Secondary succession: When succession occurs in a habitat with a previous colonization and succession history, it is called secondary succession. It is the consequence of some catastrophic event that has disrupted and altered the course of primary succession.

Preemptive colonization: It occurs by secondary invaders, when pioneers alter the condition of the habitat. They may extend the regions of pioneers or replace them.

Climax community: After the invasion of secondary invaders, habitat undergoes additional changes and succession ends with a stable assemblage of populations called the climax community.

Autotrophic succession: When gross production (P) exceeds the rate of community respiration (R), organic matter accumulates. (In many ecosystems, production is equivalent to photosynthesis, but in some, such as deep - sea hydrothermal vent communities, production is the result of chemolithotrophic metabolism).

Autotrophic succession occurs in cases where $\frac{P}{R}$ is initially greater than 1. As long as P is greater than R, biomass will accumulate during the autotrophic succession. As the $\frac{P}{R}$ ratio approaches 1, succession toward a stable community is occurring.

Example: Terrestrial cyanobacteria, Lichens.

Properties:

1. An autotrophic succession of microorganisms occurs in environments largely devoid of organic matter when there is a non-limiting supply of solar energy.
2. Autotrophic succession occurs in young pioneer communities, such as on newly exposed volcanic rock.
3. In autotrophic succession within a mineral environment, such as on bare rock, the photosynthetic pioneer organisms have minimal nutritional requirements and high tolerance to adverse environmental conditions.
4. The ability to use atmospheric nitrogen is an advantage of this type of succession. Terrestrial cyanobacteria and lichens are good examples of pioneers in this type of environment.

Heterotrophic succession: Heterotrophic succession occurs in cases where $\frac{P}{R}$ is less than 1, because consumption is greater than production. Succession in such a situation is called heterotrophic succession.

Example: Gut community.

Characteristics:

1. In heterotrophic succession, the energy flow through the system decreases with time, there is insufficient organic matter input, and the community gradually uses its stored chemical energy.
2. Heterotrophic succession is usually temporary, because it culminates in the extinction in the community when the stored energy supply is exhausted.
3. Many microbial communities involved in decompositional processes exhibit such temporary heterotrophic succession. For example, the microbial communities on a fallen log disappear after the log is completely decomposed.
4. It is possible for heterotrophic succession to lead to a stable community if there is a continuous source of allochthonous organic matter (i.e. organic matter from an external source).
5. Pioneers in a heterotrophic succession need to have above all, high metabolic and growth rates in order to stay ahead of secondary invaders.

Differences between autotrophic and heterotrophic succession:

Autotrophic succession	Heterotrophic succession
1. When gross production (P) exceeds the rate of community respiration (R) and succession occurs, it is called autotrophic succession.	1. When rate of community respiration (R) exceeds gross production (P) and succession occurs, it is called heterotrophic succession.
2. Autotrophic succession occurs in cases where $\frac{P}{R} > 1$.	2. Heterotrophic succession occurs in cases where $\frac{P}{R} < 1$.
3. It occurs in environments when there is a non-limiting supply of solar energy.	3. It occurs in environments when there is a limited supply of solar energy.
4. It is usually not temporary.	4. It is usually temporary.

- Highly efficient DNA repair mechanisms of some bacteria make them extremely radiation resistant like *Deinococcus radiodurans*.

Non-ionizing radiation: Ultraviolet light basically causes non-ionizing radiation. Solar radiation includes UV light radiation, visible light radiation and infrared radiation. The wavelength of UV radiation is 100-380 nm, but the most germicidal wavelength is 260 nm, which is also the absorption maximum of DNA.

Damages caused by non-ionizing radiation: High frequency radiation can produce changes in-

- Growth and virulence of bacteria
- Inactivation of replication mechanism
- Changes in chromosomes in the process of cell division
- DNA mutation and even damage that can cause cell death.

Adaptation mechanism:

- **Slow repair:** UV-damaged DNA can be repaired enzymatically by excision of the damaged portions, but this process requires time. The survival of the UV damaged cells can be enhanced by conditions that are adverse to rapid growth; optimal growth conditions allow for rapid expression of the UV damage with resulting cell death.
- **High visible light:** Visible light has a reactivation effect on UV-damaged cells. Part of the effect may be photochemical; blue light tends to break the UV induced thymine dimers. In addition, visible light triggers a DNA repair mechanism that is, paradoxically, not triggered by the more damaging UV radiation. So microbes exposed to UV irradiation along with high visible light intensities survive.

Visible light radiation:

Wavelength: 320 nm (violet)- 800 nm (red)

Quality of light in a habitat depends on-

- Obstructions
- Absorption
- Scattering

together called extinction

Extinction is high in ecosystem with suspended particles.

Effect of light:

1. Intensity of light influences the photosynthetic rate. Blue light penetrates further than red.
2. Some microbes show phototactic behavior, by moving towards or away from a light source. Buoyancy regulation is important in planktonic cyanobacteria.
3. Light also affects circadian rhythms in some eukaryotic microbes.

Thus light affects the survival, growth and behavior of the microbes.

Note: Circadian rhythm: Noting or pertaining to rhythmic biological cycles recurring at approximately 24-hour intervals.

Habitats:

Surface ecosystems – such as tops of leaves, ocean surface, rocks and soil surface, animal surface, airborne particles, droplets in clouds, top of snow, ice, brines with crystalline salts.

Pressure

Pressure is of three types:

1. Atmospheric pressure
2. Hydrostatic pressure and

3. Osmotolerant and osmophilic microorganisms have the capability of sensing the changes in outside osmotic pressure and reacting by rapidly degrading or polymerizing their intracellular solutes to preserve their osmotic balance.

Classification of microbes living in hypertonic habitat:

- **Osmotolerant:** They tolerate high concentration of solute (usually sugar). Example: Aspergillus, Penecillium.
- **Osmophile:** They prefer high concentration of solute (usually sugar). Example: Zygosaccharomyces rouxii.

These may cause spoilage of food preserves, syrups.

Salinity

Habitat: Salt - lake, salt spring.

Halotolerant: They tolerate high salt concentration. Example: Halobacter, Halococcus.

Challenges faced by non-halotolerant:

1. Cells dehydrate in hypertonic salt environment.
2. High salt concentration denature tertiary protein structure which is essential for enzyme activity.

Adaptation mechanisms:

1. Exclude high and toxic Na^+.
2. Build up high intracellular glycerol concentration for osmotic balance.
3. The obligate halophile, Halobacterium achieves osmotic balance with high intracellular potassium chloride (KCl). Their ribosomes need high K^+ for stability, their cell walls need high Na^+ for stability.
4. Some have bacteriorhodopsin bilayer cell membrane, which pumps out H^+ in exchange of K^+/Na^+.

Water activity

The amount of water actually available for microbial use can be expressed as the water activity (a_w).

Water is vital for growth and survival. Water activity, the index of the water actually available for microbial use, depends on the number of moles of water, the number of moles of solute, and the activity coefficients water and the particular solute. Water activity can be decreased not only by solutes (osmotic forces) but also by absorption to solid surfaces (matric forces). By definition, a_w of free distilled water is 1.0. Osmotic and metric forces usually lower a_w to some fraction of this value. Most microorganisms require a_w values above 0.96 for active metabolism, but some filamentous fungi and lichens are capable of growth at a_w values as low as 0.60. Such microorganisms are described as xerotolerant.

Xerotolerant: Xerotolerant means tolerant of dry condition. They tolerate low availability of water. They exhibit greater tolerance to desiccation.

Habitat: Atmosphere, desert soil.

Challenges faced by non-xerotolerant microbes:

1. Denature protein (hydration is necessary for tertiary protein structure).
2. Fragment nucleic acid.
3. Induce lethal mutation.

Adaptation mechanisms to survive in low water activity environment:

1. Undergoes dormant stage such as production of spores.
2. Synthesis of non-reducing sugars trehalose and sucrose. Upon desiccation these sugars form a non-crystalline glass or gum phase, that is hydrogen bonded to the protein. Thus denaturation of protein is prevented.
3. Production of gelatinous exopolysaccharide in the form of sheath, capsule and slime as desiccation protectant.

4. Rock inhabiting (lithobiontic) microbes colonize –
    - **Rock surface:** Microbes that colonize rock surfaces or rock fabric adjacent to the surfaces include lichens, which are particularly resistant to desiccation. They tend to be in moisture equilibrium with the surrounding environment.
    - **Cracks and fissures within rocks:** Cracks and fissures provide protection against desiccation and irradiation.
    - **Rock matrix:** The uppermost 1 – 3 mm of rock is microbe – free, next few mm is colonized by microbes, known as endolithic microbes. They are found both in hot deserts and cold deserts such as permafrost of Antarctica. In hot deserts, the only endolithic microbes are cyanobacteria. They are primary producers and are capable of switching their metabolic activities on and off in response to rapid changes in environmental conditions (early morning after dewfall 'on' and late morning to noon 'off').
      In cold deserts, polar endolithic communities include lichen, fungi and algae association, but are found as segregated bands within rock matrix (normal lichens are integrated, thalliod). They also lack plectenchyma, their loose filaments and cell clusters grow between rock crystals. Endolithic lichen activity results in the mobilization of iron compounds in the rock and exfoliative weathering pattern, causing a general erosion of the rock over geologic time periods. Their growth rate is also very slow. One study shows their turnover time around 20,000 years.

Movement

The movement of air and water aids the passive dispersal of microorganisms. Of equal importance is the role of movement in importing and distributing nutrients for microbial growth and in removing metabolic by – products. Factors such as solubility, diffusion, viscosity, specific gravity, porosity and the flow characteristics of the ecosystem control the movement of materials in and out of ecosystems.

Some ecosystems are characterized by extensive flow, such as in rivers, or by turbulence, such as in oceans. Other ecosystems are generally quiescent, such as ponds, or static, such as soil. Ecosystems with extensive flow or turbulence have greater mixing capacity. Materials are moved into and out of such ecosystems in part by the movement or mixing of the ecosystem. Even in quiescent and static systems, thermal convection, evapotranspiration and leaching move materials.

Diffusion

In ecosystems that lack extensive mixing, materials may move by diffusion. Diffusion results in a spreading out of a substance from its source, lowering concentration. Diffusion of materials through ecosystem depends in part of temperature. The molecular weight of the solute and the viscosity (flow characteristics) of the solvent determine in part the ability of a solution to diffuse through an ecosystem. Diffusion is often augmented by mixing due to differences in specific gravity and by thermal convection. Gaseous molecules with low specific gravities, for example tend to rise, generally moving upward through aquatic ecosystems. In the atmosphere, diffusion of gases is augmented by convection along temperature gradients, with warm gases rising and cooler gases descending through the atmosphere. The movement of gases along thermal gradients results in turbulence and mixing.

In terrestrial ecosystems, diffusion of materials is a function of porosity. Porosity refers to the number and volume of pores in a soil or sediment particle matrix. The pores, sometimes referred to as interstitial spaces, may be filled with liquid or gases. Diffusion of materials occur through the pores, and exchange rates between material in interstitial spaces and external sources affect diffusion rates and the availability of materials essential for microbial growth and activity.

1. Plate count procedures employ various media and incubation conditions. Agar is most often used as the solidifying agent because most bacteria lack the enzymes necessary for depolymerizing agar.
2. **Surface spread method:** In this method, dilutions of samples are spread on the top of the agar.
3. **Pour plate method:** In this method, the sample suspension is mixed with the agar just before the plates are poured.
4. **Roll tube method:** It is used for enumeration of obligate anaerobes, is an extension of the pour plate method. The tubes are incubated under specified conditions for a period to allow the bacteria to multiply and form microscopic colonies, after which the colonies are counted. It is assumed that each colony originated from a single bacterial cell. For this assumption to be valid, the bacteria must be widely dispersed in or on the agar. Tubes with too many colonies cannot be counted accurately because one colony may represent more than one original bacterium. Tubes with too few colonies also must be discarded from the counting procedure for statistical reasons.
5. Eosin methylene blue agar (EMB) and MacConkey's agar media are widely used to determine water quality. These media are both selective and differential. The select for the growth of Gram negative bacteria by incorporating an inhibitor of Gram positive bacteria. They differentiate bacteria capable of utilizing lactose by formation of characteristically colored colonies. On EMB ager, coliform bacteria, which are Gram negative and utilize lactose, form a characteristic colony with a green metallic sheen. Estimates of coliform counts determined in this manner are often used as an indicator of water quality and for quality control in the food industry.

- **Most probable number:** The most probable number (MPN) method, an alternative to plate count methods for determination of viable organisms, uses statistical analyses and successive dilution of the sample to reach a point of extinction.
- **Colony hybridization:** Colony hybridization is an application of nucleic acid hybridization that is combined with conventional environmental microbiological sampling and viable plating procedures. Following initial growth, bacterial colonies or phage plaques are transferred from the surfaces of the cultivation media to hybridization filters. The colonies or phage containing plaques are then lysed by alkaline or enzymatic treatment, after which hybridization is conducted. These methods depend on the ability of the target microorganisms to grow on the primary isolation medium and not to be totally overgrown by non-target populations. Growth on the isolation medium increases the number of copies of the target gene to a level detectable by a gene probe.

Determination of microbial biomass: Biomass is an important ecological parameter because, among other things, it represents the quantity of energy being stored in a particular segment of the biological community. Measurement of biomass is used to determine the standing crop of a population and the transfer of energy between trophic levels within an ecosystem. Biomass literally means 'mass of living material' and can be expressed in the unit of weight (grmas) or units of energy (calories or jules). Unfortunately, the direct measurements of microbial biomass, such as by filtration and dry weight or by centrifugation and packed cell volume measurements as practiced or pure cultures, are rarely applicable to environmental samples. These techniques tend to measure mineral and detritus particles and non-microbial biomass along with microorganisms and fail to discriminate between trophic levels, that is, between producers and consumers. Consequently, the determination of microbial biomass is often imprecise.

The techniques of determination of microbial biomass are given below:

1. **Biochemical Assays:** The most practical approach to the determination of microbial biomass is to assay for specific biochemicals that indicate the presence of microorganisms. Ideally, all the microbial biomass to be determined should have the same quantity of the biochemical being assayed, so that there is a direct correlation between the amount of the biochemical measured and the biomass of microorganisms. Also, the biochemical being assayed should be present only in the biomass to be determined. These two conditions are rarely, if ever, met, so the results of quantifying a particular biochemical must be extrapolated with caution in order to estimate the biomass of microorganisms that are present.
2. **ATP and Total Adenylate Nucleotides:** Measurements of ATP can be used to estimate microbial biomass. A factor of 250 – 286 is often used for conversion of ATP to cellular carbon for aquatic samples.

6. It is generally harmless to humans and other animals.
7. It is present in greater numbers than those of pathogens (making detection relatively easy).
8. It is easily detected by standard laboratory techniques.

Reasons for not taking common pathogens in routine microbiological examination of water:

1. Pathogens enter a water supply sporadically and they do not survive for long period of time. As a result, they could be missed in sample submitted to the laboratory.
2. If they are present in very small number, they might be escaped during detection procedure.

Number of indicator bacteria commonly found in human feces:

Indicator organisms	Cells per gram of feces (wet weight)
• Bacteroides spp.	$10^7 – 10^{11}$
• Bifidobacterium spp.	$10^7 – 10^{11}$
• *Clostridium perfringens*	$10^3 – 10^{10}$
Coliforms	
• Fecal	$10^6 – 10^9$
• Non-fecal	$10^7 – 10^9$
• Fecal streptococci	$10^5 – 10^8$

Classification of indicator organism:

Major:

1. Coliforms: 2 groups- Total coliform (TC) and Fecal coliform (FC).
2. Fecal streptococci (FS)

Minor:

- *Clostridium perfringens*
- *Pseudomonas aeruginosa*
- Staphylococci

1. Coliform as indicator organism:

There are two principle group of coliform bacteria:

Total coliform	Fecal coliform or Thermotolerant coliform
By definition, the total coliform (TC) group comprises all aerobic and facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria that ferments lactose and forms acid within 48 hours at 35^0 C.	Fecal coliforms are defined as those coliforms which respond at an elevated temperature of 44.5^0 C. Thus a more accurate name for organisms which show positive result on the FC test would be heat tolerant coliform.
Commonly found in feces, also occur naturally in unpolluted soils and waters.	Exclusively fecal in origin.
Ferment lactose with the production of acid and gas within 24-48 hours at 35^0 C.	Ferment lactose with the production of acid and gas within 24-48 hours at 44.5 ± 0.2^0 C.

Standard value of TC and FC in drinking water recommended by various authorities

- 0 – 10 total coliform cfu/100 ml of drinking water
- <1 fecal coliform cfu/100 ml of drinking water

Membrane filtration method → From note

Adaptation mechanism in low water activity

The amount of water actually available for microbial use can be expressed as the water activity (a_w).

Challenges faced by non-xerotolerant microbes:

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Ionizing radiation

Ionizing radiation: Radiations are designated as ionizing radiations if their interactions with matter produces unstable ions and free radicals that interact with living matter in a destructive manner e.g., γ rays and X rays.

Damages caused by ionizing radiation:

3. Low level radiation causes mutation.
4. High level exposure causes extensive DNA fragmentation and destroy the nucleic acid and enzymes. As a result, the microbes are killed.

Main challenges:

- Ionizing radiations are highly penetrating.
- Low level radiation causes mutation.
- High level exposure causes extensive DNA fragmentation and destroy the nucleic acid and enzymes. As a result, the microbes are killed.

Adaptive mechanisms:

- Bacterial endospores are highly resistant to gamma radiation; it takes .3-.4 million rads (Mrads) to effect a 90% kill, whereas one tenth of this dose effects the same percentage kill in most vegetative bacteria.