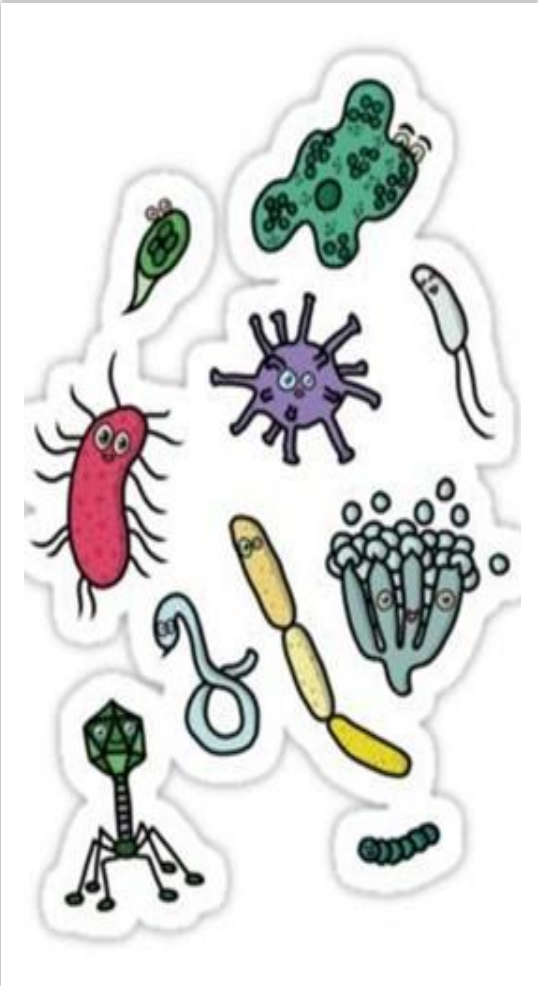
Microbe Symbiotic Relationship Book

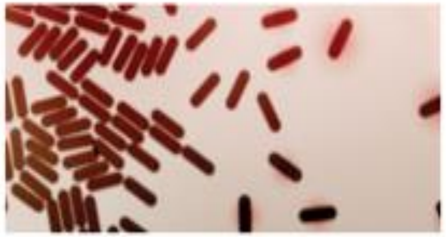
(2nd Microbiology 2023)



WHAT IS MICROBIOLOGY?

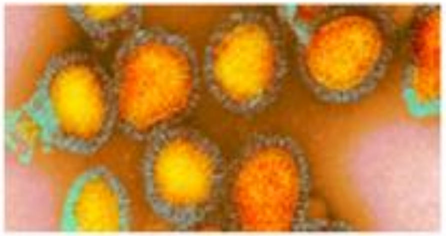
- Micro-organisms and their activities are vitally important to all processes on Earth.
- Micro-organisms matter because they affect every aspect of our lives – they are in us, on us and around us.
- Microbiology is the study of all living organisms that are too small to be visible with the naked eye.
- This includes bacteria, archaea, viruses, fungi, prions, protozoa and algae, collectively known as 'microbes'.
- These microbes play key roles in nutrient cycling, biodegradation, climate change, food spoilage, the cause and control of disease, and biotechnology.
- Microbes can be put to work in many ways: making life-saving drugs, the manufacture of biofuels, cleaning up pollution, and producing/processing food and drink.

Introducing microbes



Bacteria

More than just pathogens - can be friend or foe.

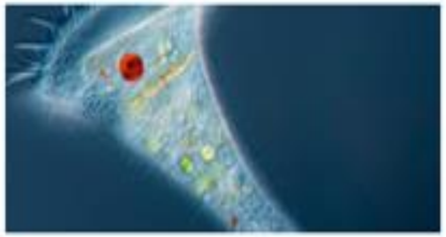


Viruses

Smallest of all the microbes, but are they alive?

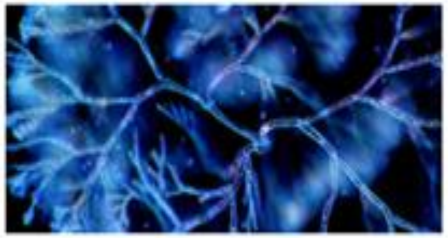
Fungi

More than just mushrooms.



Protozoa

Microbes with a taste for poo and so much more.



Algae

Microbial powerhouses essential for life.

Archaea

First found existing on the edge of life.

Microbes in the world

Microbes and the human body

Ever wondered why when we are surrounded by microbes we are not ill all the time?

Microbes and food

Food for thought – bread, chocolate, yoghurt, blue cheese and tofu are all made using microbes.

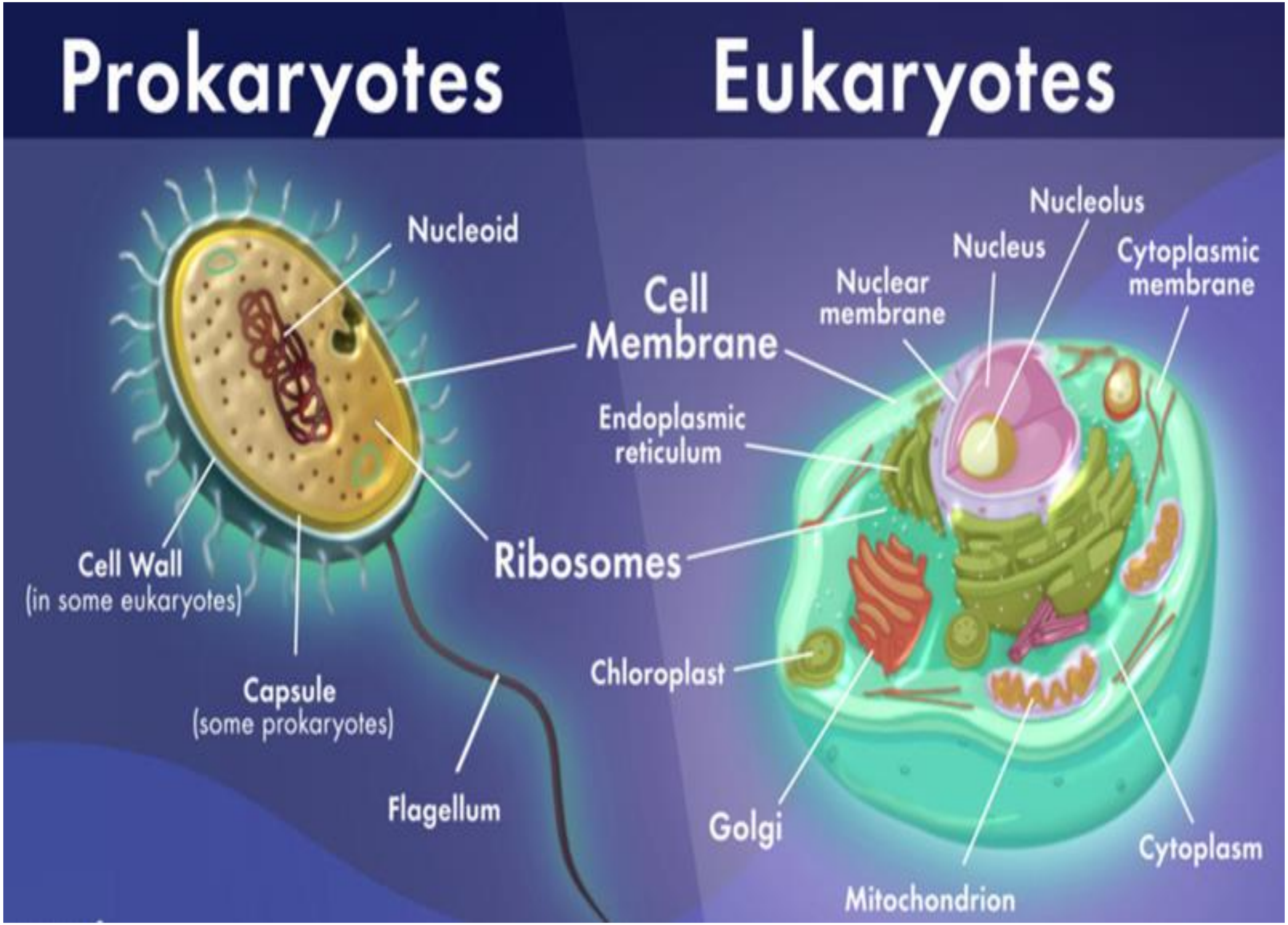
Microbes and the outdoors

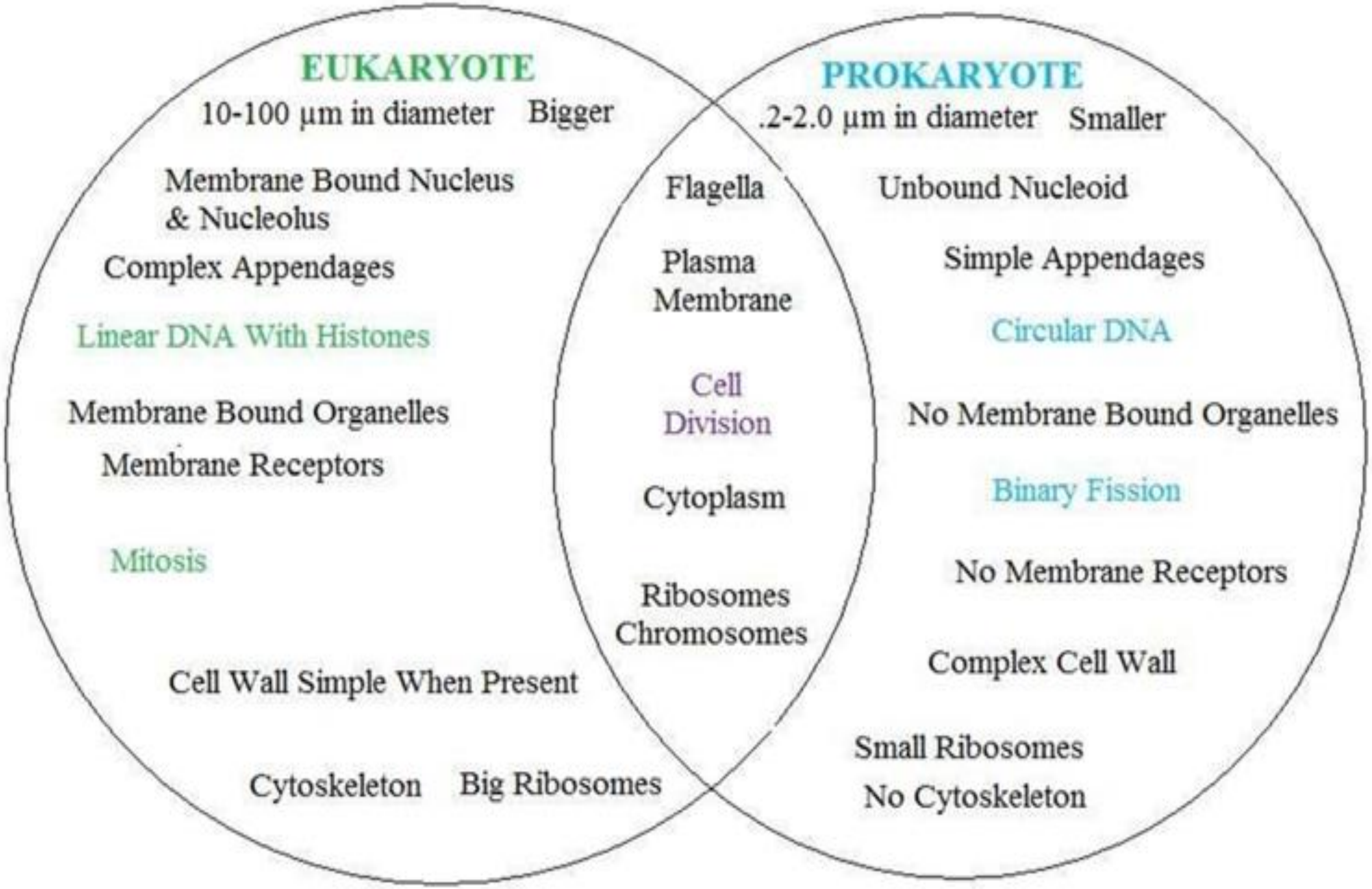
The function of microbes as tiny chemical processors is to keep the life cycles of the planet turning.

Microbes and climate change

How are microbes contributing to climate change?

- Microbiology (from Greek mīkros, "small", bios, "life"; and - logia) is the scientific study of microorganisms.
- Those being unicellular (single cell), multicellular (cell colony), or acellular (lacking cells).
- Microbiology including virology, bacteriology, protistology, mycology, immunology and parasitology.


Prokaryotes
Eukaryotes
Nucleoid
Cell Membrane
Cell Wall
(in some eukaryotes)
Capsule
(some prokaryotes)
Flagellum
Ribosomes
Nucleolus
Nucleus
Cytoplasmic membrane
Nuclear membrane
Endoplasmic reticulum
Chloroplast
Golgi
Mitochondrion
Cytoplasm


EUKARYOTE
10-100 µm in diameter Bigger
Membrane Bound Nucleus & Nucleolus
Complex Appendages
Linear DNA With Histones
Membrane Bound Organelles
Membrane Receptors
Mitosis
Cell Wall Simple When Present
Cytoskeleton Big Ribosomes
Flagella
Plasma Membrane
Cell Division
Cytoplasm
Ribosomes Chromosomes
PROKARYOTE
.2-2.0 µm in diameter Smaller
Unbound Nucleoid
Simple Appendages
Circular DNA
No Membrane Bound Organelles
Binary Fission
No Membrane Receptors
Complex Cell Wall
Small Ribosomes
No Cytoskeleton

- Historical background

- Microbiology essentially began with the development of the microscope.
- Although others may have seen microbes before him, it was Antonie van Leeuwenhoek, who was the first to provide proper documentation of his observations.
- His findings in a series of letters to the British Royal Society during the mid-1670s.
- Although his observations stimulated much interest, no one made a serious attempt either to repeat or to extend them.
- It was only later, during the 18th-century about whether life could develop.

◦ Microbes and disease

- an Italian scholar, advanced an infection that passes from one thing to another.
- A description is passed along eluded discovery until the late 1800s, when the work of many scientists, Pasteur foremost among them, determined the role of bacteria in fermentation and disease.
- Robert Koch, a German scholar, defined that a specific organism causes a specific disease.

- The period from about 1880 to 1900. Students of Pasteur, discovered a host of bacteria capable of causing specific diseases (pathogens).

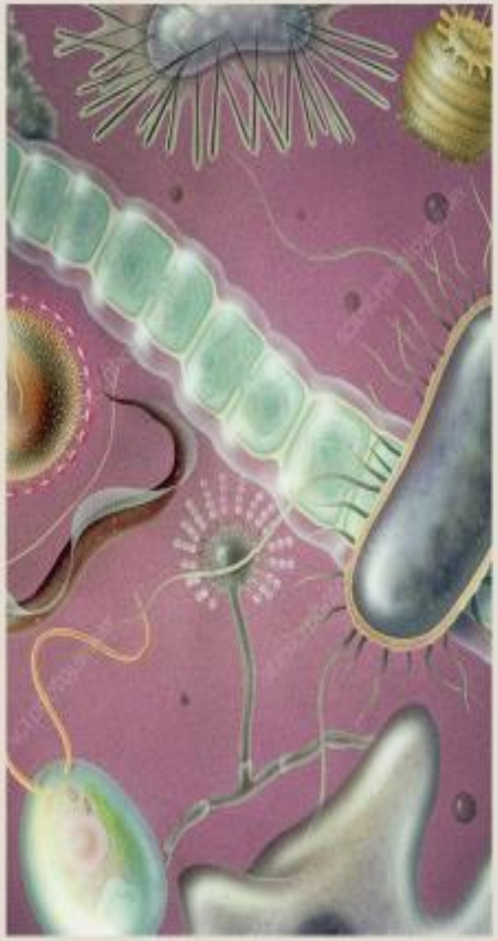
All of these developments occurred in Europe. Not until the early 1900s did microbiology become established in America.

Many microbiologists who worked in America at this time had studied microbiology or at the Pasteur Institute in Paris.

Once established in America, microbiology especially with regard to such related as biochemistry and genetics.

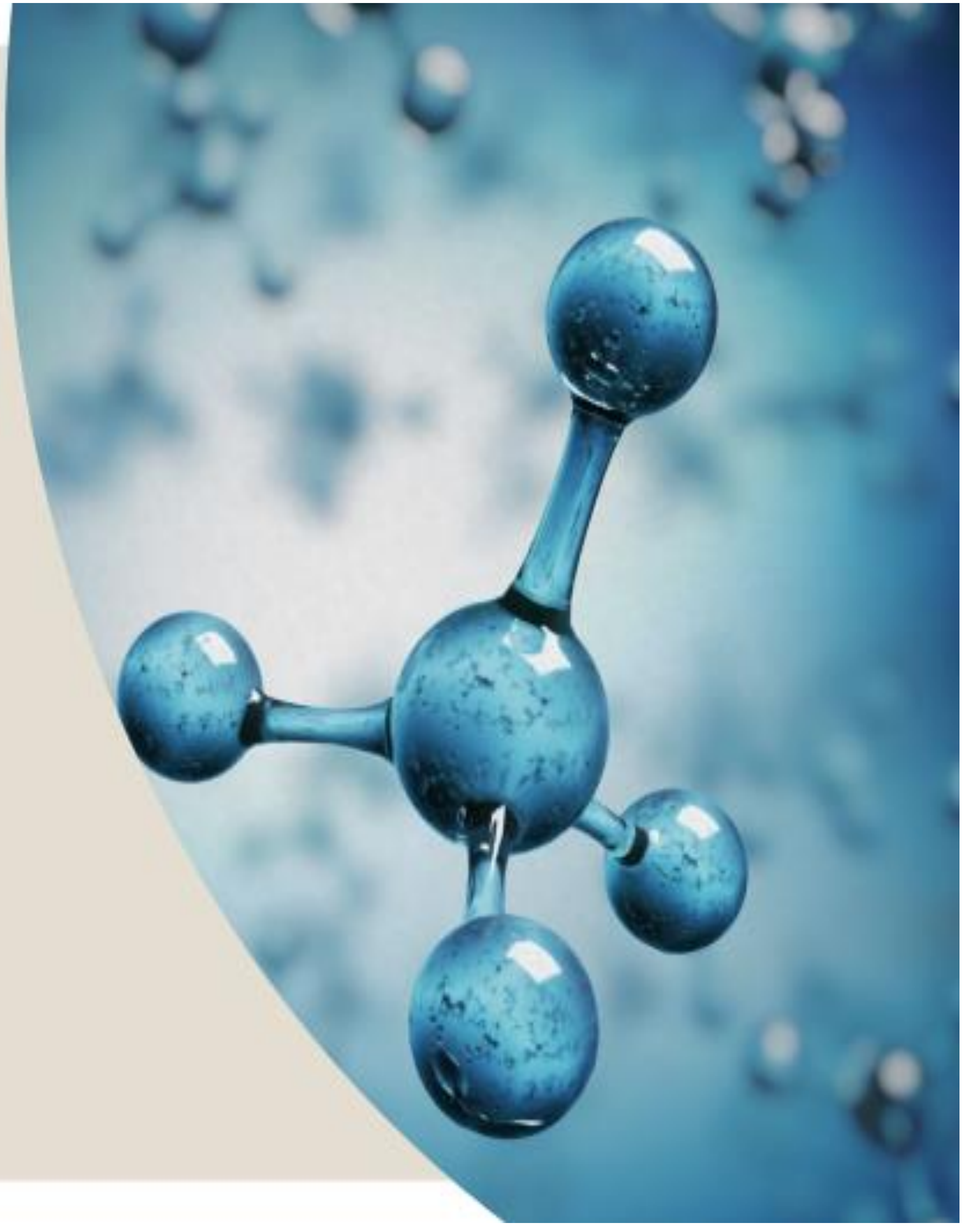
In 1923 American bacteriologist David Bergey established that science's primary reference, updated editions of which continue to be used today.

- The study of microorganisms has also advanced the knowledge of all living things.
- Microbes are easy to work with and provide a simple studying the complex processes of life; as such they have become a powerful tool for studies in genetics and metabolism at the molecular level.
- Knowledge of the basic metabolism and nutritional requirements of a pathogen, for example, often leads to a means of controlling disease or infection.



- **The six main physical factors affecting the growth of microorganisms. The factors are:**
  - 1. Water acidity
  - 2. Temperature
  - 3. pH
  - 4. Oxygen requirements
  - 5. Pressure
  - 6. Radiation.

- **Control of microorganisms is essential in order to:**
  - Prevent the transmission of diseases and infection.
  - Stop decomposition and spoilage and prevent unwanted microbial contamination.



Microorganisms are controlled by means of physical agents and chemical agents.

- Control by physical agents include:
  - high or low temperature.
  - Desiccation.
  - Osmotic pressure.
  - Radiation.
  - Filtration.
- Control by chemical agents refers to the use of:
  - Disinfectants.
  - Antiseptics.
  - Antibiotics.
  - Chemotherapeutic antimicrobial chemicals.

Basic terms used in discussing the control of microorganisms include:

1. Sterilization:

- Sterilization is the process of destroying all living organisms and viruses. A sterile object is one free of all life forms, including bacterial endospores, as well as viruses.

2. Disinfection:

- Disinfection is the elimination of microorganisms, but not necessarily endospores, from inanimate objects or surfaces.

3. Decontamination:

- Decontamination is the treatment of an object or inanimate surface to make it safe to handle.

4. Disinfectant:

- A disinfectant is an agent used to disinfect inanimate objects but generally too toxic to use on human tissues.

5. **Antiseptic:**

An antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on human tissue.

6. **Sanitizer:**

A sanitizer is an agent that reduces microbial numbers to a safe level.

7. **Antibiotic:**

An antibiotic is a metabolic product produced by one microorganism that inhibits or kills other microorganisms.

8. **Chemotherapeutic synthetic drugs:**

Synthetic chemicals that can be used therapeutically.

9. **Cidal:**

An agent that is cidal in action will kill microorganisms and viruses.

10. **Static:**

An agent that is static in action will inhibit the growth of microorganisms.

- **Sterilization processes:**

1. Heat:

A. Dry heat:

- **(hot air oven):**

  Basically, the cooking oven. but dry heat is not as effective as moist heat (i.e., higher temperatures are needed for longer periods of time). For example, 160°C /2hours or 170°C /1hour is necessary for sterilization. The dry heat oven is used for glassware, metal, and objects that will not melt.

- **Incineration:**

  Burns organisms and physically destroys them. Used for needles, inoculating wires, glassware, etc. and objects not destroyed in the incineration process.

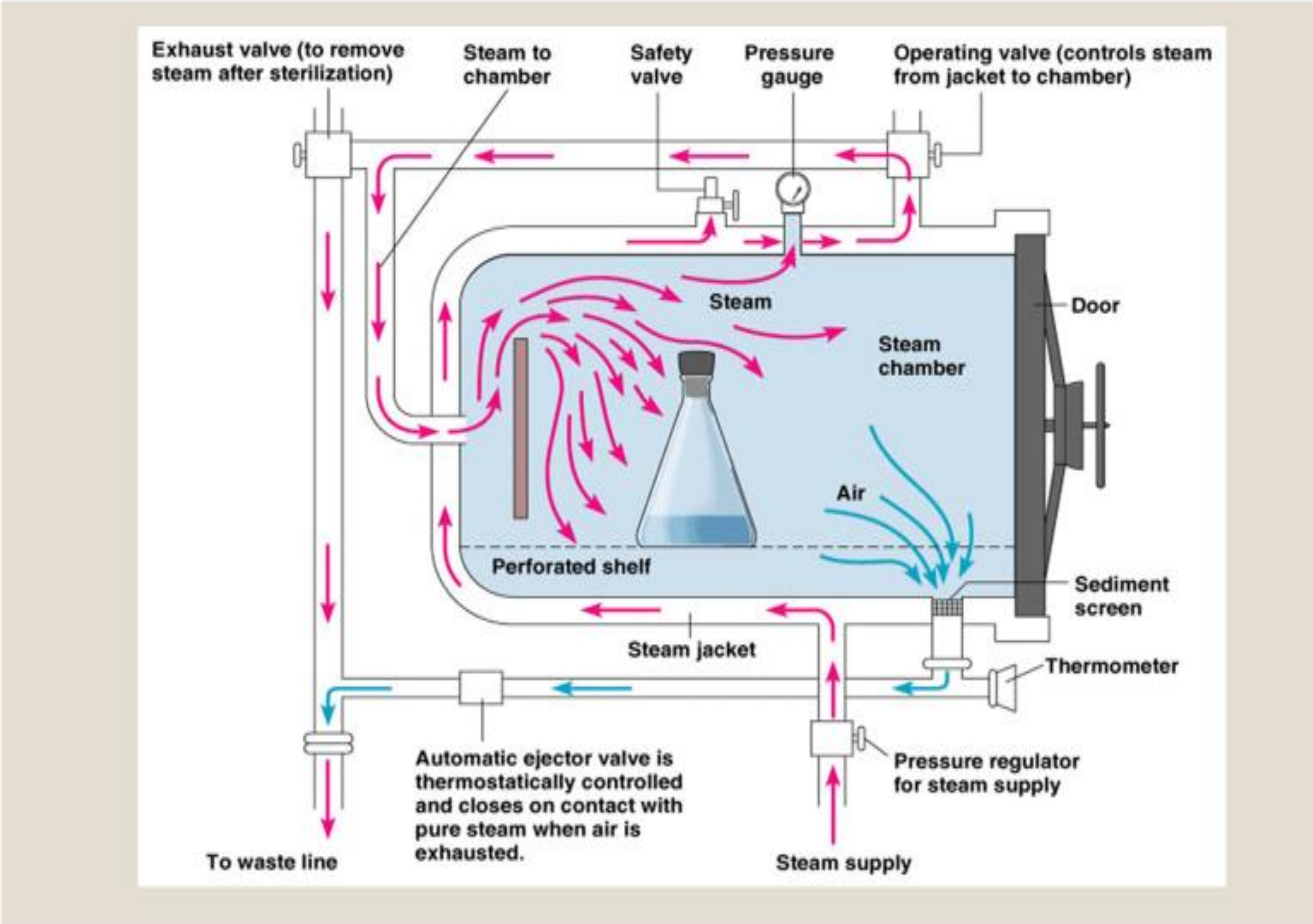
B. Wet heat:

- **Boiling:**

  100°C for 30 minutes. Kills everything except some endospores. To kill endospores, and therefore sterilize a solution, very long (>6 hours) boiling, or intermittent boiling is required.

- **Autoclaving (steam under pressure or pressure cooker):**

  Autoclaving is the most effective and most efficient means of sterilization. The usual standard temperature/pressure employed is 121°C /15 psi for 15 minutes. Autoclaving is ideal for sterilizing biohazardous waste, surgical dressings, glassware, many types of microbiologic media, liquids, and many other things.



Exhaust valve (to remove steam after sterilization)
Steam to chamber
Safety valve
Pressure gauge
Operating valve (controls steam from jacket to chamber)
Steam
Door
Steam chamber
Air
Perforated shelf
Sediment screen
Steam jacket
Thermometer
Automatic ejector valve is thermostatically controlled and closes on contact with pure steam when air is exhausted.
Pressure regulator for steam supply
To waste line
Steam supply

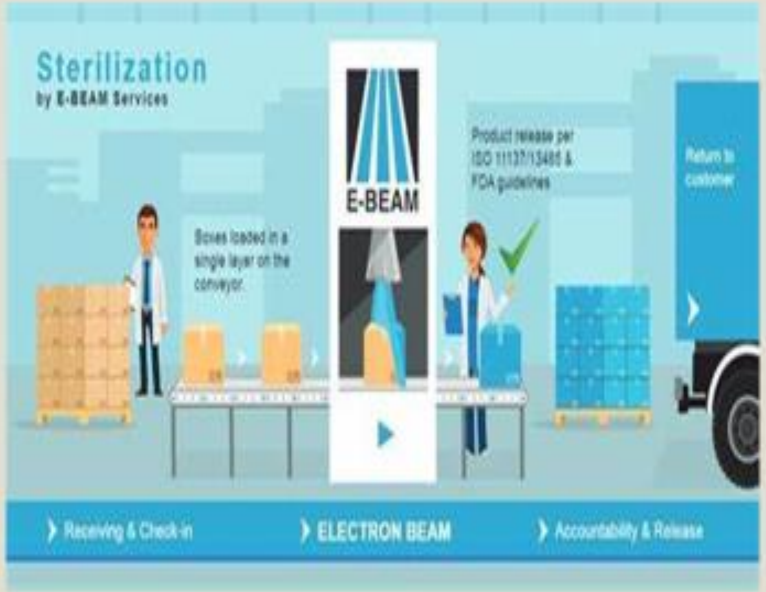
2. **Ionizing radiation:**

A: Gamma radiation:

Are forms of ionizing radiation used primarily in the health care industry, are similar in many ways to microwaves and x-rays. Gamma rays are highly effective in killing microorganisms and do not leave residues.

B: Electron beam radiation:

E-beam irradiation is like gamma radiation in that it alters various chemical and molecular bonds on contact.



3. Filtration:

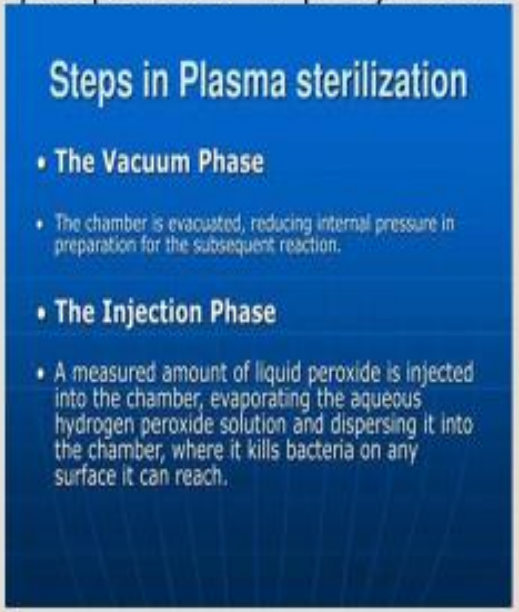
Involves the physical removal (exclusion) of all cells in a liquid or gas. It is especially important for sterilization of solutions which would be denatured by heat (e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.).

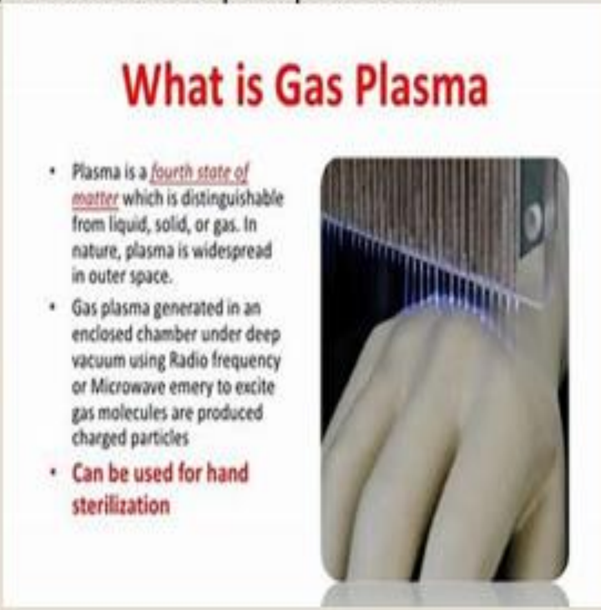
Essentially, solutions or gases are passed through a filter of sufficient pore diameter (generally 0.22 micron) to remove the smallest known bacterial cells.

4. Chemical and gas sterilization:

A. Low temperature gas plasma (LTGP):

Gas plasmas are generated in an enclosed chamber under deep vacuum using RF or microwave energy to excite the gas molecules and produce charged particles, many of which are in the form of free radicals. Plasma treatment has been used to alter the surface properties of polymers without affecting their bulk properties.



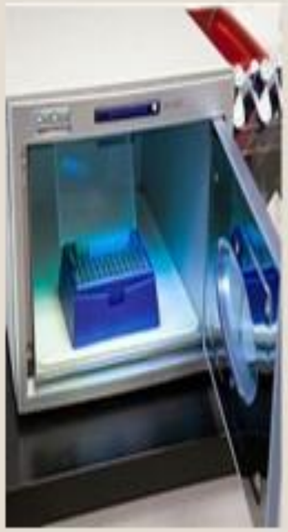


B: Ethylene oxide (ETO):

Is the most used form of chemical sterilization. Due to its low boiling point of 10.4◦C at atmospheric pressure, EtO behaves as a gas at room temperature. EtO chemically reacts with amino acids, proteins, and DNA to prevent microbial reproduction.

C: UV ozone sterilization:

Has been recently approved for use in the U.S. It uses oxygen that is subjected to an intense electrical field that separates oxygen molecules into atomic oxygen, which then combines with other oxygen molecules to form ozone.



D: Common antiseptics and disinfectants:

Chemical	Uses
Ethanol (50-70%)	Antiseptic used on skin
Isopropanol (50-70%)	Antiseptic used on skin
Formaldehyde (8%)	Disinfectant, kills endospores
Tincture of Iodine (2% I2 in 70% alcohol)	Antiseptic used on skin Disinfection of drinking water
Chlorine (Cl_2) gas	Disinfect drinking water; general disinfectant
Silver nitrate ($AgNO_3$)	General antiseptic and used in the eyes of newborns
Mercuric chloride	Disinfectant, although occasionally used as an antiseptic on skin
Detergents (e.g. quaternary ammonium compounds)	Skin antiseptics and disinfectants
Phenolic compounds (e.g. carbolic acid, lysol, hexylresorcinol, hexachlorophene)	Antiseptics at low concentrations; disinfectants at high concentrations

CULTURE MEDIA

CULTURE MEDIA

- Culture media contains nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium.

1 Classification of bacterial culture media on the basis of consistency:

1.0.1 Solid medium

1.0.2 Semisolid medium

1.0.3 Liquid (Broth) medium

1.0.1 Solid medium:

Solid medium contains agar at a concentration of 1.5-2.0%. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

1.0.2 Semisolid medium

Semisolid medium is prepared with agar at concentrations of 0.5% or less. Semisolid medium has a soft custard-like consistency and is useful for the cultivation of microaerophilic bacteria or for the determination of bacterial motility.

1.0.3 Liquid (Broth) medium

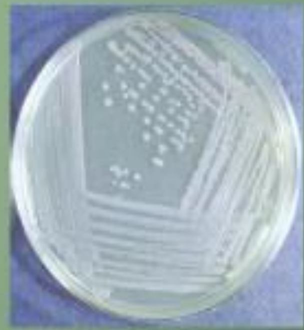
These media contain specific amounts of nutrients but don't have a trace of gelling agents such as gelatine or agar. Broth medium serves various purposes such as propagation of a large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VR broth.

2 Classification of Bacterial Culture media on the basis of purpose/ functional use/ application:

2.1 General-purpose media/ Basic media
2.2 Enriched medium (Added growth factors)
2.3 Selective and enrichment media
  2.3.1 Selective medium
  2.3.2 Enrichment culture medium
2.4 Differential/ indicator medium: differential appearance
2.5 Transport media
2.6 Anaerobic media
2.7 Assay media

2.1 General-purpose media/ Basic media:

Peptone-water, nutrient broth, and nutrient agar (NA) are considered as basal medium. These media are generally used for the primary isolation of microorganisms.



2.2 Enriched medium (Added growth factors):

Addition of extra nutrients in the form of blood, serum, egg yolk, etc, to basal medium makes enriched media.

2.3 Selective and enrichment media:

These media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogens from a mixture of bacteria.

2.3.1 Selective medium:

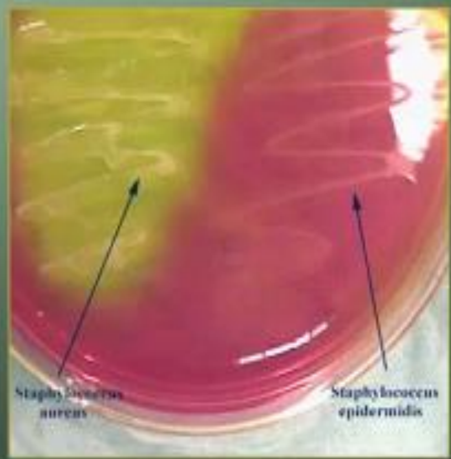
Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium is agar-based (solid) medium so that individual colonies may be isolated.

2.3.2 Enrichment culture medium:

Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment culture is typically used as a broth medium.

2.4 Differential/ indicator medium: differential appearance:

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony color.



2.5 Transport media:

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of a specimen, maintain the pathogen to commensal ratio, and inhibit the overgrowth of unwanted bacteria.

2.6 Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation-reduction potential and extra nutrients.

2.7 Assay media:

These media are used for the assay of vitamins, amino acids, and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique.

MICROBE–MICROBE INTERACTIONS:

1) CENTRAL THEMES:

• A large number of symbiotic associations occur with cyanobacteria because these organisms can provide carbon and nitrogen-containing compounds resulting from photosynthesis-driven N2 and CO2 fixation.

• Microbial interactions are complex and highly dependent on specific microorganisms involved.

• The specific environment is important in selecting for the dominant cell–cell associations.

• Cell–cell interactions where at least one partner benefits are observed in commensalism, syntrophy, symbiosis and mutualism.

• Cell interactions where one of the partners is subjected to negative effects are evident in competition, parasitism, predation, and antagonism.

• Interactions between microbes of the same species may result in developmental changes including sexual activities.

2) INTRODUCTION:

- With the development of microbial communities, the demand for nutrients and space also increases. As a result, there has been a development of different strategies to enable single-cell organisms to persist in an environment.
- Cell–cell interactions may produce cooperative effects where one or more individuals benefit, or competition between the cells may occur with an adverse effect on one or more species in the environment.
- Bacteria rarely are limited to a single type of interaction, but their response is transient and influenced by the chemical or physical environment.

Types of Microbial Interactions

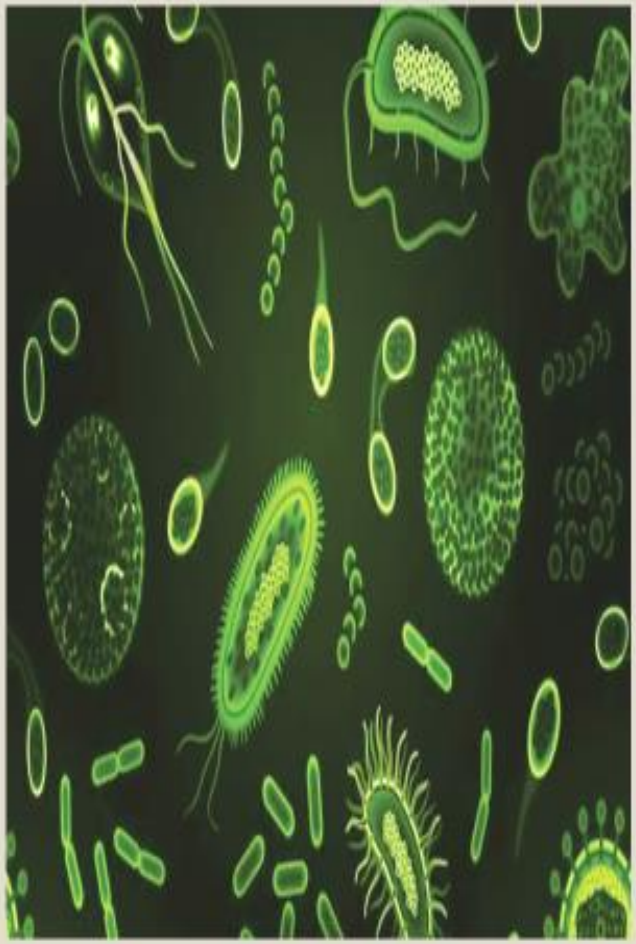
There are three types of Microbial

Interactions:

1. Positive interaction

2. Negative interaction

3. Neutral association



3) CLASSIFICATION OF MICROBIAL INTERACTIONS:

- **In general, interactions between microorganisms of different species promote either robust growth or dominance by a specific microorganism.**

3.1) Neutralism:

- Neutralism occurs when microorganisms have no effect on each other.
- Neutralism has been demonstrated in the laboratory using dual cultures of bacteria, algae, or protozoa; however, it is difficult to observe cultures in nature with neutralism.
- It is perhaps possible for neutralism to occur in natural communities if the culture density is low, the nutrient level is high, and each culture has distinct requirements for growth.
- It has been suggested that neutralism may occur in early colonization of an environment without either harmful or beneficial interactions by the microorganisms introduced.

3.2) Commensalism:

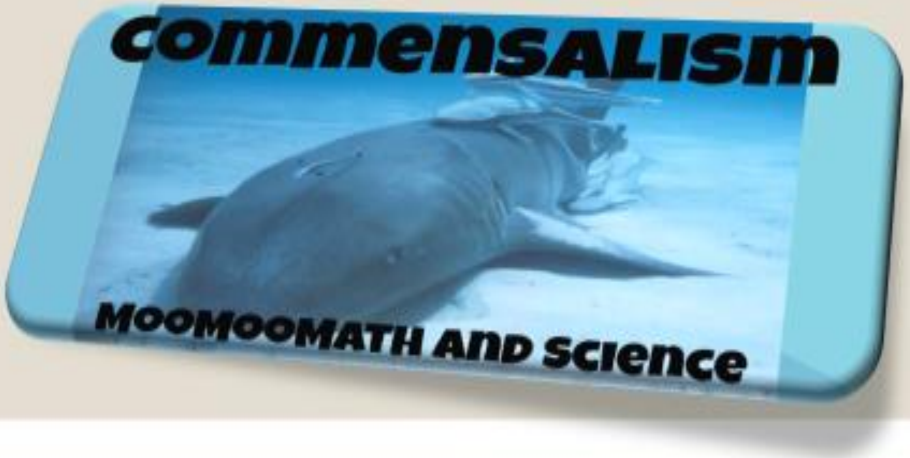
- The communalistic relationship involves two microorganisms where one partner (the commensal) while the other species (the host) is not harmed or helped. Frequently, the relationship between the commensal and the host is casual in that there is no requirement for a specific species. When the commensal is separated from the host, it can grow on its own provided the physical and chemical requirements.
- There are several situations under which commensalisms may occur between microorganisms, including the following:

(1) Cconversion of a nonmetabolizable substrate by one species to a compound that is used by a second species.

(2) One species releasing vitamins, amino acids and other growth factors that are needed by a second species.

(3) One species changing the physicochemical environment, where this activity enables a second species to grow.

(4) Commensalism may result from one species destroying toxins, changing the pH of the environment, removing molecular oxygen, or modifying the osmotic pressure of the environment to enable a second microbial species to grow.



- Additionally, there are microbial relationships of endocommensalism and exocommensalism.
- Bacteria growing on the surface of plants are known as *epiphytes*, and protozoa growing on the surface of aquatic animals are called epizoites.
- With respect to microorganisms, cyanobacteria and algal cells are known to secrete nutrients that attract bacteria to their surfaces. While many bacteria growing in the intestine of animals are in a symbiotic relationship, some bacteria do not benefit the animal host.

3.3) Competition:

- When two or more species use the same nutrients for growth, some of the populations will be compromised.
- Competition between microbial species may be attributed to availability of nitrogen source, carbon source, electron donors, electron acceptors, vitamins, light, and water.
- Competition is seen in aquatic environments were extensive phototrophic activity results in blooms of single species of diatoms or cyanobacteria.
- When succession of populations occurs, the final species could be considered to result from competition exclusion. Other examples of bacterial exclusion through competition are observed in lactic acid fermentation of foods where the environment is highly acidic.
- Competition between species occurs in various environments such as the large intestine of animals, where a single species does not dominate but a mixed population occurs because of competition for nutrients.

3.4) Parasitism:

- Parasitism occurs when one species obtains nutrients from another for the purpose of cell growth.
- Animal parasites display two types: (1) direct lifecycle that does not require an intermediate host and (2) indirect lifecycle that requires an intermediate host.
- With microorganisms, the most widespread example of obligate parasitism is with viruses that attack specific cells by a process independent of an intermediate host. Viruses are highly specialized intracellular parasites that generally kill the host cell.
- All types of microorganisms (i.e., bacteria, fungi, protozoa, algae) have viruses and one role of viruses is to destroy susceptible populations. Scientists suggest that viruses are responsible for killing about 20% of the ocean's bacteria.
- The interaction between a microbial parasite and the host may either kill the host or result in a stable relationship without death to the host.
- Sometimes is carried on the chromosome of the host cell with no apparent harm to the host, and many bacteria harbor proviruses. Pathogenic bacteria or fungi may attack and kill appropriate animals or plant hosts.

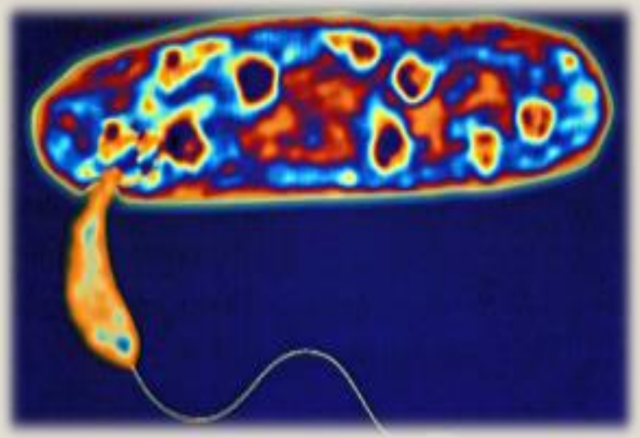
3.5) Predation:

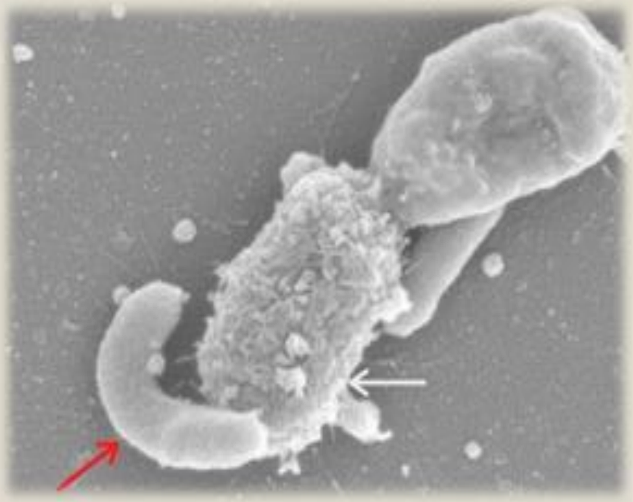
- Predatory microorganisms specifically target other microorganisms for materials that enable the predator to survive.

- In the world of eukaryotes, it is common that the larger animal eats the smaller one; however, with microorganisms the predator may be smaller than the prey.

- Some have suggested that the evolution of the predator was to satisfy a need for cycling of organic carbon or nitrogen compounds. These unique prokaryotes are present in nature where they inhabit soil, aquatic environments, and wastewater.

- They cannot grow outside the prey, or they are facultative, in that they also grow independent of the prey.

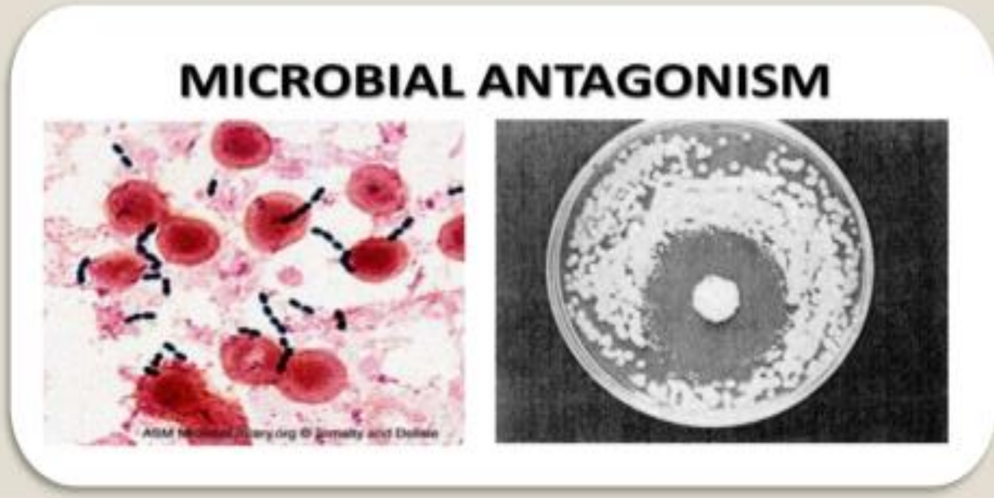




3.6) Antagonism (Amensalism):

- Competition between species resulting from detrimental products or activities is commonly known as antagonism.

- Antagonistic behaviour commonly focuses on exclusion of an organism from growing on a specific site not because space is required for the dominant bacteria but to exclude the other bacterium from utilizing limiting nutrients. Several different processes that account for successful antagonism, and many of these require close contact between bacteria.



INTERACTIONS BETWEEN MICROORGANISMS AND PLANTS

1 <u>CENTRAL THEMES</u>

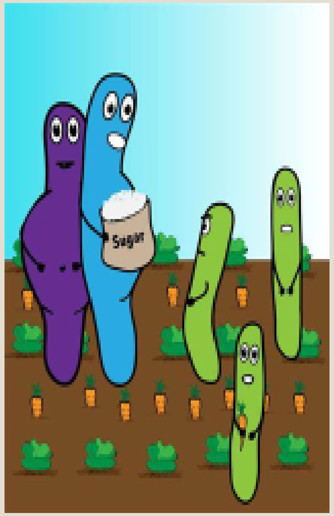
• Cyanobacteria have symbiotic relationships with several different cellular systems. This may be due in part to the fact that cyanobacteria provide carbon materials resulting from photosynthesis to the other partner.

• The rhizosphere is an environment where microbial activities contribute to plant growth. Bacteria and fungi grow on organic compounds released from the plant root and produce plant-line hormones.

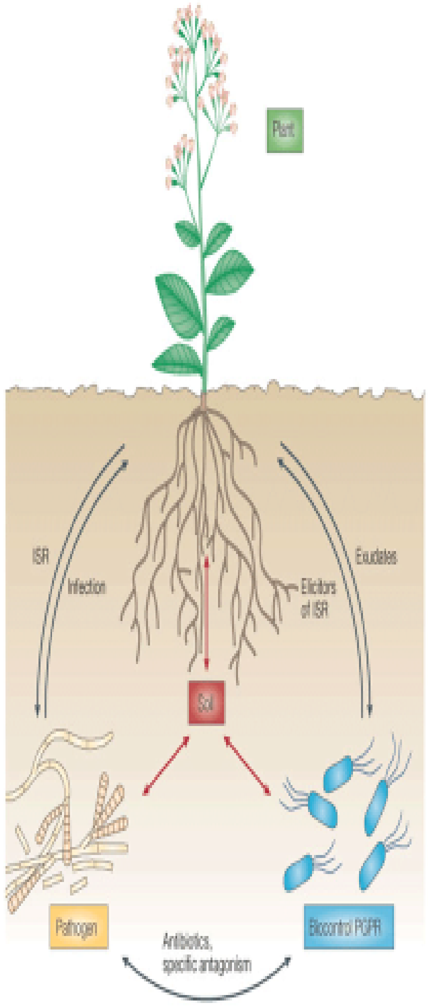
• There are several types of mycorrhiza associations where fungi establish a persistent relationship with plant roots. In these relationships, fungi obtain minerals from the environment to support plant growth, and the plant root provides carbon compounds for the fungal cells to grow.

• Atmospheric nitrogen may be reduced to ammonia by cyanobacteria and other free-living prokaryotes. Nitrogen fixation is also observed, with rhizobia and frankia producing nodules on appropriate plant roots.

• A few species of bacteria and fungi are important plant pathogens infecting vascular tissues or decomposition of plant cell walls. Gall production on plants is attributed to infection by Agrobacterium tumefaciens that contains the Ti plasmid.



- Earth is richly populated with plants, and many different types of microorganisms grow in close association with them. While microbial activities detrimental to plant growth may be the most obvious, microorganisms are also beneficial to plants.
- As reflected in Figure, microorganisms may provide some nutritional benefit to a plant host, and this would result in increased plant growth. Over time different interactions between microorganisms (bacteria, fungi, or algae) and plants have been identified, and several of these activities are indicated in Figure. Perhaps one of the most obvious environments for microbe–plant interactions occurs in soil.



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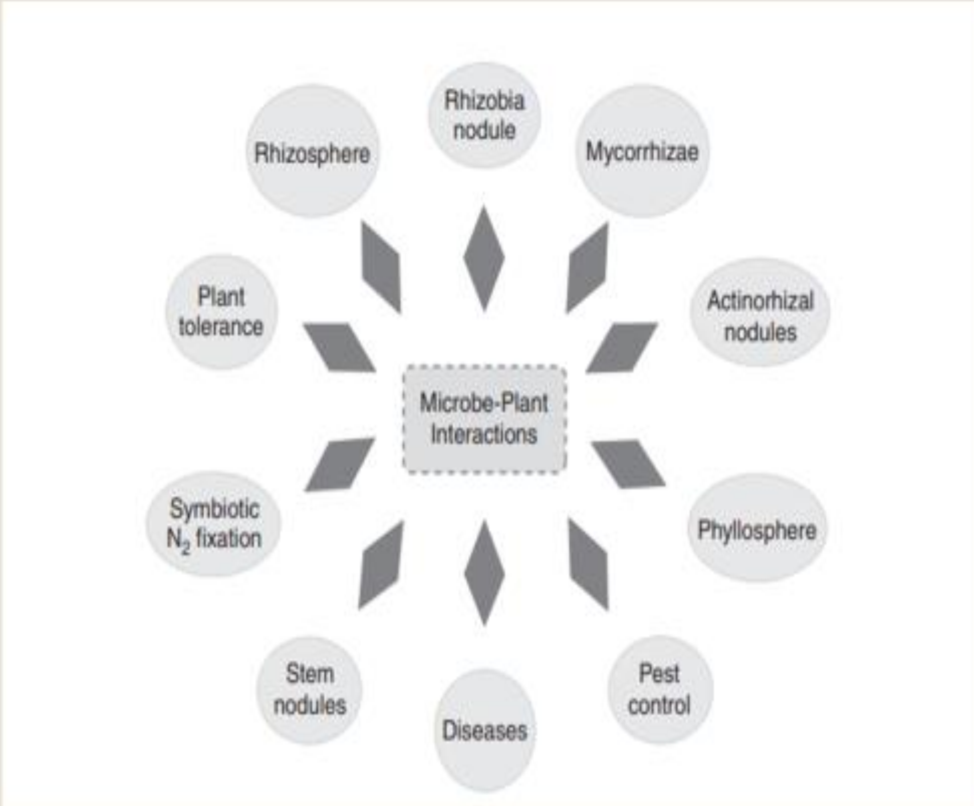
Model indicating various plant–microbe interactions

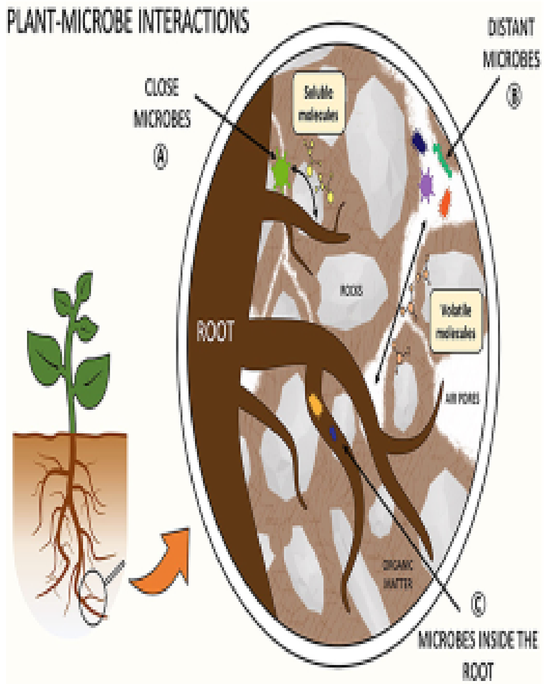


SYMBIOTIC ASSOCIATIONS WITH CYANOBACTERIA

- Cyanbobacteria are broadly distributed in nature and form symbiotic relationships with many different organisms.
- Cyanobacteria enhance their survival by establishing an association with a biological partner.
- Cyanobacteria are found in a variety of environments, including those in symbiotic associations with plants.
- *Azolla* is an aquatic fern that contains bilobed leaves attached to a stem and is found floating in freshwater. This tiny plant has a symbiotic association with *Anabaena azollae* where the cyanobacteria fixes atmospheric nitrogen and *Azolla* provides carbohydrates.
- The cyanobacteria grow as a microcolony in the plant. Nutrient exchanges appeared.
- *Azolla* has long been used to enrich the nitrogen level of rice fields and is often used as a fertilizer known as "green manure."

INTERACTIONS IN THE RHIZOSPHERE

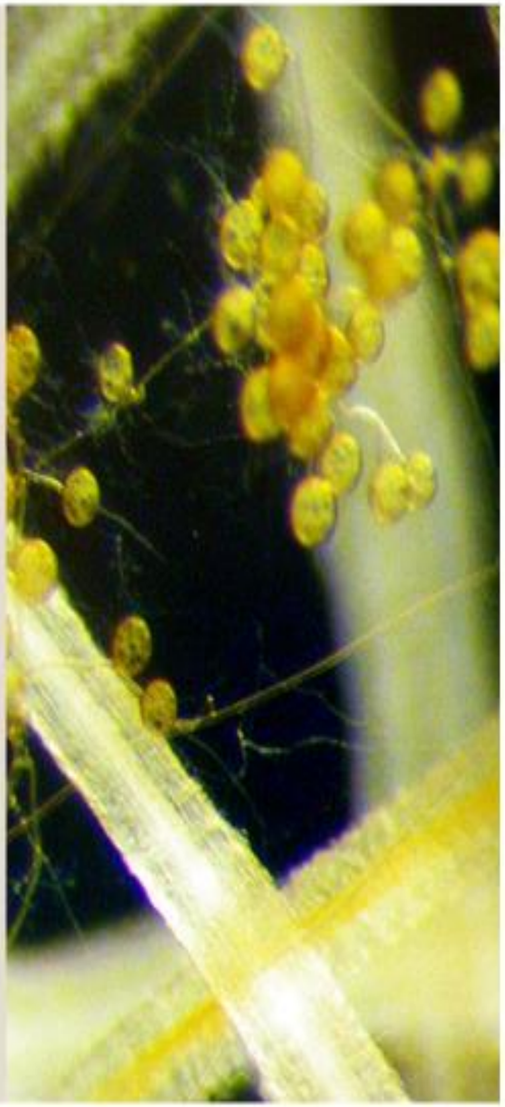
- The soil layer that is within a few millimeters from the root system is the rhizosphere, which consists of soil and all the biological agents present in the soil.
- In practical terms, rhizosphere refers to the soil remaining on roots after a plant has been removed from the soil and gently shaken.
- This designation includes bacteria, fungi, algae, protozoans, and soil animals.
- Organisms of the rhizosphere are collectively referred to as rhizospheric microflora and grow in a complex community.
- The microflora is characteristic for a specific cultivar, and greatest microflora activities are observed when plants are flowering.
- Soil bacteria that stain Gram-negative are more abundant than Gram-positive bacteria in the rhizosphere.



- The microorganisms on the root surface are present as microcolonies occupying a unique environment, and this environment is termed the rhizoplane. Rhizoplane microorganisms are present as microcolonies on the root covering only 10–30% of the root surface.
- This association of microorganisms is not uniformly distributed along the surface of the root, but microorganisms are found especially at the junction of the epidermal cell and at the base of root hairs.
- These areas represent regions where rhizodeposition is especially high. The microorganisms are not carried by extension of the plant root because fungi grow at a rate of 3 mm/day.
- For pea plants, the movement of *Pseudomonas fluorescens* is only 3 cm in 1 week, while the root elongates by 10–20 cm in a week.
- Additionally, the root extends through airspaces in the soil and bacteria moving through the soil are unable to keep up with the elongating root.

MYCORRHIZAE

- Rhizospheric interactions of terrestrial fungi are influenced by the number of microorganisms, type of microorganisms, and specific plant root exudates.
- While many of these fungal interactions are highly transient, some fungi in the rhizoplane may establish a mutualistic relationship with the root.
- In 1885, this stable fungus–root relationship was first designated as mycorrhizae by Albert Bernard Frank, and over the years the description has been expanded to include both endomycorrhizae and ectotrophic mycorrhizae.
- Growth on the exterior of the root is characteristic of ectotrophic mycorrhizae, while growth inside the root is attributed to the endomycorrhizae or endotrophic mycorrhizae.



NITROGEN-FIXING BACTERIA AND HIGHER PLANTS

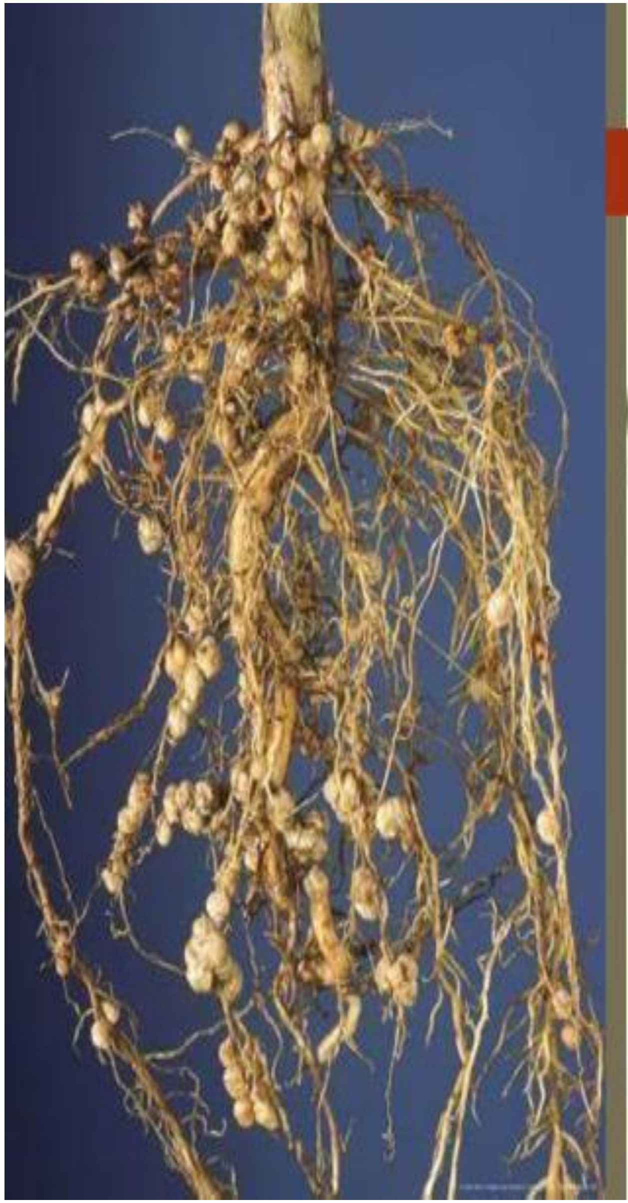
- The enzyme system for nitrogen fixation is found only in the prokaryotes, and in the case of symbiotic nitrogen fixation in plants, there is considerable specificity between the legume symbiont and bacteria for the stable association.
- Specific bacteria infect either root hairs or stems of plants, and the most common association is the bacteria– root association. This association is highly beneficial for both the bacteria and the plant.
- The plant provides the carbon and energy source for the bacteria to grow, while the bacteria fix nitrogen with the production of amino acids for plant growth.

1 Root Associations:

- The association of nodules with roots of leguminous plants is one of the oldest reports in microbial ecology. In 1888, Martinus Beijerinck isolated bacteria from the root nodules of legume plants and established the nitrogen-fixing capability of these bacteria when associated with plants.
- The bacteria that grow symbiotically with roots of legumes are frequently referred to as rhizobia, and currently these bacteria are assigned to four designated genera.
- The establishment of root nodules requires bacteria that can compete in the rhizosphere as well as establish a symbiotic relationship with roots. An important aspect for rhizospheric and symbiotic bacteria is iron nutrition.

- Bacteria are specific for a legume species, and formation of the root nodule is the result of a unique differentiation process.
- 1. The legume root secretes specific chemicals known as flavonoids, and these signaling molecules attract rhizobia growing in the rhizosphere.
- 2. The flavonoids also induce transcription of nod genes in the rhizobial genome to produce lipochitin oligosaccharides, called Nod factors.
- 3. The plant root recognizes the chemical structure of the Nod factors, and these lipochitin oligosaccharide molecules are taken up by legume receptor kinases. The Nod factors activate the plant hair roots, and this recognition is responsible in part for the specificity between the host and legumes.
- 4. The symbiotic bacteria attach onto the root hairs and enter into the root by a process known as root infection.

- 5. The bacteria produce a lipopolysaccharide capsule that enables the rhizobia to evade plant defence systems and enter the root by a structure designated as the infection thread.
- 6. Phytohormones are produced by bacteria to stimulate root cell division, and the plant root expands quickly to produce a nodule.
- 7. Bacteria in the root nodule adjust to the low-nitrogen environment and the reduced oxygen level in the nodule. In some cases the bacteria differentiate to produce rounded cells or cells with a "Y" shape. These differentiated bacteria are bacteroids, and these cellular units are important in fixing nitrogen.



- A photograph of soybean roots with nodules present on the root hairs. Nitrogen fixation in the soybean nodule is attributed to the bacteroids, differentiated rhizobial cells, present in the symbiosomes.
- Because the nitrogenase enzyme is oxygen-sensitive, leghemoglobin in the nodule reduces the level of free O2 to enable nitrogen fixation. The product of nitrogen fixation is the production of glutamine, and this amino acid is transported by way of the root hairs to regions of protein synthesis.
- Most commonly there is a high level of specificity between bacteria and the legume to establish a symbiotic state. However, some soil rhizobia may nodulate several legume species, and this may be attributed to lateral gene transfer of Nod factors between different rhizobia.

Actinorhizal Nodules:

- Another plant-root symbiosis associated with nitrogen fixation is the actinorhizal symbiosis.
- At least 25 genera of plants from eight plant families will interact with bacteria known as *Frankia* to produce a fibrous root nodule. Frankia are widely distributed in soils and grow in multicellular arrangements typical of actinomycetes. The Frankia – root interaction results in an actinorhizal nodule by a highly ordered process similar to legume nodule development.
- The plants involved are woody shrubs or small trees that are found worldwide, and Russian olive is one of the more common trees found in the United States.
- Plant growth attributed to nitrogen fixation by actinorhizal plants was first observed by Woronin in 1866. The taxonomy of Frankia strains is not well established because the bacteria had been difficult to grow in the laboratory.

2 Stem Associations

- In tropical and subtropical regions, aquatic legume species have nitrogen-fixing nodules on their stems. Generally nodules are produced on the submerged stems or on stems at the air–water interface.
- These bacteria are interesting because they also contain bacteriochlorophyll a and have the capability to carry out limited anoxygenic photosynthesis.
- Stem-nodulating bacteria will fix N2 explanta, while bacteria that nodulate roots will fix nitrogen only when growing symbiotically.
- Additionally, under appropriate conditions nodules have been observed on the lower stems of field beans, clover, peanuts, and soybeans.
- Nitrogen fixed by the bacterium–stem relationship is important for plant growth because the soils where plants are nodulated are nitrogen-deficient.
- Bacteria associated with nitrogen-fixing nodules on stems are undergoing classification, and a species that nodulates clover stems has more recently been identified as *Methylobacterium nodulans*.

BACTERIA SUPPORTING PLANT GROWTH:
1 Production of Hormones

- Many rhizospheric microorganisms produce chemicals that stimulate plant growth, and these chemicals have a molecular structure similar to that of plant hormones.
- Many bacteria produce plant-stimulating compounds, including auxins, gibberellins, cytokinins, ethylene, and abscisic acid. Additionally, many rhizospheric fungi and numerous rhizospheric bacteria produce the plant hormone ethylene.
- These plant-like hormones are produced by free-living, symbiotic, and pathogenic strains of rhizospheric microorganisms. While microorganisms use plant hormones to initiate root symbiosis, the benefit from plant hormone production by saprophytic microorganisms is not readily apparent.

2 Growth-Promoting Rhizobacteria

- The most numerous microorganisms in the soil are bacteria, and these organisms may influence mycorrhizal growth.
- Scientists recognize the importance of soil bacteria and have called them plant-growth-promoting rhizobacteria (PGPR) or simply growth-promoting bacteria.
- Certain bacteria are proposed to support the establishment of a fungus– root relationship, and they are collectively referred to as mycorrhization helper bacteria (MHB).
- Not all bacteria in the soil stimulate mycorrhizae formation, but the organisms most commonly recognized for stimulating it are fluorescent *Pseudomonas*.
- It appears that metabolic products of bacteria support the establishment of mycorrhizae; however, details of this activity remain unresolved. It is quite likely that some of the same bacteria are being studied in MHB and PGPR systems because fluorescent bacteria of the genus Pseudomonas are important in both groups.

DETRIMENTAL ACTIVITIES OF MICROORGANISMS ON PLANTS

- **1 Fungal Parasites**
- Several fungi and a small number of bacteria are pathogenic for plants. Fungi of all taxonomic classes are capable of causing epidemic plant diseases that produce considerable economic loss and hardship for humans.
- Disease-producing fungi may be considered as parasites on plants where the infected plant provides nutrients for fungal growth. Some of the fungi are obligate parasites, while others are facultative with fungal growth also from nutrients in the soil.

The pattern followed for disease formation in plants is generally as follows:

1. Spores or vegetative cells of fungicome in contact with the plant.

2. The fungi penetrates the surface through wounds, stomata, and other openings in the plant cell.

3. An infection occurs with the fungi growing in either the intracellular or intercellular region.

4. Other tissues are invaded, and the pathogen is spread throughout the plant.

5. Depending on the pathogen, death of the plant may be observed in a few days or a few weeks

2 Bacterial Pathogens

- The bacteria-producing diseases in plants generally display a number of hydrolytic enzymes for penetration of the plant surface, and growth in the plant is generally intercellular between the parenchyma cells.
- As the disease progresses in the plant, bacterial distribution may become systemic as a result of migration into the vascular tissue. In some cases, the bacteria release pectinase that hydrolyzes the plant cell walls.
- In other instances, cells of bacteria may concentrate in the sieve cells and the reduction of flow in the xylem or phloem results in plant death. As a response to the bacterial infection, there is an oxidative burst by the plant to inhibit the invading pathogen.
- Successful plant pathogens have several virulenceenhancing factors, including assimilation of iron and the production of superoxide dismutase in response to oxidative response. Dominique Expert has been a pioneer in the study of virulence-associated siderophore production by phytopathogenic bacteria and is highlighted here.

- The bacterium *Agrobacterium tumefaciens* produces tumors in a diverse group of plants at the root–stem interface that is called the “crown” of the plant. Thus, the disease has been called “crown gall.”
- The microorganism is an aerobic Gram negative bacterium that is widely distributed in soils where it grows on a variety of sugars and organic acids.
- The infection is initiated by a wound to the plant that enables bacterial cells to attach to the plant by either pili or capsular material.
- The *A. tumefaciens* cell does not enter the plant cell; rather, a large plasmid is transferred from the bacterium to the plant. This plasmid is designated as Ti because it is responsible for tumor induction in plants. The Ti genes are transferred into the plant genome with the production of plant hormones, auxins, and cytokinins.

3. Rhizosphere Activities and Plant Diseases

- A series of complex relationships in the rhizosphere influence the growth and survival of plant pathogens in the soil.
- The rhizosphere activities important for plant diseases that have a lifecycle in the soil.
- While the interaction between the pathogen and roots of the host are relatively obvious, the root exudates of the host are a key component in the infectious process.
- Cells sloughed from the roots and the root exudates may directly inhibit growth of the pathogens or may stimulate the growth of saprophytic microorganisms that may have an antagonistic effect on the pathogen.
- Thus, important factors influencing the establishment of a disease process in plants is a reflection of the health of the plant and the extent that the rhizosphere supports a highly diverse microbial population.

FUNGI PROMOTING INCREASED HEAT TOLERANCE IN PLANTS

- A three-way symbiotic system has resulted in increasing the thermal resistance of certain green plants.
- When the fungus *Curvularia protuberate* infects plant, the plant is capable of growing in soil that has a temperature of 65° C.
- However, for this heat tolerance, the fungus must also be infected with a dsRNA virus, and this virus has been designated as*Curvularia* thermal tolerance virus (CThTV).
- This physiological response´ in plants is not restricted to infected this plant because increased thermal tolerance of tomato, and other plants, occurs when plants are infected by the *Curvularia* fungus carrying CThTV. T
- his novel way symbiosis provides a unique perspective on mutualism found in the ecosystem

BIOCONTROL OF PESTS AND PATHOGENS

- Throughout time, the control of insects and agents responsible for disease production in plants has been achieved through the use of chemicals. However, an increased concern for the addition of chemicals into the environment has prompted scientists to explore the use of biological agents to control or prevent the growth of pathogens.
- Plant-growth-promoting rhizobacteria (PGPR) produce a variety of antibiotic compounds that inhibit a variety of Gram-negative and Gram-positive soil bacteria, including some that may be phytopathogenic.
- In one case, antibiotic synthesis by growth-promoting *pseudomonads* results in the production of some metabolites, which are effective against pathogenic fungus.
- The application of certain strains of *Pseudomonas* to fields growing potatoes has been helpful in increasing the yield of potatoes. It has been proposed that bacteria produce iron in the environment and pathogenic fungi are unable to acquire iron for growth.

LIVING TOGETHER: MICROBIAL COMMUNITIES

- **Microbial Community Ecology:**

The last decade of microbial community ecology has been dominated by studies of species diversity and an ever-increasing number of habitats.

The discovery that we can grow less than 1% of the species of bacteria that are present in the environment using standard microbiological culture techniques has led to studies dominated by cultureindependent techniques that employ molecular phylogenetic analyses. For example, our previous concept that a few species of bacteria live in the human mouth.

BIOMATS AND BIOFILMS

- This might make you think of the scum on your teeth in the morning or the slimy feel of the cats' water bowls if you don't change the water.
- Biofilms are communities of microorganisms embedded in a matrix, usually adhering to a surface. Organisms gain much from living in the structured environment of a biofilm community.
- Living in houses protects us from the weather (desiccation, freezing, UV damage, etc.); living in biofilms protects microorganisms from similar threats, as well as stressors more specific to microorganisms, such as pH extremes.

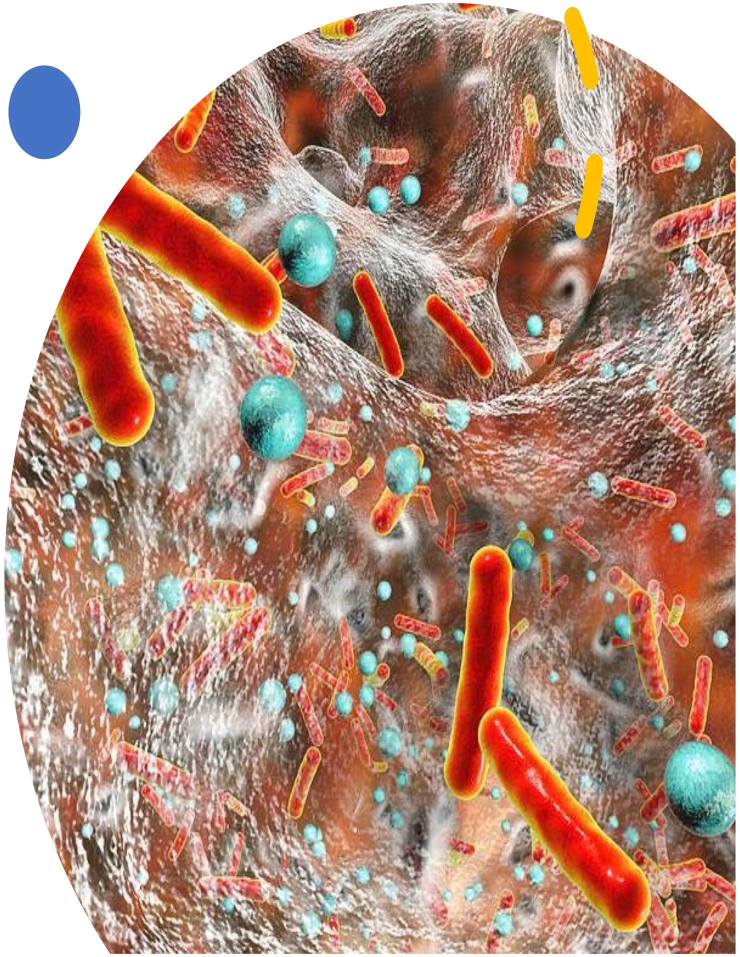
- **Biofilms can shield microorganisms from a variety of harmful substances, including toxic metals, salts, antibiotics, and predators.**
- **The biofilms aid in transmission, but also protect the bacteria from predation.**
- **Microbial biofilms also have important implications for humans. Early in the 1980s, researchers discovered that bacteria such as *staphylococci* inhabited medical devices and were protected from antibiotics by their biofilms.**
- **This discovery provided a powerful motivation for humans to study biofilms, and researchers have pieced together key information about how biofilms form that will allow us to better combat microbial diseases that are resistant to treatment because of their biofilm nature.**

- Biofilm morphology has been characterized as colonies or pellicles (free-floating).

Four stages of growth and development have been characterized:

- • Planktonic cells adhere, reversibly, to a surface or interface.
- • Cells multiply and form a nonreversible attachment.
- • The biofilm community grows, adding to the three -dimensionality of the community.
- • As the biofilm reaches maturity, parts detach in a dispersal stage the initiates new biofilms.

- In different environmental conditions diverse kinds of biofilms form. Key environmental factors that affect the kind of biofilm formed include fluid shear and the amount and kind of nutrients available.
- Working together, engineers and biologists have shed light on the viscoelastic and hydrogel characteristics of biofilms.
- In the case of biofilms characterized as hydrogels, the biofilm polymer is hydrated with water, forming a viscous jelly-like matrix.
- Fluid shear can deform the viscous matrix, and the biofilm will break under the shear forces if its tensile strength is exceeded.



COLONIZATION AND RECOLONIZATION BY MICROORGANISMS

- **Today many studies examine how microorganisms colonize entirely new habitats such as freshly cooled lava, newborn guts, and emerging leaves.**
- **Of critical importance for humans is how microorganisms colonize wounds, medical devices and implants, transplants, contact lenses, and other materials.**
- **These microbial colonization events often lead to pathogenic processes, and their study can lead to new insights into how to control microbial pathogenesis.**
- **Of less personal concern is the microbial colonization of water pipes, stainless steel, and other inanimate parts of our lives.**
- **On the other hand, some microbial colonization events are very helpful to humans, such as the colonization of plant roots by nitrogen - fixing bacteria and mycorrhizal fungi that facilitate plant growth.**

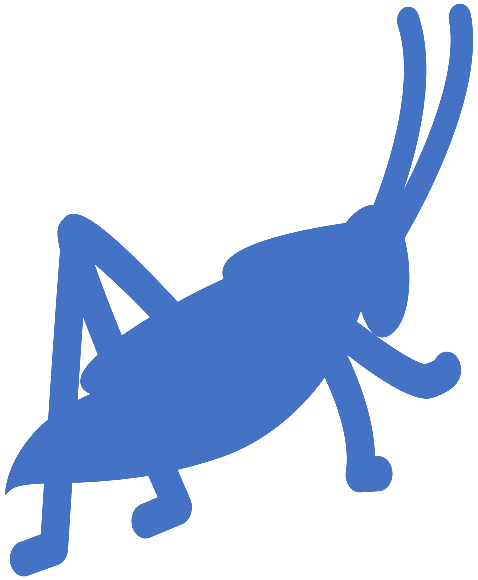
1 Case Study: Colonization of the Sterile Newborn Gut

- An ideal environment in which to study microbial colonization is that of the human newborn gut, which is sterile at birth, but colonized within hours, eventually developing a community of 400 aerobic and anaerobic species. Colonization of the gut is influenced by maternal and environmental factors. The mother's diet, stress level, bacterial flora, and mode of delivery are strong controllers of the nature of the newborn's initial microbial flora. Babies delivered by Caesarean section (C section) show a pattern of colonization by bacteria from the hospital environment.
- Breastfeeding produces an infant gut flora different from that seen in formula-fed babies. Mothers who breastfeed transmit *lactobacilli* and *bifidobacteria* in much greater numbers than those in Enterobacteriaceae, while babies fed formula develop a community dominated by Enterobacteriaceae that develops more quickly than does the community in breastfed babies.

2 Case Study: Undesirable Colonization Factors in Disease

- Some microbial colonization is not welcome. The gastrointestinal tract is a hostile place for invading pathogenic microorganisms, which encounter stomach acid, bile salts, and the barrier presented by the intestinal epithelial cells.
- Invaders have developed several strategies for evading defense mechanisms of the host in order to successfully colonize the GI tract. Indeed, transport through a host can lead to key gene expression changes in the pathogens, which allow for successful colonization of the next host.

DISPERSAL, SUCCESSION, AND STABILITY



- Compared to plant and animal communities, microbial communities have a much greater diversity on several levels.
- They can be a mixture of bacterial and archaeal species and eukaryotes; they can be autotrophs or heterotrophs or both at different times in their lifecycle; or they may or may not need oxygen.
- These factors contribute complexity to modeling what structures microbial communities and their succession over time. Thus, we cannot simply apply plant or animal successional models to microorganisms.
- Some pioneering researchers are studying changes in species richness and resource availability over time and have used these data to propose models for microbial succession.

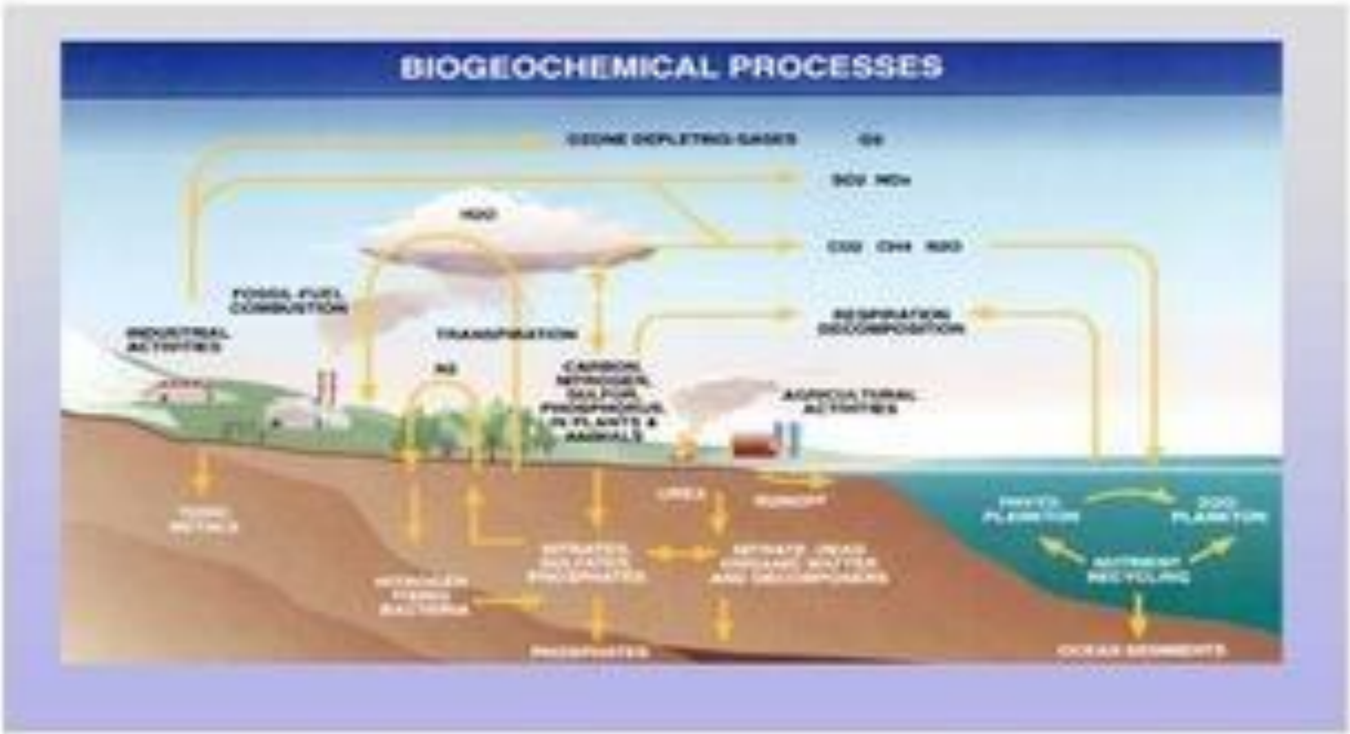
Case Study: Dispersal and Succession in the Oceans Whale Falls as Dispersal Agents between Vents

- When whales die and fall to the ocean floor, forming the basis for fascinating experiments in colonization, dispersal, and succession of bacterial communities.
- The fat-rich bone marrow of whale falls is a super media for microorganisms.
- Bacterial decomposition of the whale bone lipids releases sulfides that feed other organisms.
- Other bacteria have taken up residence in small worms called *Osedax*, assisting the worms in breaking down the marrow fat.

SPECIES DIVERSITY

- When we talk about species diversity, we are actually talking about multiple concepts, including the number of species (richness) and their relative environment or community.
- Even such a simple definition is complicated in describing microbial species diversity.
- Ernst Mayr's biological species concept defines a species as a group of interbreeding populations and is applied to eukaryotic organisms.
- In bacterial and archaeal microbial species, a species is commonly defined in terms of the degree of genetic relatedness based on DNA-DNA hybridization.
- Organisms whose purified genomic DNA hybridizes at >70% are deemed to be the same species. Two organisms are characterized as the same species if they share 97% (some researchers suggest 99% as the cutoff) 16S rRNA sequence identity

MICROBIAL PROCESSES CONTRIBUTING TO BIOGEOCHEMICAL CYCLES



- • Microorganisms are active participants in elemental cycling because they couple energy flow in redox reactions to biosynthesis.
- • Microorganisms have an indispensable role in global carbon cycling.
- • Bacteria are important in the global nitrogen cycle in that they fix nitrogen, produce amino acids from different nitrogen sources, and participate in denitrification.
- • Microorganisms mobilize phosphorus in the environment through the use of enzymes to release inorganic phosphate from phosphate esters and phosphonates.
- • Bacteria and fungi have developed siderophore systems to solubilize and import iron from environmental sources.
- • Microorganisms are active in cycling of sulfur, manganese, selenium, and numerous other redox-active elements.
- • The movement of hydrogen as H+ in membrane activities, the metabolism of organic compounds with hydrogen, and the metabolism of H2 are important contributions of bacteria.

- While geomicrobiology is the study of microorganism as influenced by the geologic environment and geochemical processes.
- Biogeochemistry is the systems approach to study atmospheric, environmental, and Earth sciences.
- Biogeochemistry studies the composition of the biosphere by examining the relevant nutrient cycles and the impact of energy relationships on the natural environment.
- As a result of diverse metabolic activities, microorganisms have a great influence on chemicals in the environment including elements used to produce cellular biopolymers.
- Microorganisms have considerable influence on the biogeochemical cycling of elements in both anaerobic and aerobic environments.

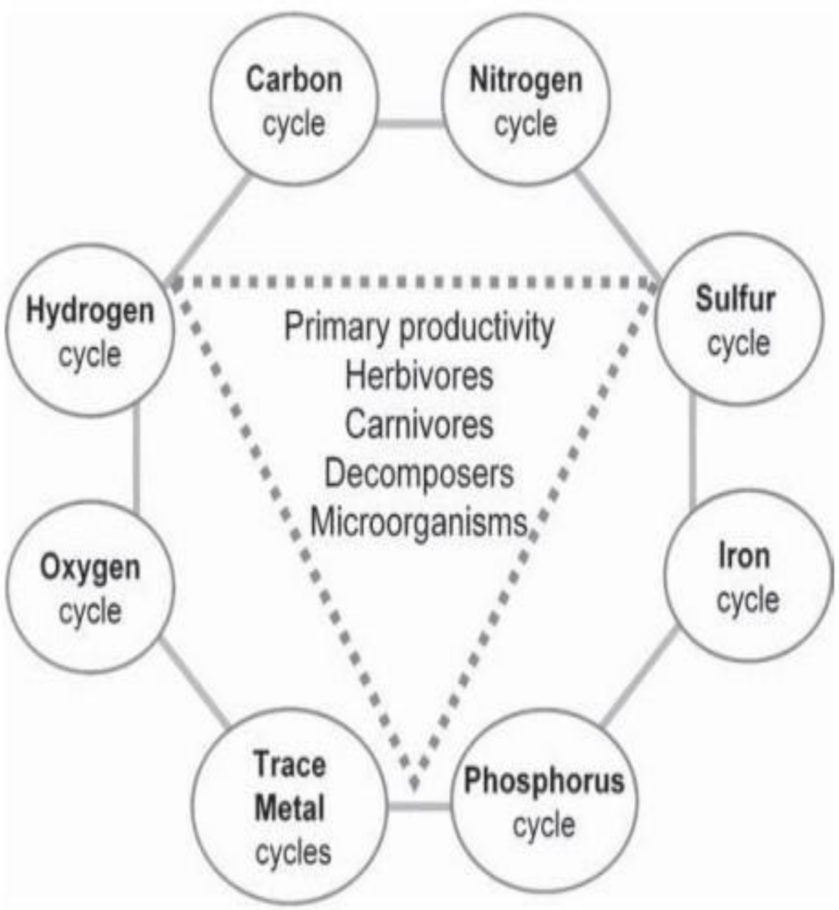


Figure 10.2. Microbial interaction with several biogeochemical cycles. The collective activities of microorganisms stimulate the cycling of elements through oxidation–reduction reactions.

- The importance of hydrogen, oxygen, carbon, nitrogen, phosphorus, and sulfur in cells of algae and bacteria is reflected by their abundance (see Table 10.1).

TABLE 10.1. Major Elements Present in the Cells of Microorganisms

	Abundance (g/100 g Dry Weight Cells)	
Element	Algae	Bacteria
Hydrogen	7.2	8.0
Oxygen	48.3	20.0
Carbon	33.6	50.0
Nitrogen	6.2	15.0
Sulfur	1.1	1.1
Phosphorus	0.8	3.2

Source: Barton et al. (2007).

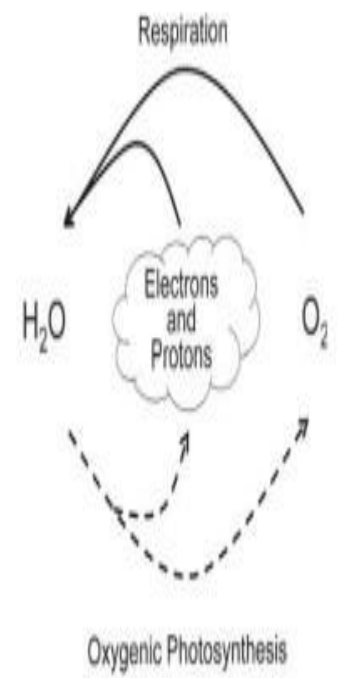
- Microorganisms acquire carbon, nitrogen, sulfur, and phosphorus from the environment to make carbohydrates, proteins, nucleic acids, and other organic molecules; in addition, these elements are released back into the environment on death of the microorganisms.
- This assimilation of nutrients to couple the release of nutrients from cells with cell decay is one component of nutrient cycling.
- However, there is another dimension of nutrient cycling that reflects the wide capability of bacteria to couple metabolism of inorganic nitrogen and sulfur compounds to energy production for biosynthesis.
- Heterotrophic microorganisms obtain energy from the metabolism of organic compounds, and chemolithotrophic sulfur or nitrogen bacteria use inorganic compounds as either electron donors or electron acceptors.

ENERGY FLOW

- All life forms, including microorganisms, require energy to grow, and the processing of energy from the surrounding environment is one of the areas known as bioenergetics.
- Energy transformation from molecular interactions to cellular response follows the laws of thermodynamics, and **Josiah Gibbs** established a procedure for predicting the activity of a reaction.
- The free energy of a reaction is expressed as ΔG (G for Gibbs), with a negative ΔG indicating that the reaction can occur spontaneously and a positive ΔG indicating that energy is required to make the reaction proceed.

OXYGEN AND CARBON CYCLING

- Water is the source of oxygen for O2, and the aerobic atmosphere of Earth is produced by continuous photosynthetic processes.
- When O2 production initially occurred on Earth, there were no aerobic organisms and molecular oxygen accumulated.
- A collection of biological systems evolved to consume O2 through respiration, and currently there is equilibrium between O2 production and O2 consumption.
- When compared to other elements, the oxygen cycle (Figure) is relatively simple.



- With carbon as a principal element in cell systems, it would follow that distribution, fluxes, and reserves of carbon would be of global interest. Microorganisms have an important role in all aspects of the carbon cycle.
- Primary producers are the organisms that fix carbon dioxide, and while higher plants are important in terrestrial areas, algae and cyanobacteria account for primary productivity in marine environments.
- Nonphotosynthetic fixation of carbon dioxide by chemolithotrophic or heterotrophic bacteria accounts for relatively small amounts of carbon transferred from the atmosphere to biomass.

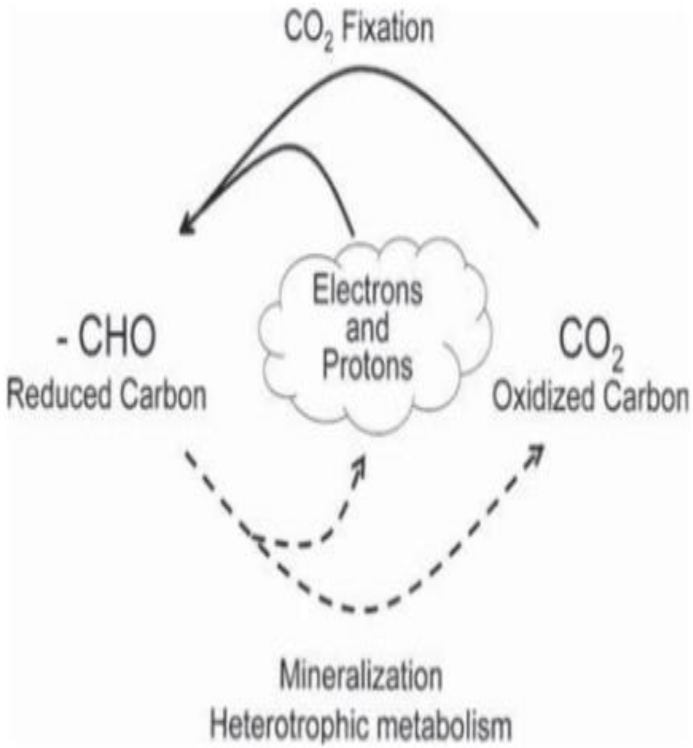


Figure 10.6. Redox reactions associated with the carbon cycle requires the cycling of electrons.