrients for microbial growth and help prevent the detachment of cells on dynamic surfaces, such as in flowing systems.
- Biofilm formation can lead to a diversity as a function of the biofilm depth. For example if the O_2 concentration in the inner layers of the biofilm is depleted. This would open up to a new habitat niche for anoxic microorganisms.

Fun fact!!

The human mouth has 700 phylotypes (microbes with overall similarity)

Biofilm growth

Stages of biofilm growth

- Random collision of the cell to the intended surface leads to attachment. Initial attachment of the cell occurs with the help of cell appendages such as pili or flagella and also by using surface proteins.
    - Example: *Pseudomonas fluorescens* uses LapA protein for initial adhesion
- Subsequent to initial attachment, the cell starts expressing the biofilm specific genes. These genes encode proteins that synthesize intercellular signaling molecules and initiate production of polysaccharides that form the EPS.
- These polysaccharides are used for matrix formation. Ideally, upon initial adhesion to the surface, switching from planktonic to biofilm mode is initiated by production of the chemical "cyclic di-guanosine monophosphate".
- This compound modulates gene expression and enzyme activity, such as by binding to transcriptional regulator, mRNA (known as riboswitches), and specific proteins to alter enzyme activity.
- For example, c-di-GMP binds to proteins that reduce the flagellar motor activity, regulates generation of cell surface proteins required for attachment, and mediates the synthesis of extracellular matrix polysaccharides of the biofilm.

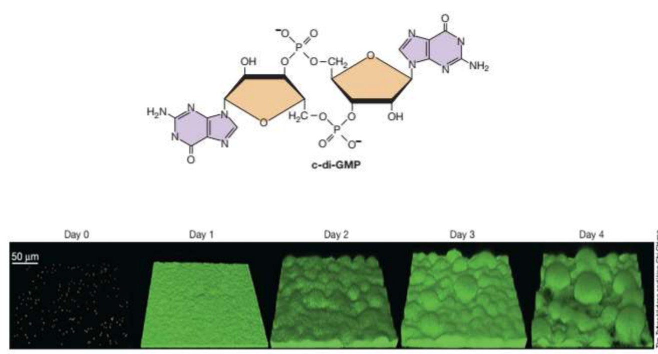

Fig. 2: (a) Structure of the c-di-GMP (b) *Pseudomonas. aeruginosa* biofilm formation over a glass slide in controlled laboratory environment. The final mushroom shaped matured biofilms are around 0.1 mm tall

Biofilm growth

The stages of biofilm development as depicted in adjoining figure. It is a cyclic process that occurs in a stage-specific and progressive manner.

1. The process is initiated following surface contact by single planktonic cells followed by attach to the substratum via the cell pole or via the flagellum (step I), followed by longitudinal attachment.
2. Transition to the irreversible coincides with a reduction in flagella reversal rates, reduction in flagella gene expression and the production of biofilm matrix components (Step –II).
3. Biofilm maturation stages are identified by the appearance of cell clusters that are several cells thick and are embedded in the biofilm matrix (maturation-I stage)
4. Complete maturation into microcolonies (maturation-II stage)(26, 43).
5. Dispersion has been reported to coincide with the decrease in and degradation of matrix components, with dispersed cells being motile and demonstrating increased drug susceptibility relative to biofilm cells. The biofilm matrix is shown in beige.

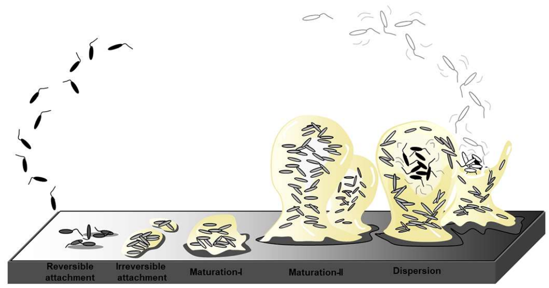

Fig. 3: Stages of biofilm growth
(**Source:** Sauer K, Stoodley P, Goeres DM, Hall-Stoodley L, Burmølle M, Stewart PS, Bjarnsholt T. The biofilm life cycle: expanding the conceptual model of biofilm formation. Nat Rev Microbiol. 2022 Oct;20(10):608-620. doi: 10.1038/s41579-022-00767-0. Epub 2022 Aug 3. PMID: 35922483; PMCID: PMC9841534.)

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Biofilm growth

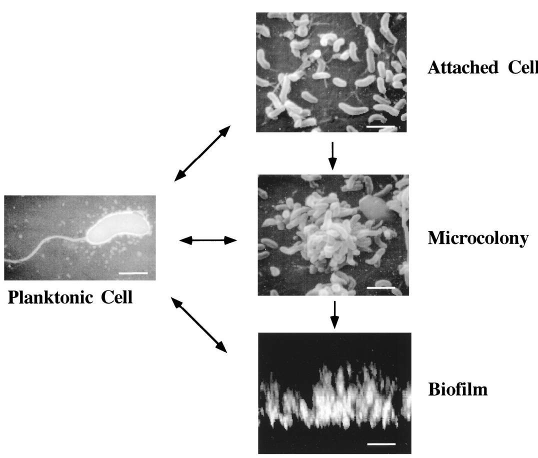

Fig. 4: (a) biofilm formation by *Vibrio cholerae* by Transmission electron microscopy image (scale 1 μM) Stages of biofilm growth
(**Source: Sauer** K, Stoodley P, Goeres DM, Hall-Stoodley L, Burmølle M, Stewart PS, Bjarnsholt T. The biofilm life cycle: expanding the conceptual model of biofilm formation. Nat Rev Microbiol. 2022 Oct;20(10):608-620. doi: 10.1038/s41579-022-00767-0. Epub 2022 Aug 3. PMID: 35922483; PMCID: PMC9841534.)

Fig. 4: (b) Biofilm in pipes
(**Source**: https://waterandhealth.org/safe-drinking-water/biofilms-drinking-water-quality/)

Fig. 4: (c) Biofilm in pipes
(**Source**: https://www.biologicalwasteexpert.com/blog/biofilm-based-wastewater-treatment)

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Biofilm growth: intercellular activities

- Biofilm development is greatly affected by intercellular activities.
- Quorum sensing: biofilm formation can be enhanced by excretion of signaling molecules. For example, the cells of *P. aeruginosa*, secrete "**acyl homoserine lactone**", which is a major intercellular signaling molecule.
- This signaling lactones can regulate the expression of genes related to biofilm formation in the new *P. aeruginosa,* that intercepts this lactone molecule.
- The signaling molecules can also aid in delocalization and act as a response to changes in environment. For example, in *Pseudomonas fluorescens*, lower phosphate levels act lead to reduce c-di-GMP production that reduces the LapA localization on the outer membranes.
- The LapA protein is responsible for the surface adhesion of the cells. Thus reducing the LapA localization on the outer surface reduces the biofilm formation. Further reduction in PO_4^{2-} leads to further reduction of c-di-GMP production which again triggers production of protease that cleaves LapA protein.

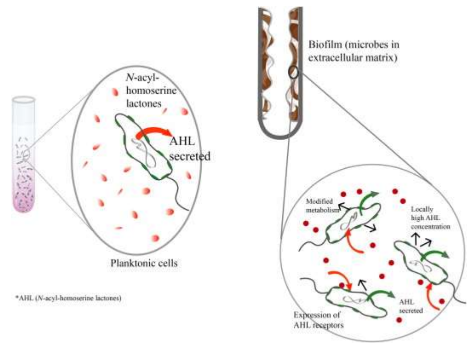


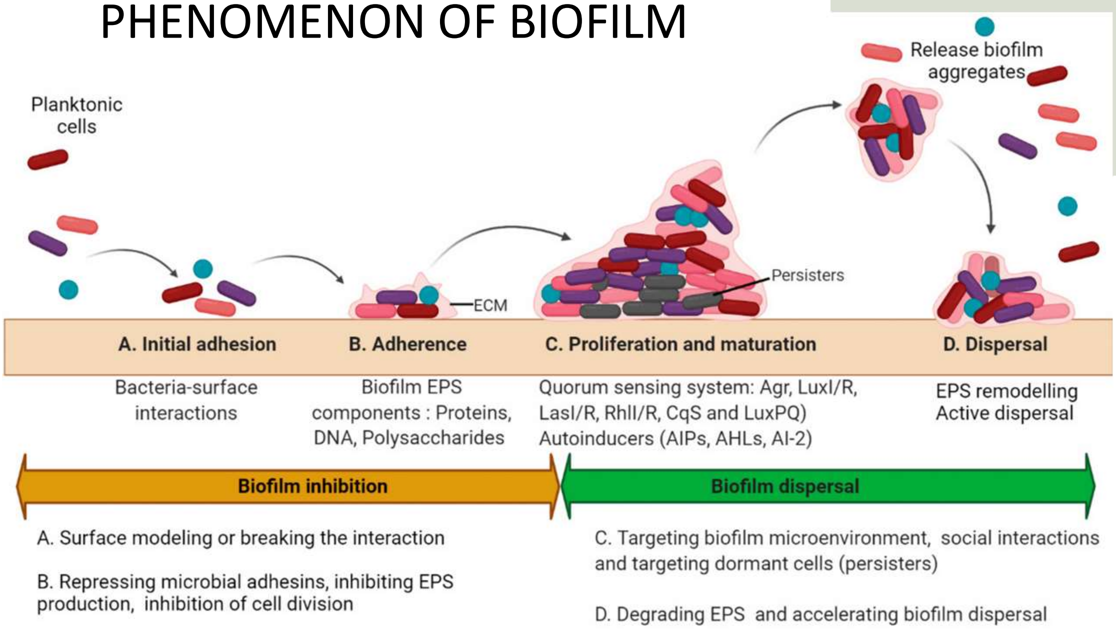
Fig. 5: Role of Acyl homoserine lactone in quorum sensing. (**Source:** Bueno, Juan. "Antimicrobial models in nanotechnology: from the selection to application in the control and treatment of infectious diseases." In *Nanotechnology in diagnosis, treatment and prophylaxis of infectious diseases*, pp. 19-38. Academic Press, 2015.)

BIOFILM CONTROL

BIOFILMS

Biofilms are complex communities of microorganisms, primarily bacteria, that adhere to surfaces and form a protective matrix of extracellular polymeric substances (EPS). These EPS consist of a combination of polysaccharides, proteins, and DNA, which create a slimy and protective environment for the microorganisms within the biofilm. Biofilms are ubiquitous in nature and can be found in various environments, including natural ecosystems, industrial settings, and within the human body.

PHENOMENON OF BIOFILM



Source: https://www.mdpi.com/2076-0817/11/3/292

ADVANTAGES OF BIOFILMS

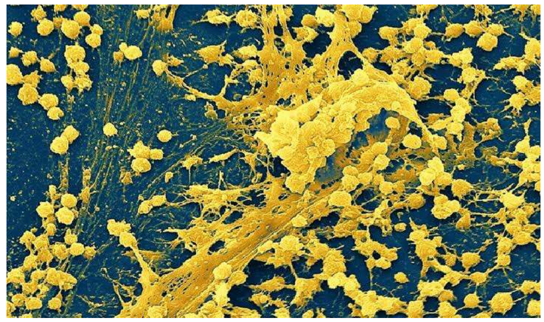
- Biological Symbiosis: In natural ecosystems, biofilms play crucial roles in nutrient cycling and ecosystem stability. They can form beneficial relationships with plants, helping with nutrient uptake and enhancing plant growth
- Wastewater Treatment: In wastewater treatment plants, biofilms are utilized to break down organic matter and pollutants, contributing to the purification of water before it is released back into the environment
- Bioremediation: Biofilms can be engineered to degrade environmental contaminants, such as oil spills or chemical pollutants, making them valuable tools in bioremediation efforts
- Medical Applications: In the medical field, biofilms can be harnessed for beneficial purposes. For example, they are used in the production of certain antibiotics and in the development of probiotics that support a healthy gut microbiome
- Food Production: In food processing, biofilms are involved in the fermentation of various products, including yoghurt, cheese, and beer, contributing to their flavor and texture

DISADVANTAGES OF BIOFILMS

- Infections: One of the most well-known negative aspects of biofilms is their role in causing persistent infections. Biofilm-associated bacteria can be highly resistant to antibiotics and immune system attacks, making infections difficult to treat
- Dental Plaque: Dental plaque is a biofilm that forms on teeth, leading to tooth decay and gum disease if not properly managed through oral hygiene practices
- Industrial Equipment Damage: Biofilms can accumulate on surfaces in industrial settings, such as pipes and machinery, causing corrosion and reducing the efficiency of equipment

CURRENT
METHODS OF BIOFILM CONTROL

- Chemical disinfectants are commonly used to control biofilms.
- Mechanical removal through brushing, scraping, and flushing can be effective.
- Biofilm-resistant materials are being developed to prevent biofilm formation.



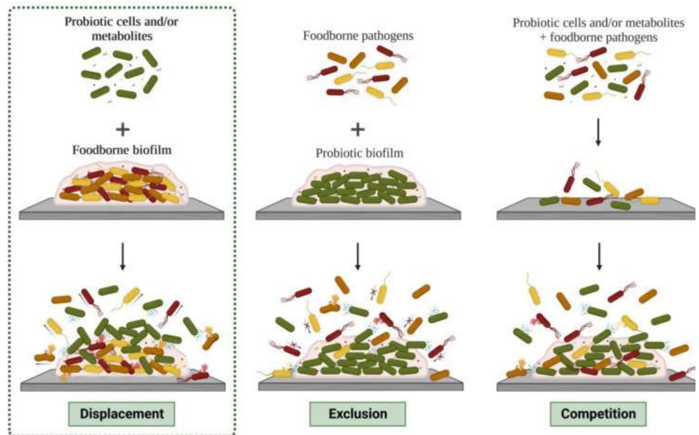
BIOFILM RESISTANT SURFACES

Includes surfaces inherently resistant to biofilm formation or that can be treated with coatings and surface modifications to prevent bacterial attachment and growth

- In the passive strategies (physical modifications), attachment is impeded by controlling surface properties
- Examples include modifying properties like surface hydrophobicity and surface electrostatic charge
- The active strategies (chemical modifications) inhibit biofilm formation by interfering with the proliferation and metabolism of microbes by ion coatings, biocides or antibiotics on the surface
- Example: Chelators act as biofilm inhibitors by hindering metal ions' role in biofilm production
- Silver slats, metallic silver and silver nanoparticles are antibacterial agents that work against E.coli and S. aureus

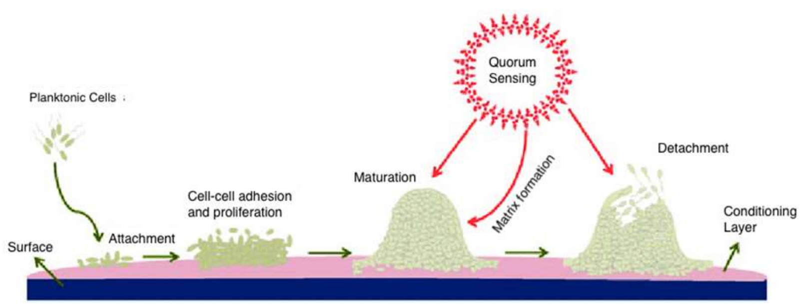
PROBIOTICS

- Some probiotics, like lactic acid bacteria (LAB), can prevent cell attachment and control biofilm formation by pathogens
- This antagonistic activity may be due to competition for nutrients and adhesion sites or the release of antimicrobial metabolites such as bacteriocins, biosurfactants, organic acids, hydrogen peroxide, and inhibitory exopolysaccharides



Source: HTTPs://www.ncbi.nlm.nih.gov/pmc/articles/PMC10135146/#:~:text=Probiotics%20as%20an%20Anti%2DBiofilm,3%2C18%2C32%5D.

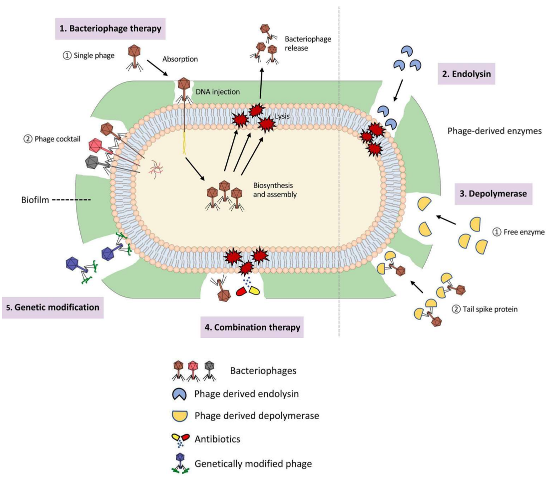
QUOROM SENSING



- Quorum sensing (QS) is a cell-to-cell communication system that regulates many bacterial behaviors, including biofilm formation
- Quorum quenching (QQ) inhibits the production or signaling of QS molecules
- Quorum quenching (QQ) is a promising approach to control biofilm formation and treat biofilm-based infections
- Example: Gram-negative pathogenic bacteria Vibrio cholerae uses quorum sensing for virulence during a cholera infection

Source: https://link.springer.com/chapter/10.1007/978-981-32-9409-7_3

BACTERIOPHAGES



- Bacteriophages are viruses that specifically infect and kill bacteria
- Phages can be used to disrupt and inhibit biofilm matrix
- Phages have several advantages over traditional antibiotics, including:
  - High specificity
  - Self-replicating
  - Easy Production
- Example: Phage T4 can cause infection and replicate within E. coli biofilms, disrupting the biofilm matrix

Source: https://www.frontiersin.org/articles/10.3389/fmicb.2022.825828/full#:~:text=Phage%2Dbased%20treatment%20is%20capable,%2Ddependent%20during%20self%2Dreplication.

CHALLENGES OF BIOFILM CONTROL

- Role of extracellular substances: Microorganisms in biofilms have specialized mechanisms for adhering to surfaces, making it difficult to prevent their initial attachment. Once attached, they produce extracellular polymeric substances (EPS), stabilizing their attachment
- Increased antimicrobial resistance: A mature biofilm's structure and chemical composition provides a barrier that protects embedded cells from antimicrobials. This resistance is partly due to the EPS matrix, which acts as a physical barrier and limits the penetration of antimicrobial agents
- Role of quorum sensing on biofilm formation: Disrupting quorum sensing is a potential control strategy but presents challenges including specificity, resistance, delivery, variability in QS systems, environmental factors, ethics, and regulatory approval

CHALLENGES OF BIOFILM CONTROL

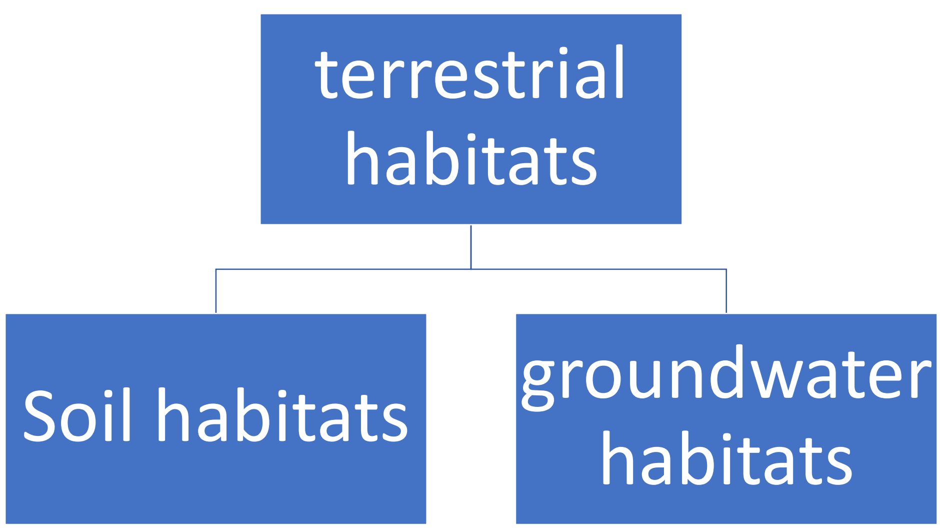
- Biofilm formation in Complex Structures

Research explores biofilm formation in complex environments with varying surface roughness. Mathematical models help understand the impact of surface topography on biofilm development. The spacing between roughness blocks and their height are critical factors influencing biofilm growth. Experimental studies also confirm that surface topography, particularly the depth and size of cavities, plays a significant role in biofilm accumulation.

- Mixed Species Interaction

Mixed-species biofilms are common in natural environments, and understanding inter-species interactions within them is vital for biofilm control. Mathematical models help overcome experimental challenges. One model studied Streptococcus gordonii and Porphyromonas gingivalis biofilm formation, revealing a detrimental effect of S. gordonii on P. gingivalis due to the production of hydrogen peroxide. Another model explored mutualism vs. exploitation in microbial communities, showing that cooperative bacteria can persist under specific conditions, offering insights into biofilm pattern formation.

Terrestrial habitats



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Soil habitats

- For convenience, the soil is divided broadly into two types: mineral soils, ideally developed during weather of rocks (igneous and metamorphic) and organic soils comprised of sedimentary deposits.
- We do not find a clear demarcation of these two distinct soil types in nature. The soils found in nature is a mix of the two broad types.
- The bacterial activity has a distinct effect on the soil. Microbial actions produce organic acids which dissolve the calcium compounds, CO_2 produced during the microbial respiration mix with the soil moisture to form H_2CO_3 which erodes the soil minerals.
- Physico-chemical agents co-weather the soil matrix (freezing and thawing, wind and water erosive action etc.)
- Plant roots also create crevices in rocks and root excretions in the rhizosphere have high microbial abundance.
- Such soil formation continues downwards and eventually builds a soil profile.

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Soil habitats

Factors that affect microbial consortia development in soils

- Nutrients such as nitrogen and phosphorus
- Water availability: Water may exists as thin films in the crevices, absorbed onto the surface.
- Water mixes with minerals and forms a “soil solution”. Soils which are well drained have atmospheric O_2 penetration while soils with poor drainage properties are water flooded.
- Either cases gives rise to very distinct niche suitable for diverse sets of microbes. Even for waterlogged soils, the quick consumption of O_2 changes the aquatic micro-environment from oxic to anoxic/anaerobic zone. This shifts the microbial abundance towards anoxic microbes from aerobic microbes.

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Soil physiology and important soil properties

- The soil as we see it comprises of certain zones:
  - a) Top most soil layer
  - b) Unsaturated or vadose zone
  - c) Saturated zone (aquifers)
- Each of these zones act as a porous media which again can be divided into three phases
  1. Solid phase: contains the inorganic minerals + organic compounds
  2. Liquid phase: Water/solution phase
  3. Gas phase: the trapped gases and gases liberated from physico-chemical and biochemical processes

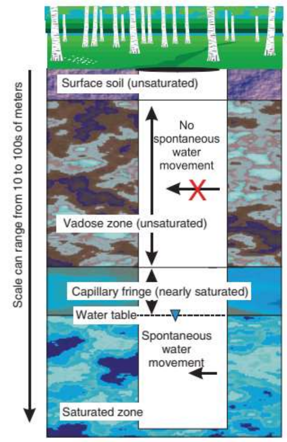

Fig. 5: Cross-section of subsurface.

(**Source:** Pepper , I. L. , Gerba , C. P. , Brusseau , M. L. (2006) Environmental and Pollution Science, 2e. Academic Press, San Diego, CA.)

Soil physiology: Vadose and saturated zones

Vadose zone

- The vadose zone is unsaturated and has majorly unweathered parent rock material.
- Carbon and micronutrient availability is low *(oligotrophic).*
- Thickness of vadose zone varies and is a function of *'n'* number of factors. Primarily, the depth of the strata below the saturated zone (the bedrock) decides how shallow/deep the aquifer is. It is also a function of rainfall received in the region, porosity of the top soil etc.
- In case of arid regions these vadose zones can be several 100 m thick

Saturated zones

- Common names aquifers. Comprised of porous parent material in water saturated conditions.
- **The boundary between vadose zone and saturated zone is known as capillary fringe**
- Shallow, intermediate (<300 m) and deep aquifers (>= 300 m)

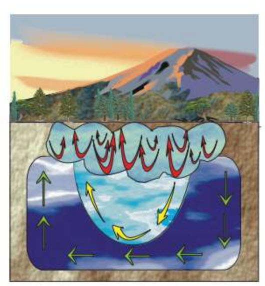

Fig. 6: Types of Aquifer diagrams

(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

Soil physiology: Vadose and saturated zones

Soil composition: Typically 40-50% on volume basis. Among this solid fraction 5% is organic matter.

Main inorganic minerals are Si and O. Soil is further classified into sand (0.05 – 2 mm), silt (0.002- 0.05 mm) or clay (<0.002 mm) based on particle size. Texture of a soil is defined by the percent of the sand, silt and clay present by weight proportions) example: sandy clay or silty sand. Soil particles are aggregated by present polysaccharides, metabolites bind the primary inorganic particles together. Fungal hyphae and plant roots also hold soil together (secondary aggregates).

In between the primary particles and the secondary aggregates there are voids wherein water is present (inter-aggregate pores). There is also intra-aggregate pores.

Water movements through clayey soil is slower than sandy soils even though the overall number of pores are more. This is owing to the extremely small pore size that hinders flow of the soil water solution.

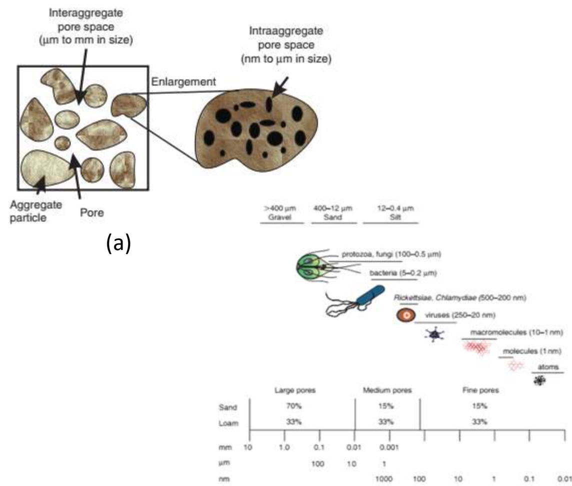

Fig. 6 (a): Types of Aquifer diagrams (b) size comparison of soil particle with organisms

(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

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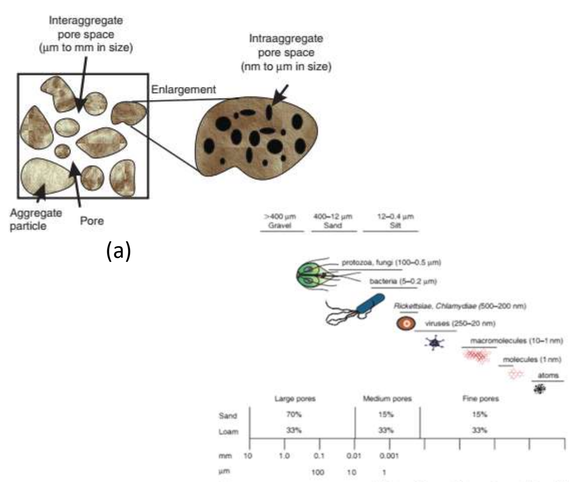

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Soil physiology: different horizons

Soil profile with respect to generation of different horizons

1. Dark organic-rich layer, known as the O horizon,
2. A lighter colored layer, designated as the A horizon, where some humified organic matter accumulates.
3. The third layer is the E horizon because it is characterized by eluviation (process of removal or transport of nutrients and inorganics out of the A horizon).
4. The next zone is B horizon, which is related illuviation. Illuviation is the deposition of the substances from the E horizon into the B horizon.
5. Beneath the B horizon is the C horizon, which contains the parent material from which the soil was derived.
6. The C horizon is generally unweathered parent material and marks the transition between a soil and the vadose zone.

Although this is common to most soils, not all soils contain each of these horizons.

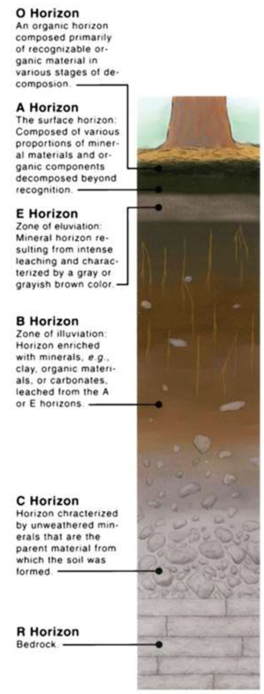

Fig. 7: Soil profiles illustrating soil horizon

(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

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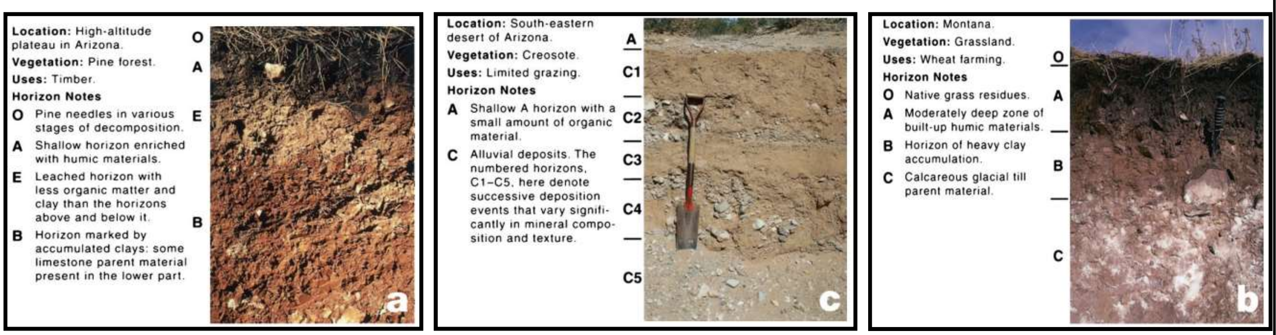
Soil physiology: different horizons



(a) (b) (c)

Fig. 8: Further examples of Soil profiles illustrating soil horizon shown in (a) High altitude (b) Grassland and (c) desert zones

(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

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Soil physico-chemical properties

- Cation exchange capacity: Soils are negatively charged owing to the structure of the clay. The clay contains inorganic lattices of Si and Al oxides
  a) **Isomorphic substitutions:** Substitution of a divalent cation such as Mg^{+2} for a trivalent Al^{+3} cation can result in loss of a positive charge thus leading to over all negative charge (
  b) **Ionization:** Hydroxyl groups at the edge of such soil lattice can ionize thus leading to –ve charge development

$$Al - OH \rightarrow Al - O^{-} + H^{+}$$

- The capacity of the soil to hold on to cations is called the cation exchange capacity (CEC). These cations are held by the negatively charged clay and organic matter particles in the soil through electrostatic forces (negative soil particles attract the positive cations). The cations on the CEC of the soil particles are easily exchangeable with other cations and as a result, they are plant available. Thus, the CEC of a soil represents the total amount of exchangeable cations that the soil can adsorb.
- The cations in the matrix can be replaced by the cations in the soil solution. This is governed by the adsorption affinity.

$$Al^{3+} > Ca^{2+} = Mg^{2+} > K^{+} = NH_4^{+} > Na^{+}$$

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Soil physico-chemical properties

- The CEC affects how the biomolecules move in the soil matrix.
- For example, the positively charged cations which are essential micronutrients can be more bioavailable owning to higher CEC. Also, the higher CEC soils may favor more immobilization of microbes thereby enhancing colony formation (**Though in principal both microbes and soil are –vely charged !!)**
- Soil pH: affects the solubility of the chemicals in the soil (ideally dissociation constant pK and pH are factors affecting ionization of chemicals in soil solution).

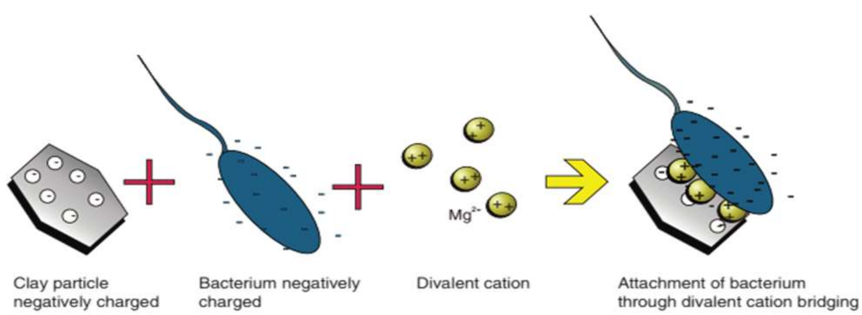

Fig. 9: mechanism of microbe attachment to soil particle
(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

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Soil organic matter

- Soil organic matter consists of
  1) Live biomass, including animals, plants, microbes (bacteria, archaea, fungi., algae,
  2) Dead and decaying biomass
  3) **Humic substances: heterogenous polymers which are formed during the decay of plant animal and microbial biomass.**
- The humic substances serve as slow releasing source of carbon and nutrients for the indigenous slow-growing microbes of the soil (known as autochthonous organisms)
- These are 3-D highly complex organic compounds with a mol. Wt. 700 – 300,000 Da. Has both hydrophilic and hydrophobic tails which can fold in a way to expose the hydrophilic part thus forming an inner hydrophobic layer. This enables humic substances to 'sorb' non-polar compounds from soil.

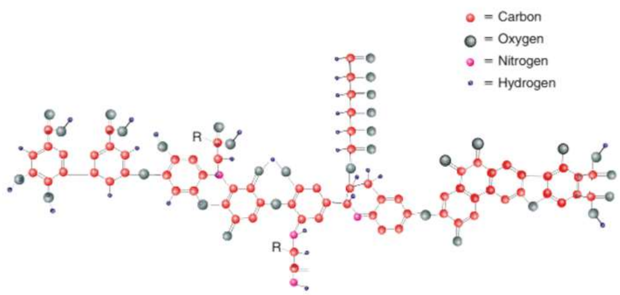

Fig. 10: humic acid structure

(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

Soil water

- Soil water is a constant changing matrix with soil as well as organic matter
- It is often referred to as representation of the soil chemistry as we can trace the activities through the influx and efflux of solutes in water.
- ***Soil water potential (SWP): It is the work done per unit quantity necessary to transfer an infinitesimal amount of water from a specified elevation and pressure to another point in the porous medium.*** It is also expressed as the potential energy per unit volume, mass or weight of soil water with respect to free water (the water on the surface). This is function of gravity and matric forces (cohesive and adhesive forces).
- Stronger SWP may result in presence of pore water against the force of gravity.
- Water is more bioavailable at any SWP above – 0.1 atm because the water becomes more tightly bound by matric and capillary forces

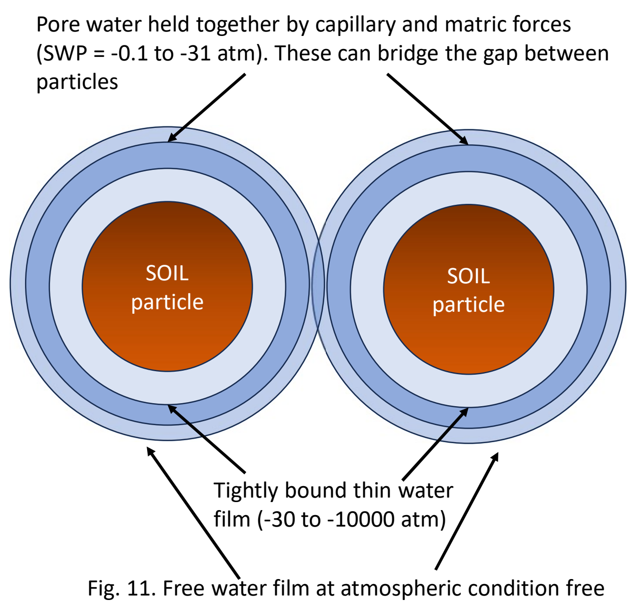

Fig. 11. Free water film at atmospheric condition free to move 0 to -0.5 atm

Soil atmosphere

- Soil atmosphere: Well aerated soils are as good as atmosphere and constituents are nearly same.
- The field capacity is the amount of water remaining in the soil a few days after having been wetted and after free drainage has ceased. The matric potential at this soil moisture condition is around - 1/10 to – 1/3 bar. **This means these soils have both adequate moisture as well as O_2 content to favor aerobic growth**
- *Microenvironments in such soils with field capacity might have some anaerobic growth.*
- Respiration in both aerobic as well as anaerobic zones of soil forms CO_2 which changes the pH and in turn changes the redox conditions in the soil. Such changes can in turn affect the availability of terminal electron acceptors required for respiration.

Microbial communities in the surface soil

- Algae
- Bacteria
- Protozoa
- Fungi
- Archaea
- Viruses or phages (viruses which serve as parasites to the above organisms)
- Additional microbial load due to animal movements, bird droppings etc.

bacteria

- Present in as high numbers as 10^7 to 10^8 cells per gram of soil.
- Anaerobic microbes rarely found in the top surface soil.
- Two types of broad division:
  1. Oligotrophs: slow growing low nutrient requiring micro-organisms surviving on the carbon present/added to the soil through decay of biomass
  2. Copiotrophs: the ones that are present in the soil in dormant -> active -> dormant state following a famine-> feast -> famine substrate condition.
- Oligotrophs and copiotrophs can exist in succession.
- How many types of bacteria may be present in soil?
- *To the tune of thousands types of species*

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bacteria

Abundant culturable bacteria in soil

Organism	Characteristics	Function
Arthrobacter	Heterotrophic, aerobic, gram variable; up to 40% of culturable soil bacteria	Nutrient cycling and biodegradation
Streptomyces	Gram-positive, heterotrophic, aerobic actinomycete; 5–20% of culturable bacteria	Nutrient cycling and biodegradation; antibiotic production, e.g., *Streptomyces scabies*
Pseudomonas	Gram-negative heterotroph, aerobic or facultatively anaerobic; possess wide array of enzyme systems; 10–20% of culturable bacteria	Nutrient cycling and biodegradation, including recalcitrant organics; biocontrol agent
Bacillus	Gram-positive aerobic heterotroph; produce endospores; 2–10% of culturable soil bacteria	Nutrient cycling and biodegradation; biocontrol agent, e.g., *Bacillus thuringiensis*

Examples of autotrophic soil bacteria

Organism	Characteristics	Function
Nitrosomonas	Gram negative, aerobe	Converts $NH_4^+ \rightarrow NO_2^-$ (first step of nitrification)
Nitrobacter	Gram negative, aerobe	Converts $NO_2^- \rightarrow NO_3^-$ (second step of nitrification)
Acidothiobacillus	Gram negative, aerobe	Oxidizes $S \rightarrow SO_4^{2-}$ (sulfur oxidation)
Acidothiobacillus denitrificans	Gram negative, facultative anaerobe	Oxidizes $S \rightarrow SO_4^{2-}$; functions as a denitrifier
Acidothiobacillus ferrooxidans	Gram negative, aerobe	Oxidizes $Fe^{2+} \rightarrow Fe^{3+}$

Examples of heterotrophic soil bacteria

Organism	Characteristics	Function
Actinomycetes, e.g., *Streptomyces*	Gram positive, aerobic, filamentous	Produce geosmins ("earthy odor") and antibiotics
Bacillus	Gram positive, aerobic, spore former	Carbon cycling, production of insecticides and antibiotics
Clostridium	Gram positive, anaerobic, spore former	Carbon cycling (fermentation), toxin production
Methanotrophs, e.g., *Methylosinus*	Gram negative, aerobic	Methane oxidizers that can cometabolize trichloroethene (TCE) using methane monooxygenase
Ralstonia Eutrophus	Gram negative, aerobic	2,4-D degradation via plasmid pJP4
Rhizobium	Gram negative, aerobic	Fixes nitrogen symbiotically with legumes
Frankia	Gram positive, aerobic	Fixes nitrogen symbiotically with nonlegumes
Agrobacterium	Gram negative, aerobic	Important plant pathogen, causes crown gall disease

Actinomycetes

- Actinomycetes colonies are highly filamentous and represent fungal growth as they have a network which looks like the fungal hyphae (though at a much smaller scale).
- Gram positive bacteria
- The actinomycetes can metabolize a variety of substrates similar to bacteria. Ideally these are also gram positive bacterial species which are saprophytes capable of degrading important plant and animal remains. These also have a high guanine and cytosine content in their cells.
- Actinomycetes are important sources for antibiotic production.
- Highly effective for degrading more complex substance such as chitin, cellulose and hemicellulose.

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Fungi

- Fungi numbers range in $10^5 – 10^6$ in number in the soil. Yeast, The anaerobic fungi are found to the tune of 10^3 per gram of soil.
- The fungi play important role by being the first responders towards the decay of dead biomass. This is owing to the higher tolerance of acidic pH as compared to bacteria ().
- Fungi can degrade cellulose and lignin in addition to the other important plant polymers.
- Fungi such as white rot fungi are plant parasite. Common ground fungi genera such as *Penicillium* and *Aspergillus* recycle nutrients to and back from soil.
- Fungal hyphae also contribute to soil formation and soil structure as the network holds on to the soil particle thus reducing soil erosion.

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Comparison

Characteristic	Bacteria	Actinomycetes	Fungi
Numbers	Most numerous	Intermediate	Least numerous
Biomass	Bacteria and actinomycetes have similar biomass		Largest biomass
Degree of branching	Slight	Filamentous, but some fragment to individual cells	Extensive filamentous forms
Aerial mycelium	Absent	Present	Present
Growth in liquid culture	Yes—turbidity	Yes—pellets	Yes—pellets
Growth rate	Exponential	Cubic	Cubic
Cell wall	Murein, teichoic acid, and lipopolysaccharide	Murein, teichoic acid, and lipopolysaccharide	Chitin or cellulose
Complex fruiting bodies	Absent	Simple	Complex
Competitiveness for simple organics	Most competitive	Least competitive	Intermediate
Fix N	Yes	Yes	No
Aerobic	Aerobic, anaerobic	Mostly aerobic	Aerobic except yeast
Moisture stress	Least tolerant	Intermediate	Most tolerant
Optimum pH	6–8	6–8	6–8
Competitive pH	6–8	>8	<5
Competitiveness in soil	All soils	Dominate dry, high-pH soils	Dominate low-pH soils

Algae & protozoa

ALGAE

- Ideally dominates the first 10 cm of any soil. Instances of hemicellulose deeper growth owing to capability to switch towards heterotrophic growth. Density of around 5000 – 10000 cells per g of soil.
- Algae and cyanobacteria are the first set of microbes that can be found in desert, volcanic or any other soils with low/nil organic. Together, these micro-photoautotrophs contribute to the formation of top soil.
- Produces organic biomass through phototrophic pathways. This builds the organic content of the soil. Production of carbonic acid helps in mineralization and rock weathering.
- Algae produces extracellular polysaccharides which adds on to organic matter and provides adhesive action to loose soil particles.
- Examples of algal species in soil: *Chlamydomonas* found in acidic soil, *Botrydiopsis,* found in neutral and alkaline soils, *Porphyridium,* found in nutrient rich soils etc.

PROTOZOA

- Most abundant protozoa in soil is amoeba.
- The protozoa prey on the microbes for their metabolism.
- Number ranges between 30000 to 350000 per g of soil. Instances 1.6×10^6 per g of soil.

Soil habitats: Subsurface rising research field

- Recent investigations have indicated that microbial presence can be found as deep as 3 km below the earth's surface: Crust thickness: 30-70 km (continental crust)
- Typical sub-surface conditions: nutrient depleted, high temperatures and anoxic in nature.'
- Case study: a particular strain identified in fissure water: *Desulforudis audaxviator*. This organism is capable of using H_2 as electron donor and is capable of CO_2 fixation. It also has genes for nitrogen fixation which means it can continue to metabolize using inorganic forms of carbon, nitrogen and H_2 as electron donor. These are ideally chemolithotrophs.
- Doubling time of such subsurface microbes can vary between days to centuries.

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Aquatic habitats

- Study of aquatic environments are important from environmental engineering point of view.
- More than 70% of earth's surface is covered with water. Microorganisms are key component in aquatic environment.
- There are three aquatic environments:
  1. Inland surface water
  2. Seas
  3. Groundwater
- There are four types of habitat possible in these environment
  a) Planktonic
  b) biofilm
  c) Sediment
  d) Microbial mat

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Planktonic growth

- Planktonic growth refers to microbial growth in suspension.
- Phytoplankton: phototrophic eukaryotes (algae) and prokaryotes (cyanobacteria)
- Bacterioplanktons: heterotrophic bacteria
- Zoo planktons: protozoa

Primary production is 50-60 Pg (10^{15} g) of carbon per year. This is 50% primary production on a global scale.

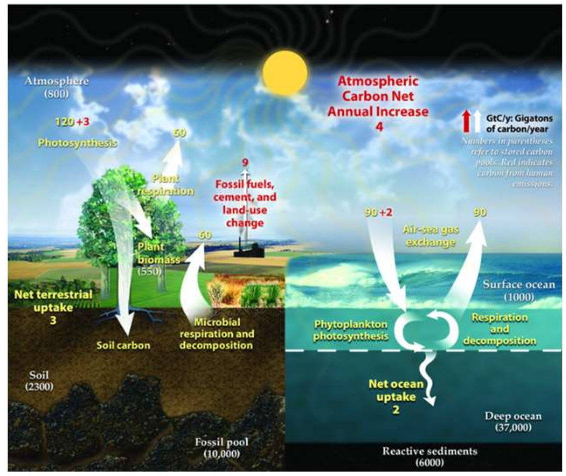

Fig. 12: Planktonic contribution to carbon Picture credit: US Department of Energy

(**Source:** https://owlcation.com/stem/What-are-phytoplankton)

Primary production in planktonic phase

- Primary production in the ocean is 50-60 Pg (10^{15} g) of carbon per year. This is 50% primary production on a global scale.
- Oceans have low primary productivity in terms of microbial biomass present, this is owing to the low nutrient conditions.
- Coastal areas have more nutrients abundance due to outflow from agricultural zones. This creates dead zones in ocean.

Fresh water bodies undergo eutrophication at such high levels of nutrients.

"In shallow marine waters such as marine bays and inlets, nutrient inputs can actually lead to the waters becoming intermittently anoxic from the removal of O_2 by respiration and the production of H_2S by sulfate-reducing bacteria. An extensive region (6000– 7000 square miles) of oxygen depletion in the Gulf of Mexico is associated with high loads of nitrogen and phosphorus carried in by the Mississippi River from agricultural runoff in the Mississippi Valley. This region, called the Gulf of Mexico Dead Zone, contributes to the loss and impairment of fish and benthic sea life that sustain major seafood industries in this region. The Gulf of Mexico experiences other ecological problems as well, as we examine now."

Fig. 13: Dead zones in Gulf of Mexico. Picture credit: Robert Simmon, NASA

(**Source:** https://www.scientificamerican.com/article/ocean-dead-zones/)

Secondary production in planktonic phase

- The secondary production starts with the microfauna.
- Zooplankton eats algae and in turn is eaten by bigger sized organisms.
- *Fun fact: the fish that we consume usually takes 4-5 trophic levels to be produced.*
- the Dissolved organic matter released by primary production is used by the microfauna to produce more organic biomass while a fraction goes mineralizes to CO_2
- DOM is also released by this heterotrophic microbes.
- Another contribution of DOM comes from the dead cells/organisms in the aquatic habitat as well as the excretion of higher organisms

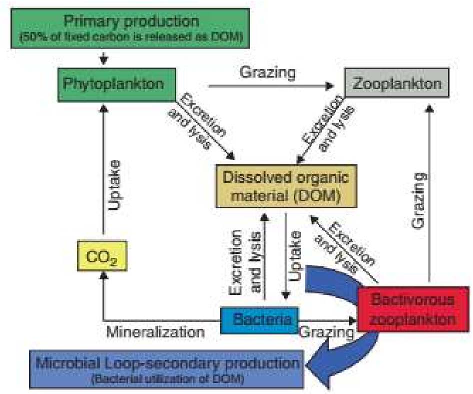

Fig. 14 DOM cycling in different trophic levels

((**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

Benthic zone

- A zone of transition between the water column and the mineral subsurface.
- The zone has settleable organic matter, minerals and water. This is an a ideal concoction for higher microbial growth (to the tune of 5 times).
- Oxygen found in the upper layers as the deeper layers the oxygen depletion is faster. Aerobic → facultative → methanogenic.
- The microenvironmental niches may have alternate oxic and anoxic microorganisms.
- Example: organic acids produced by anaerobes serve as electron donors for strictly anaerobic organisms that produce CH_4. The CH_4 thus produced serves as the food for methanotrophs which utilize the methane in presence of oxygen to produce CO_2.

Microbial mats

- Microbial mats or deep layer sediments can play a vital role in nutrient recycling.
- These mats are extremely rich and dense biofilm layers.
- Photolithotrophic and/or chemolithotrophic growth.
- Such growth in addition to nutrient diffusion from outside the 'mat' region creates a gradient driven diversification in niche development.
- Most abundant mat developers: **Cyanobacteria**
- The thickness of such mats can extend between several millimetres to centimetres
- Abundance in hot geysers, hypersaline lakes, marine estuaries

Fig. 15 Microbial mat collected by NASA scientists from a hypersaline environment

(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

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Cyanobacterial mats

- Cyanobacteria and other phototrophic bacteria are the primary producers.
- The microbial mats are only found in aquatic ecosystems wherein grazers (higher order herbivores).
- The chemical and biological nature changes diurnally. In day photosynthesis enhances the O_2 levels in the photic layer (upper layers) and active sulphate reduction happens in the bottom layers forming H_2S.
- The zone where O_2 and H_2S mix, there can be synergy between the phototrophic and the chemolithotrophic sulphur bacteria which consume the substances rapidly.
- In absence of daylight, the entire mat turns anoxic and H_2S concentration enhances.
- Example: sulphur oxidising bacteria *Chloroflexus* and *Roseiflexus* use H_2S as electron donor

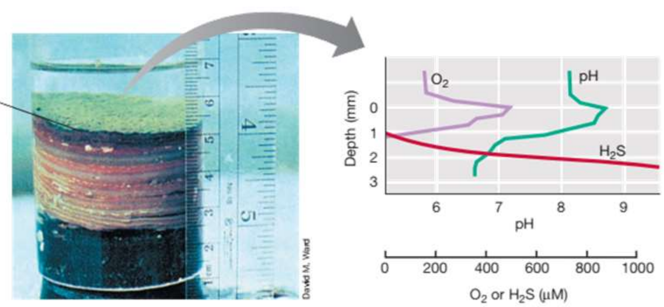

Fig. 16 mat specimen collected from alkaline Yellowstone National Park, USA.

(**Source:** Environmental Microbiology- 2nd edition. R. M. Maier, I. L. Pepper, C. P. Gerba.)

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Aquatic environment: Fresh water environment

- Study of fresh water microbes known as microlimnology
- Freshwater bodies with high primary production rates generate high biomass yield.
- This high yield followed by generation of dead biomass/ consumption of these biomass generated by other microbes which leads to faster O_2 consumption. Such faster O_2 consumption can lead to anaerobic conditions in the water body.
- Apart from the photoautotrophic organisms the chemoautotrophic organisms which use H_2S or elemental sulphur as electron donor can also fix mineralized form of carbon (CO_2) to produce new biomass.
- This biomass generation further adds to the load of oxygen stress as the biomass is utilized as a carbon source by underwater grazers and other zooplanktonic/ microbial mat microbes.
- Annual turnover of lakes due to seasonal water movement produces recycling of nutrients from the bottom surface to the upper layers.

Freshwater seasonal stratification

- The upper zone is called epilimnion, while the micro limnology lower zone is called hypolimnion,
- The middle zone, characterized by a rapid change in temperature is known as thermocline,
- Because water is most dense at 4°C, temperature-induced density stratification occurs at the thermocline in the summer and the winter. In the summer, the epilimnion, which is heated by sunlight, is typically warm and oxygen rich (Fig. 6.10 A). This zone is usually characterized by intensive primary productivity that can deplete the epilimnion of mineral nutrients, resulting in nutrient limiting conditions.
- The characteristics of the hypolimnion are the reverse epilimnion. It has low temperature and oxygen levels, lack of light penetration, and a high mineral nutrient content.
- As colder weather approaches, the warm waters of the epilimnion cool until they reach the temperature, and consequently the density, of the hypolimnion. When this happens the thermocline breaks down and allows mixing of the epilimnion and the hypolimnion.
- In the winter a layer of ice forms at the top of the lake and the epilimnion is formed in the region of 0°C (ice layer) to 4°C (Fig. 6.10 B).
- The hypolimnion remains at 4°C or warmer, and again a thermocline is formed and no mixing occurs. In the spring, as the lake thaws and the two zones reach a similar temperature, mixing occurs once again.
- In essence, the turnover and mixing of these two layers allow reoxygenation of the hypolimnion and replenishment of mineral nutrients in the epilimnion. epilimnion.

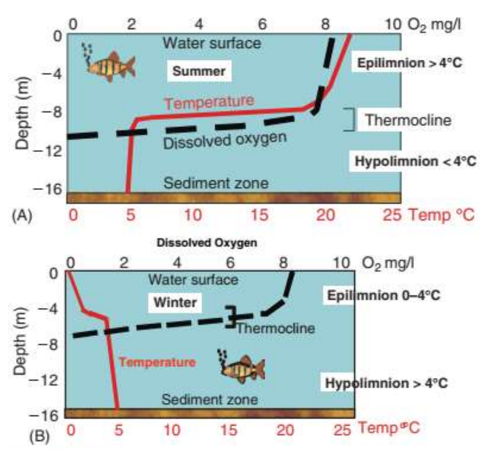

FIGURE 6.10 Idealized profiles of temperature and oxygen in a temperate region, eutrophic lake during the summer (A) and winter (B). Stratification is due to thermal warming of the upper layers in the summer months. Cooling of the upper layer in the fall and early winter breaks the mixing barrier and allows the sediment zone to be reoxygenated. Adapted from Wetzel, 1983.

Case studies

Assignment time:

- Group 3: Make a presentation on the deep water horizon catastrophe
- Group 4: Make a presentation on the occurrence of the oxygen minimum zones

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