Ministry of Higher Education
and Scientific Research
University of Baghdad
College of Science
Department of Biology

Theoretical soil and water microbiology
2020-2021

المرحلة الرابعة – الدراستين الصباحية والمسائية

الفصل الدراسي الثاني

تدريسي المادة:

أ.م.د. سناء رحمن عليوي م.د. نسرين هادي عودة

Soil Microbiology*:* It is the branch of science/ microbiology concerned with soil-inhabiting microorganisms, their functions, and activities.

Soil is one of the world's most important natural resources. Together with air and water, it is the basis for life on planet earth. Soil can be defined as a thin layer of material covering the earth's surface and is formed from the interactions between five factors called **soil forming factors** includes topography of the land, the organisms present in the environment, the climate, the parent material, and the time.

Soil profile

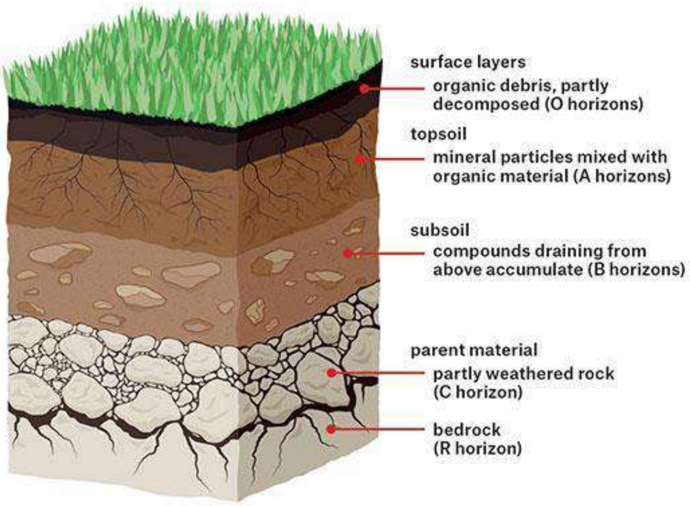
A vertical cross-section of the soil, made of layers running parallel to the surface. These layers are known as **soil horizons,** each layer of soil has distinct features. These horizons are represented by letters O, A, E, C, B and R. **(Figure-1)**

1. O horizon

The O horizon is the upper layer of the topsoil which is mainly composed of organic materials such as dried leaves, grasses, dead leaves, small rocks, fallen trees, and other decomposed organic matter. This horizon of soil is often black brown or dark brown in color, and this is mainly because of the presence of organic content.

2. A horizon (Topsoil)

It is commonly referred to as the topsoil and typically ranges from 8-20 cm in thickness. It is a mineral horizon that formed at the surface or below an O-horizon and is characterized by an accumulation of organic matter mixed with the mineral fraction. As they are rich in organic matter, giving them a darker color than deeper layers. It has a large amount of sand and less clay.



(Figure-1): Soil profile

3. B horizon (Subsoil)

known as the zone of accumulation because chemicals such as iron, aluminum leached from the A horizon accumulate here. The accumulation of organic matter, chemical substances, and mineral particles in the lower horizons of soil from the upper horizons because of the downward movement of water is called **illuviation.** The B horizon has a lighter color, less organic matter and more clay than the A horizon.

4. C horizon (Parent Soil)

It lacks the properties of the A and B horizons because it is influenced less by the soil-forming processes. It is usually the parent material of the soil. The layer indicated with the absence of organic matter and microbial activities.

5. R horizon (Rocky bed)

The R horizon is the underlying bedrock, such as limestone, sandstone, or granite. It is found beneath the C horizon.

Soil texture and soil structure

Soil texture and soil structure are both unique properties of the soil that have a profound effect on the behavior of soil, such as water holding capacity, nutrient retention and supply, drainage, and nutrient leaching.

Soil texture is one of the most important physical properties of soil. Soil texture is the proportion of sand, silt, and clay particles that make up soil, these particles are distinguished solely by size. Table.1 illustrated soil particles size.

Table (1) Soil Texture Particles size

Type of Soil Particles	Size range/mm
Sand	**0.05 to 2.0**
Silt	**0.002 to 0.05**
Clay	**less than 0.002**

The texture of the soil is important because it determines soil characteristics that affect plant growth, from these characteristics are water-holding capacity, permeability and soil workability.

Types of soil

1. Sandy Soil

Has a large particle size. This soil is known as the poorest type of soil for agriculture and growing plants as they have very low nutritional value and poor water holding capacity.

2.Silt Soil

It is known to have much smaller particles compared to sandy soil and is made up of rock and other mineral particles, which are smaller than sand and larger than clay. It is the smooth and fine quality of the soil that holds water better than sand. The silt soil is more fertile compared to the other three types of soil. Therefore, it is also used in agricultural practices to improve soil fertility.

3.Clay Soil

Is mainly composed of the smallest particles of soil, which are densely packed with very little, or no airspace and they effectively retain water. This soil is not suitable for growing plants as it is harder for moisture and air to penetrate the soil.

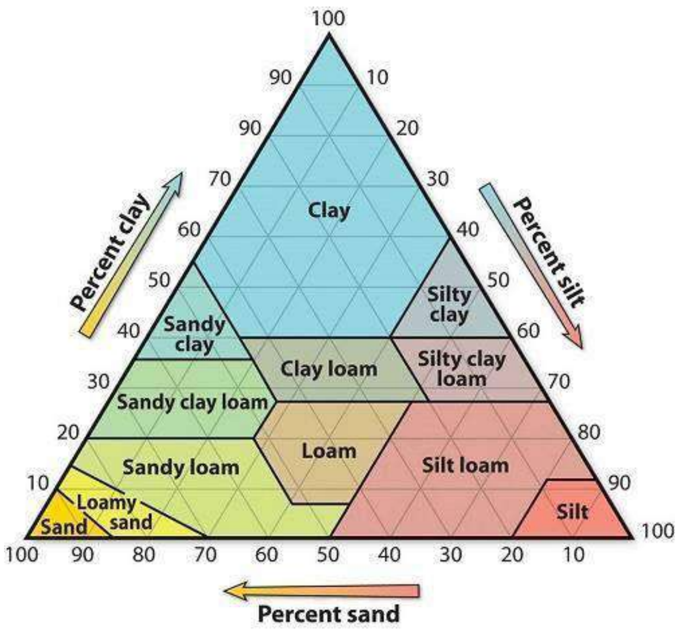
4.Loamy Soil

It is a combination of sand, silt and clay such that the beneficial properties from each are included. It can retain moisture and nutrients; hence, it is more suitable for farming. This soil is also referred to as agricultural soil.

Textural triangle

Textural class a grouping of soils based upon the relative proportion of sand, clay and silt . Soil with the finest texture is called clay soil, while soil with the coarsest texture is called sands. However, a soil that has a relatively even mixture of sand, silt, and clay and exhibits the properties from each separate is called loam.

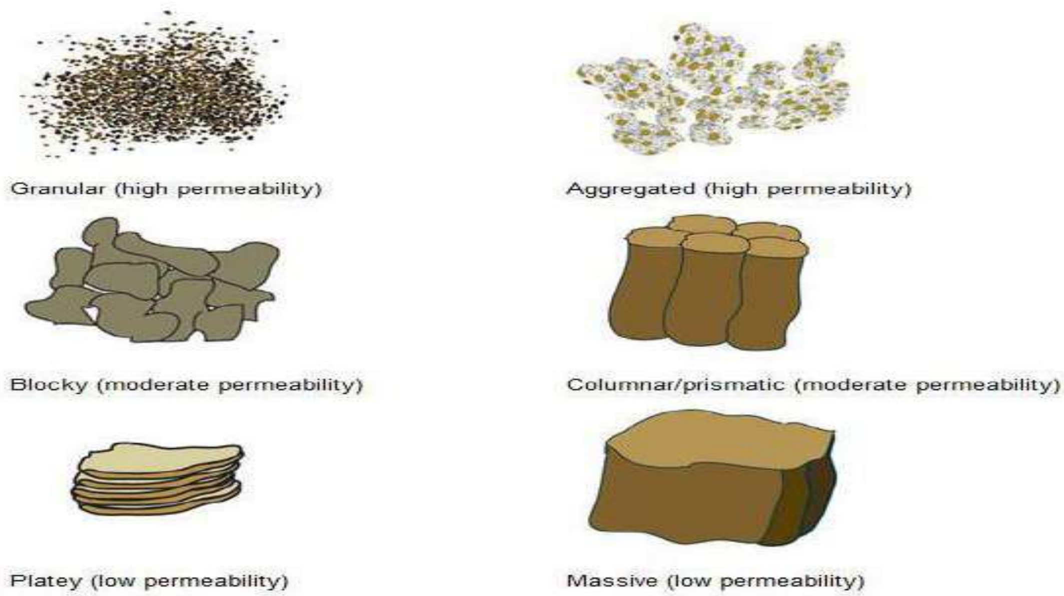
There are different types of loams, based upon which soil separate is most abundantly present. the textural triangle used to determine the soil type (**Figure-2**)



(Figure – 2) Soil texture triangle for determination of soil

Soil structure: is the arrangement of the soil particles into aggregates of various sizes and shapes. Aggregates that occur naturally in the soil are referred to as peds.

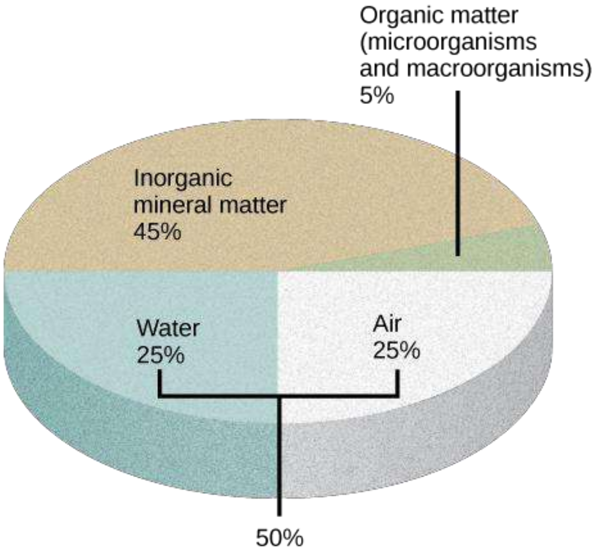
Good structure is important as it allows air movement through the soil. Soil air and water are vital for healthy plant growth and nutrient supply. The various types of soil structures are provided in **Figure.3**



(Figure – 3) Types of soil structures

Soil composition

The basic components of soil are minerals, organic matter, water and air. The typical soil consists of approximately 45% mineral, 5% organic matter, 25% water, and 25 % air. (**Figure. 4).** Good healthy soil has sufficient air, water, minerals and organic material to promote and sustain plant life.



(Figure -4) The four major components of soil

• Inorganic materials

In most soil types of inorganic materials (mineral particles) represent about 45% of total soil volume. The inorganic material of soil is composed of rock, slowly broken down by weathering and biogeochemical factors into smaller particles that vary in size. Soil particles that are 0.005 to 2 mm in diameter are sand. Soil particles between 0.002 and 0.05 mm are called silt, and even smaller particles, less than 0.002 mm in diameter, are called clay. Some soils have no dominant particle size, containing a mixture of sand, silt, and humus; these soils are called loams.

Based on its chemical nature the mineral portion of soil can be divided into two groups:

1- Silicate minerals

Minerals are composed of silicate groups. They are the largest and most important class of minerals and makeup approximately 90 % of Earth's crust, quartz is the most common silicate minerals.

2- Non – Silicate minerals

Minerals that are not composed of silicate groups. Makeup only 10% of Earth's crust such as copper, silver and gold.

- **Water and Air**

Soil particles pack loosely, forming a soil structure filled with pore spaces, these pores contain soil solution (water) and gas (air). Water and air in soil vary significantly with soil texture, weather, and plant uptake of water, but their percentage together in most of the soil types is about 50 % of total soil volume.

- **Soil water:**

Comes from rain, snow, dew and irrigation. Soil water serves as a solvent and a carrier of nutrients for plant growth. The microorganisms inhabiting the soil also require water for their metabolic activities. Percentage of soil – water is about 25 % total volume of soil.

Soil water amount affected by many factors:

1. Porosity:

Soil porosity refers to the space between soil particles, which consists of various amounts of water and air, porosity depends on both soil texture and structure, for example, fine soil has small but numerous pores than coarse soil. A coarse soil has bigger particles than fine soil, but it has less porosity. Water can be held tighter in small pores than in large ones, so fine soils can hold more water than coarse soil.

2. Infiltration:

Water infiltration refers to the movement of water from the soil surface to the soil profile. Soil texture – structure, slope, and gravitation have the largest impact on filtration rate, water moves by gravity into the open pore space in the soil, the size of soil particles and their spacing determine how much water can flow in.

3. Permeability:

Soil permeability refers to the movement of air and water through the soil, which is important because it affects the supply of root – zoon air, moisture, and nutrients available for plant uptake. Pore size and the number of pores closely relate to soil texture and structure, influence soil permeability.

Sandy soils are known to have high permeability, which results in high infiltration rates and good drainage. Clay textured soils have small pore spaces that cause water to drain slowly through the soil. Clay soils are known to have low permeability, which results in low infiltration rates and poor drainage.

Soil air:

Apart from soil pores which not occupied with water are filled with air. Compared with atmospheric air, the soil is lower in oxygen and higher in carbon dioxide, because CO_2 is continuously recycled by microorganisms during the process of decomposition of organic matter.

Soil air comes from the external atmosphere and contains nitrogen, oxygen, CO_2, and water vapor (CO_2> O^2).

CO_2 in soil air is 0.3 more than atmosphere air (0.03%). Soil aeration plays important role in plant growth, microbial population, and microbial activity in the soil. A good aerated soil types lead to complete oxidation of organic matter and characterized with high redox potential capacity, which offers e^- and H^+ donor and acceptors, results in thriving of aerobic and facultative microorganisms, but poor aerated soil types (saturated soils), which featured by low redox potential capacity cause continues to reduce of NO^{3-} and SO_4^{2+} and accumulation of some harmfully intermediate like CH_4, that affected soil fertility and increase of anaerobic microorganisms population.

Organic matter

Soil organic matter (SOM) is the fraction of the soil that consists of plant or animal tissue in various stages of breakdown (decomposition) and microorganisms and their excretions. During the decomposition process, microorganisms convert the carbon structures of fresh residues into transformed carbon products in the soil. There are many different types of organic molecules in the soil. Some are simple molecules that have been synthesized directly from plants or other living organisms. These relatively simple chemicals, such as sugars, amino acids, and cellulose are readily consumed by many organisms. For this reason, they do not remain in the soil for a long time. Other chemicals such as resins and waxes also come directly from plants but are more difficult for soil organisms to break down. Generally, the proportion of SOM in the soil ranging from 3 – 5 % of total soil volume.

Non – living organic matter can be considered to exist in two distinct pools:

1. **Non-humic Substances:** substances are released directly from cells of fresh residues, such as proteins, amino acids, sugars, and starches. This part of soil organic matter is easily decomposed fraction.

2. **Humic Substances:** Successive decomposition of dead material and modified organic matter results in the formation of a more complex organic matter called humus. This process is called humification. Because of the complex structure of humic substances, humus cannot be used by many microorganisms as an energy source and remains in the soil for a relatively long time. Humus affects soil properties. As it slowly decomposes, it colors the soil darker; increases soil aggregation and aggregate stability; increases water and nutrient retention which increases soil fertility and allows the growth of the plant, about 35-55 percent of the non-living part of organic matter is humus.

• Soil Living organic matter (Soil Biota)

Soil biota consists of micro-organisms (bacteria, fungi, archaea and algae), soil animals (protozoa, nematodes, mites, springtails, spiders, insects, and earthworms) and plants. Microorganisms form a very small fraction of soil mass and occupy a volume of less than 1%, in the upper layer of soil (topsoil up to 10 – 30 cm depth i.e., Horizon A), the microbial population is very high which decreases with depth of soil. Each organism or group of organisms is responsible for a specific change/ transformation in the soil. The final effect of various activities of microorganisms in the soil is to make the soil fit for the growth and development of higher plants.

Living organisms present in the soil are grouped into two categories as follow:

1. Soil flora (microflora) e.g. Bacteria, Fungi, Actinomycetes, and Algae.
2. Soil fauna animals include macrofauna (e.g., Earthworms), mesofauna (e.g., Mites), microfauna (e.g., protozoa, nematodes).

Soil Microflora

1- Bacteria:

These are unicellular organisms without organelles or nuclei they are one of the simplest forms of life. The size ranges from 1-5 microns. The shape varies from cocci (round shaped) to bacilli (rod shaped) and to spiral.

In terms of population, they are probably the most numerous microbes whose population ranges from a few hundred to 3 billion per gram of soil. They are very versatile in their metabolic activities some can use simple inorganic materials as an energy source while others are heterotrophic. Some bacteria need oxygen for their respiration others are anaerobic and some can adapt to the presence or absence of oxygen.

Based on their origin soil bacteria are classified as:

- Indigenous (true resident), or autochthonous.
- Invaders or allochthonous.
- Transient

Importance:

1. Are very important in the general decomposition of organic matter in the soil.
2. They carry out specific functions important in nutrient cycling such as nitrification.
3. A group of bacteria is important in nitrogen fixation- conversion of atmospheric nitrogen to plant available forms.
4. Biotransformation of chemicals and biogas formation.

Examples of some soil bacteria: *Agrobacterium, Arthrobacter, Bacillus, Alcaligenes, Erwinia, Clostridium, Nitrosomonas, Nitrobacter, Rhizobium and Thiobacillus.*

2- Fungi

They have well developed organelles including nuclei, mitochondria, they are more developed than bacteria. The most important characteristic of fungi is the possession of a filamentous body consisting of strands of hyphae. They about 5 µm in diameter. Almost all fungi are heterotrophic in nature and all are aerobic thus they do not occur in a diverse environment as bacteria.

Importance:

- Production of fungistatic products including antibiotics.
- Degrade some of the tough plant residues, like lignin.
- They are important in processes leading to humus formation.
- Contributes to soil aggregation that protects soil particles from weathering effects.
- Support soil microenvironments bio balance by their feeding practices on protozoa and nematodes.
- Some fungi form a symbiotic association with roots of higher plants (Mycorrhiza).

Some of soil fungi are: *Alternaria, Aspergillus, Cladosporium, Helimenthosporium, Humicola, Fusarium and Phytophthora.*

3- Actinomycetes

Structurally, these organisms lie between bacteria and fungi, they bear similarity to bacteria in terms of cell size and structure characteristics, and they are filamentous organisms like fungi. They are the next populous in the soil after bacteria, the number ranging from 10^5 - $4x10^6$ cell/g of soil the organisms prefer moist and well aerated soil. They are sensitive to acidic conditions with optimum pH ranging from 6 – 7.5.

Importance

- They are important in the decomposition of organic matter, like cellulose, protein, and fats.
- Some actinomycetes produce antibiotics e.g., *Streptomyces sp.*
- Contribute to humus formation.

The important members of actinomycetes are:*Actinomyces, Streptomyces, Nocordia, Micromonospora, Actinoplans and Thermoactinomycetes.*

4- Algae

Algae grow where an adequate amount of moisture and light present due to their need for the photosynthesis process, most of them prefer growth in neutral to alkaline soil (pH 7 – 10). The prominent genera in soil are ***Anabaena, Nostoc, Calothrix*** **and** ***Oscillotoria.***

Algae roles in the soil can be listed as the following:

1- Revolutionized the field of agriculture due to their photosynthetic capacity, which acts as a source of carbonic and nitrogenous organic matter in the soil.

2- Many algae species used commercially as biofertilizers.

3- Soil algae used in the reclamation of sodas soil and alkaline soil types.

Rhizosphere

The rhizosphere is the narrow region of soil around the plant root that is influenced by several factors like the root exudates and the associated soil microorganisms. As plant roots grow through the soil, they release water soluble compounds such as amino acids, sugars and organic acids that supply food for the microorganisms. The food supply means microbiological activity in the rhizosphere is much greater than in soil away from plant roots. In return, the microorganisms provide nutrients for the plants. All this activity makes the rhizosphere the most dynamic environment in the soil.

The rhizosphere can be divided into three zones:

1- The endorhizosphere is the plant's internal tissues includes portions of the cortex and endodermis in which microbes can occupy the "free space between cells.

2- Rhizoplane is the medial zone directly adjacent to the root.

3- Exorhizosphere which extends from the rhizoplane out into the soil.

The rhizospheric M.O. have either beneficial or harmful effects on the development of plants.

Possible effects of rhizospheric M.O are:

- The M.O. catalyze the reactions in the rhizosphere and produce CO_2 and form organic acids that in turn solubilize the minerals to uptake by plants.

- Aerobic bacteria utilize O_2 and produce CO_2, therefore lower O_2 and increase CO_2 tension that reduce roots elongation and nutrient and water intake.

- Some rhizospheric M.O. secretes plant regulators such as indole acetic acid, gibberellins and Cytokinins.

- Rhizospheric M.O. promote plant nutrition as bacteria like *Rhizobium* act as phosphate solubilizing bacteria increase the availability of accumulated phosphate, increase the efficiency of biological nitrogen fixation and render availability of iron and zinc through the production of plant growth-promoting substances.

- Rhizospheric M.O. protects plants against pathogens indirectly by control of pathogens (biocontrol) via synthesis of antibiotics or secondary metabolite-mediated induced systemic resistance.
- The products of rhizospheric zone M.O. metabolism sometimes have toxic effects on plants developing, these termed the phytotoxins.

Element cycles

Earth's biosphere can be thought of as a sealed container into which nothing new is ever added except the energy from the Sun. Since new matter can never be created, it is essential that living things be able to reuse the existing matter again and again.

The six most common elements in organic molecules (carbon, nitrogen, hydrogen, oxygen, phosphorus, and sulfur) take a variety of chemical forms. They may be stored for long or short periods in the atmosphere, on land, in water, or beneath the Earth's surface, as well as in the bodies of living organisms. Geologic processes such as weathering of rocks, erosion and water drainage, all play a role in this recycling of materials, as do interactions among organisms. The ways in which an element moves between its various living and non-living forms and locations is called **a biogeochemical cycle.**

The most important **biogeochemical cycles** are the **carbon cycle**, **nitrogen cycle, oxygen cycle, phosphorus cycle, sulfur cycle** and **the water cycle.**

Carbon cycle

The carbon cycle is one of several recycling processes, but it may be the most important process since carbon is known to be a basic building block of life, carbon is the basis of carbohydrates, proteins, lipids, and nucleic acids all of which form the basis of life on Earth. All the carbon we currently have on earth is the same amount we have always had. The carbon cycle is nature's way of reusing carbon atoms, which travel from the atmosphere into organisms in the earth and then back into the atmosphere over and over again. Carbon occurs in nature into two main states: complex carbonated organic compounds and inorganic carbon, most of the inorganic carbon is in form of **CO_2.** CO_2 makes up only about 0.04% of the atmospheric gases, on Earth most carbon is stored in rocks and sediments, while the rest is located in the ocean, atmosphere, and in living organisms. These are the reservoirs, through which carbon cycles. (figure - 1)

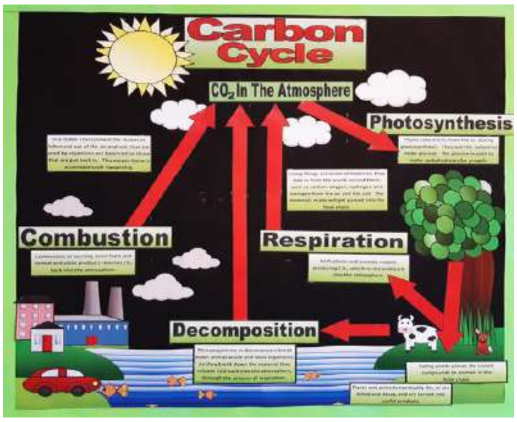


Figure -1: The carbon cycle.

Steps of the Carbon Cycle

There are different steps by which carbon is moved from one form to another, the major of the Carbon Cycle is photosynthesis, respiration, decomposition and the combustion of fossil fuels.

1. **Photosynthesis**

Carbon exists in the atmosphere as carbon dioxide (CO2). It first enters the ecological food web (the connected network of producers and consumers) when photosynthetic organisms, (such as Plants, algae, and a group of bacteria called cyanobacteria), absorb carbon dioxide, the plants then fix or capture the carbon dioxide and can convert it into simple sugars like glucose through the photosynthesis. Plants store and use this sugar to grow and reproduce. When plants are eaten by animals, their carbon is passed on to those animals. Since animals cannot make their own food, they must get their carbon either directly by eating plants or indirectly by eating animals that have eaten plants.

2. **Respiration.**

Respiration is the next step in the cycle, it occurs in plants, animals, and even decomposers. The animal respiration is taking in oxygen (and releasing carbon dioxide) and oxidizing its food (or burning it with oxygen) in order to release the energy the food contains. So the carbon is returned to the atmosphere as carbon dioxide. Carbon atoms that started out as components of carbon dioxide molecules have passed through the body of living organisms and been returned to the atmosphere, ready to be recycled again.

3. **Decomposition.**

Decomposition is the process by which dead organic substances are broken down into simpler organic or inorganic matter such as carbon dioxide, water, simple sugars and mineral salts. The organisms that decompose organic matter are called decomposers. Decomposers (fungi, bacteria, actinomycetes and invertebrates such as worm) are microorganisms can feed on the rotting remains of plants and animals. It is their job to consume both waste products and dead matter, they also return carbon dioxide to the atmosphere by respiration. Decomposers not only play a key role in the carbon cycle, but also break down, remove, and recycle what might be called nature's garbage.

The rate of decomposition is affected by soil temperature, moisture, aeration and food availability.

Generally, decomposition is either aerobic and anaerobic.

- **Aerobic decomposition**

Aerobic decomposition is the process by which organic materials decompose in the presence of oxygen. Most heterotrophic microbes easily utilize aerobically soil organic compounds to get their energy and nutrients.

$$\text{Organic compound} + O2 \xrightarrow{\text{Mineralization}} CO2 + H_2O + \text{energy} + \text{inorganic nutrients}$$

Immobilization

recycled again to atmosphere **Assimilated by living organisms**

Mineralization: Is the process by which organic matter is decomposed to release simpler, inorganic compound (e.g., CO_2, NH_4, SO_4, Mg).

Immobilization: The nutrients that are converted into biomass become temporarily "tied up" from nutrient recycling until the organism dies, at which time the **C** released back into the environment via decomposition.

- **Anaerobic decomposition.**

The anaerobic decomposition (O_2 free) is a complex biochemical reaction carried out in a number of steps by several types of microorganisms, during the process a gas mainly composed of methane and CO_2 is produced.

There are four basic steps of anaerobic decomposition. (Figure-2)

1) hydrolysis.

2) acidogenesis (Fermentation).

3) acetogenesis.

4) methanogenesis.

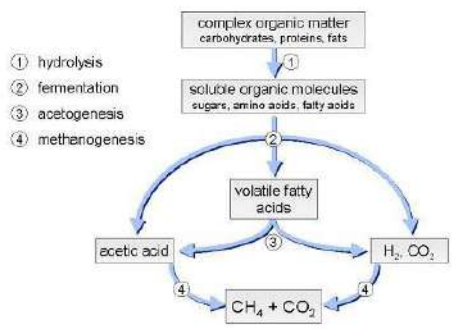


Figure-2: Anaerobic decomposition steps.

Hydrolysis: Complex organic matter (such as protein, carbohydrates) is decomposed into simple soluble organic molecules (such as amino acids, sugar) by water and enzymes. The enzymes are exoenzymes (protease, cellulase) from several bacteria and fungi.

Acidogenesis (Fermentation): During acidogenesis, soluble monomers are converted into small organic compounds, such as short chain (volatile) acids (propionic, formic, lactic, butyric, succinic acids), ketones (glycerol, acetone), and alcohols (ethanol, methanol).

Acetogenesis: The acidogenesis intermediates are attacked by acetogenic bacteria; the products from acetogenesis include acetic acid, CO_2, and H_2.

Several bacteria contribute to acetogenesis, including *Clostridium spp., peptococcus anaerobes, lactobacillus,* and *actinomyces.*

Methanogenesis: The last phase of anaerobic digestion is the methanogenesis phase.Several reactions take place using the intermediate products from the other phases, with the main product being methane. Accomplished by certain soil bacteria known as **Methanogenic bacteria.** Methanogenic bacteria are a part of the carbon cycle, anaerobically, they convert the acetic acid made by acetogenic bacteria to CH_4, CO_2, or convert alcohol (methanol or ethanol) to CH_4, or derived CH_4 from reduction CO_2.

$$\mathbf{2CH_3CH2OH + CO_2 \rightarrow 2CH_3COOH + CH_4}$$

$$\mathbf{CH_3COOH \rightarrow CH_4 + CO_2}$$

$$\mathbf{CH_3OH \rightarrow CH_4 + H_2O}$$

$$\mathbf{CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O}$$

Main soil methanogenic bacteria are:

Methanococcus, Methanobacteria and Methanosarcina.

To complete the recycling pattern another group of methane bacterium called **Methanotrophes** can reoxidize released **CH_4** again to **CO_2**, like ***Methylomonas.*** This conversion also yields water and energy.

Combustion.

A process of burning fossil fuel, carbon in coal, oil and natural gas returns to the atmosphere as CO_2 when these fossil fuels are burned.

Other soil autotrophic bacteria can participate in the cycling of carbon by oxidizing carbon monoxide CO. This gas is relatively rare under ordinary conditions, released from some activities, commonly from partial combustion. Extremely poisonous for most aerobic organisms, including man, it is used as a source of energy and carbon by at least one bacterial species Carboxydomonas that oxidize CO to CO_2.

There is an increase of CO_2 in the atmosphere by about one – third, and it continues to rise. Like to CO_2 methane concentration is likewise increasing about 1% per year, as the result of human activities (combustion of fossil fuel. The term greenhouse gases describe the ability of these gases to trap heat within Earth's atmosphere, leading to an increase planet’s temperature, a phenomenon known as the greenhouse effect. Soil microorganisms play a role in the generation of each of these gases.

Nitrogen cycle

Nitrogen is a naturally occurring element that is essential for growth and reproduction in both plants and animals because nitrogen is a component of proteins and nucleic acids, that are essential to life on Earth. Although 78 % by volume of the atmosphere is nitrogen gas, this abundant reservoir exists in a form unusable by most organisms. Through a series of microbial transformations, however, nitrogen is made available to organisms, which in turn ultimately sustains all organism's life.

Nitrogen gas exists in nature in both organic and inorganic forms. The movement of $\mathbf{N_2}$ between the atmosphere, terrestrial and marine ecosystems in different forms is described by the **Nitrogen Cycle**, microorganisms are the key element in the cycle, provide different forms of nitrogen compounds by their metabolic activities.

In general, the nitrogen cycle has several steps: (Figure-1)

1. Nitrogen fixation.
2. Ammonification.
3. Nitrification.
4. Denitrification.

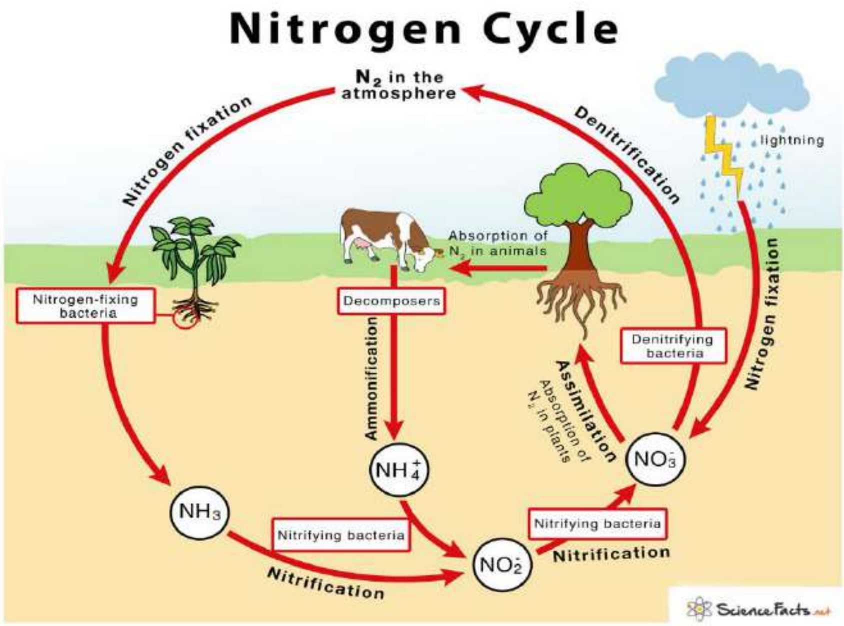


Figure-1: Nitrogen cycle steps.

1. Nitrogen fixation

Nitrogen fixation is natural process, it occurs either biologically or non-biologically by which the (N_2) in the atmosphere is converted into ammonia or related nitrogenous compounds that can be assimilated by plants.

Types of Nitrogen Fixation

- **Atmospheric fixation:** A natural phenomenon where the energy of lightning breaks the nitrogen molecules into nitrogen oxides and is then used by plants.

- **Industrial nitrogen fixation:** This is a man-made alternative that aids in nitrogen fixation using ammonia. atmospheric nitrogen and hydrogen (usually derived from natural gas or petroleum) can be combined to form ammonia (NH_3), which is converted into various fertilizers such as urea.

- **Biological nitrogen fixation (BNF):** Most nitrogen fixation occurs naturally in the soil by microorganisms called diazotrophs that are diverse groups of prokaryotes including free – living and symbiotic bacteria, cyanobacteria and actinomycetes. Common nitrogen fixing microorganism is given in table (1), and the phenomenon of this activity is known as diazotrophy.

Table -1: N_2 fixing microorganisms

Nitrogen fixing microorganisms					
Free living M.O.				**Symbiotic M.O.**	
Aerobic	Anaerobic	Facultative Anaerobic	Photosynthetic	Legumes	*Non-legumes*
Azotobacter, Azomonas and *Beijerinckia*	*Clostridium pasteurianum* and *Desulfovibrio*	*Aerobacter* and *Klebsiella*	*Rhodomicrobium* and *Rhodopseudomonas*	*Rhizobium*	*Frankia*

The biological conversion of atmospheric nitrogen taken place with the help of an enzyme called nitrogenase, which combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by bacteria to make their own organic compounds. Nitrogenase is a metalloprotein that consists of two proteins – an iron protein and a molybdenum-iron protein but sometimes contains vanadium instead of molybdenum.

The nitrogenase enzyme is highly sensitive to oxygen. It is inactivated when exposed to oxygen, although this is not a problem for anaerobic bacteria, it could be a major problem for the aerobic species, but These organisms have various methods to overcome the problem. For example, *Azotobacter* species have the highest known rate of respiratory metabolism of any organism, so they might protect the enzyme by maintaining a very low level of oxygen in their cells.

The fixation process requires such a large amount of energy (ATP), it needs about 16 moles of adenosine triphosphate (ATP) for the reduction of each mole of nitrogen and they obtain this energy by oxidation of organic molecules. Free-living microorganisms which are non-photosynthetic in nature, receive these molecules from other organisms, while photosynthetic microorganisms, such as cyanobacteria, use sugars synthesized in the process of photosynthesis. Symbiotic nitrogen-fixing microorganisms obtain these compounds from their host plants.

Symbiotic N_2 fixation microorganisms

Rhizobium is the most well-known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of nodules where the nitrogen fixation takes place. The bacterium's enzyme system supplies a constant source of reduced nitrogen to the host plant and the plant provides nutrients and energy for the activities of the bacterium. In the soil the bacteria are free living and motile, feeding on the remains of dead organisms. Free living rhizobia cannot fix nitrogen and they have a different shape from the bacteria found in root nodules. They are regular in structure, appearing as straight rods; in root nodules the nitrogen-fixing form exists as irregular cells called bacteroids which are often club and Y-shaped.

Root nodule formation

The actual process of nodulation is a very coordinated effort between legume and the *Rhizobium* in soil. One *Rhizobium* strain can infect certain species of legumes but not others Specificity genes determine which *Rhizobium* strain infects which legume.

The initial interaction between the host plant and free-living rhizobia is the release of a variety of chemicals by the root cells into the soil such as **flavonoids**, rhizobia which colonize the soil in the near of the root hair will respond to the flavonoids, so the bacteria attracted to the root hair and attach themselves to the root hair surface and secrete specific nod factor, these stimulate the hair to curl. Rhizobia then invade the root through the hair tip which they induce the formation of an **infection thread**.

Rhizobium multiplies within the infection thread which it grows through the root hair cells and penetrates through several layers of cortical cell and then rupture, the bacteria released from infection thread into the cytoplasm of the host cell and after the bacteria undergo alteration morphologically into larger forms called **Bacteroids**, cortex cells in turn rapidly divided to form a tumor like nodules of Bacteroids – packed cells, each nodule contains thousands of bacteroids. The nodules begin to fix nitrogen by the nitrogenase that catalyzes the conversion of atmospheric nitrogen to ammonia.

The nitrogenase complex is sensitive to oxygen, becoming inactivated when exposed to it. *Rhizobium* controls oxygen levels in the nodule with **leghaemoglobin**. This red, iron-containing protein has a similar function to that of haemoglobin, binding to oxygen. This provides sufficient oxygen for the metabolic functions of the bacteroids but prevents the accumulation of free oxygen that would destroy the activity of nitrogenase.

Free living N_2 fixation microorganisms

Many heterotrophic or autotrophic microorganisms live in the soil and fix nitrogen without direct interaction with other organisms. The heterotrophic M.O. must find their own source of energy to fix N_2, typically by oxidizing organic molecules released by other organisms or from decomposition. There are some free-living organisms that have chemolithotrophic capabilities and can thereby utilize inorganic compounds as a source of energy. *Azotobacter* is best example of free -living aerobic nitrogen fixers.

2. Ammonification

Before we know what ammonification is, we first must understand proteolysis. Plants use the ammonia produced by symbiotic or non - symbiotic N_2 fixation microorganisms to make their nitrogen- containing compounds (such as proteins) then the animals eat the plants and the plant protein convert into animals' protein. upon death, plants and animals undergo microbial decay in the soil and the nitrogen released. Thus, the process of enzymatic break down of protein by the M.O. with the help of proteolysis enzyme is known as proteolysis.

$$\text{Protein} \xrightarrow{\textbf{Proteases}} \text{peptides} \xrightarrow{\textbf{peptidases}} \text{amino acids}$$

The most active microorganisms responsible for proteolysis are ***Pseudomonas, Bacillus, Proteus, Penicillium*** **and** ***Alternaria.***

Amino acids released during proteolysis undergo deamination in which amino group (NH_2) is removed. Thus, the process of deamination which leads to the production of ammonia or ammonium (NH_4^+) is known as **ammonification.** Ammonification usually occurs under aerobic conditions (known as oxidative deamination), it is represented as follows:

$$\underset{\textbf{Amino acid}}{RCHNH_2COOH} + O_2 \xrightarrow{\textbf{deaminase}} \underset{\textbf{Keto acid}}{RCOCOOH} + \underset{\textbf{Ammonia}}{NH_3} + \text{energy}$$

Ammonification is commonly brought about by ***Proteus, Micrococcus*** **and** ***Clostridium.***

3. Nitrification

In soil the liberated ammonia during ammonification is rapidly oxidized to nitrate by some soil highly specialized bacterial groups of strictly aerobic chemolithotrophs, this oxidation process termed nitrification, which occurs in two stages and each stage is performed by different bacteria as follows:

Stage 1: Oxidation of ammonia (NH_3) to nitrite (NO_2^-) and this process is known as **Nitrosofication.**

$$2\ NH_3 + 3O_2 \longrightarrow 2\ NO_2^- + 4H^+ + 2\ H_2O + \text{energy}$$

This stage performed by soil bacteria such as ***Nitrosomonas, Nitrosococcus*** **and** ***Nitrosovibrio.***

Stage 2: In this stage, nitrite is oxidized to nitrate by nitrite oxidizing - bacteria such as *Nitrobacter, Nitrococcus* and *Nitrospira* and several fungi such as *Penicillium* and *Aspergillus.*

$$2NO_2^- + O_2 \longrightarrow 2\ NO_3 + \text{energy}$$

The formed nitrate utilizes by plants as their primary source of N_2 nutrition. Nitrate that is not used for plant nutrition is susceptible to reduce to ammonia or nitrogen gas or lost through leaching depending on soil conditions.

Factors affecting nitrification

- **Soil pH:** Nitrification occurs over a wide pH range in soil, the optimal pH has been estimated to be between pH 6.5 to 8.8. Nitrification rates are slower in acid soils.
- **Soil Temperature:** The optimal temperature for nitrification ranges from 30° C – 35°C.
- **Soil aeration:** Since nitrification is an aerobic process, oxygen is necessary because it affects the M.O involved in the process.
- **Soil Moisture**: Nitrifying bacteria are sensitive to changes in soil water content since it influences the abundance and activity of bacteria.

4. Denitrification

Several heterotrophic bacteria can convert nitrate to nitrite and to ammonia, this is a reverse process of nitrification known as **denitrification.**

Nitrate reduction normally occurs under anaerobic soil conditions (waterlogged soil). Denitrification (dissimilatory nitrate reduction) consists of four steps and each step is carried out by an array of enzymes. Nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase are the four main enzymes involved in denitrification, the overall process of denitrification as follows:

$$\underset{\text{Nitrate}}{NO_3} \xrightarrow{\text{Nitrate reductase}} \underset{\text{Nitrite}}{NO_2} \xrightarrow{\text{Nitrite reductase}} \underset{\text{Nitric oxide}}{NO} \xrightarrow[\text{reductase}]{\text{Nitric oxide}} N_2O \text{ Nitrous oxide}$$

$$N_2O \xrightarrow{\text{Nitrous oxide reductase}} N_2$$

The most important denitrifying bacteria are ***Thiobacillus denitrificans, Micrococcus denitrificans, Pseudomonas*** **and** ***Achromobacter.***

Denitrification also called assimilatory nitrate reduction which leading to the production of ammonia as some of the M. O assimilate ammonia for the synthesis of amino acids and proteins.

Denitrification leads to the loss of nitrogen from the soil which results in depletion of an essential nutrient for plant growth, therefore it is an undesirable process for soil fertility and agricultural productivity, but it has major ecological importance since without denitrification the supply of nitrogen including N_2 of the atmosphere would have not got depleted and NO_3 (which are toxic) would have accumulated in the soil and water.

Sulfur cycle

Sulfur an essential element for the macromolecules of living things, is one of the components that make up proteins and vitamins. Proteins consist of amino acids that contain sulfur atoms. Sulfur is important for the functioning of proteins and enzymes that necessary for plants and animals.

Most of the earth's sulfur is tied up in rocks and salts or buried deep in the ocean in oceanic sediments. Sulfur occurs in nature and in the soil as organic forms such as proteins and inorganic forms such as sulfate, these elements undergo alteration between organic and inorganic forms, and between oxidative and reductive states.

The sulfur cycle is biogeochemical cycle in which the sulfur moves between rocks, waterways and living systems.

The sulfur is released by the weathering of rocks, once sulfur is exposed to the air, it combines with oxygen, and becomes sulfate (SO_4^{2-}). Plants and microbes assimilate sulfate and convert it into organic forms. As animals consume plants, the sulfur is moved through the food chain and released when animals and plants die and decompose. Sulfur also is released into the atmosphere by the burning of fossil fuels and volcanic activities.

The Role of Microorganisms in the Sulfur Cycle

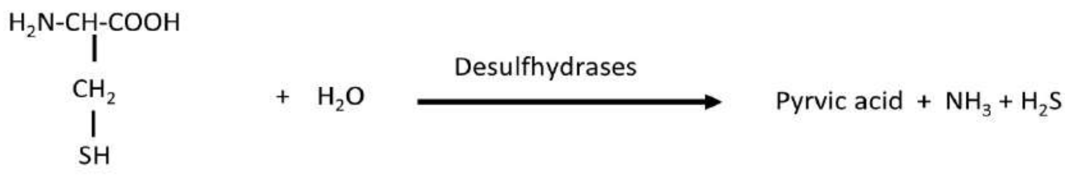
Microorganisms play an important role in the sulfur cycle as these organisms have specialized enzyme systems and mechanisms to form different sulfur compounds.

Sulfur is microbiologically metabolized in soil through different transformation processes:

- Mineralization (decomposition) of organic sulfur into inorganic forms, such as hydrogen sulfide (H_2S) and elemental sulfur.
- Oxidation of hydrogen sulfide, elemental sulfur (S°) and related compounds to sulfate.
- Reduction of sulfate to sulfide.
- Microbial immobilization of the sulfur compounds and subsequent incorporation intothe organic form of sulfur.

- **Mineralization (decomposition) of organic sulfur compounds**

Decomposition of large organic sulphur compounds to smaller units and finally into inorganic compounds by the microorganisms, as given below.



Cysteine

- **Oxidation of inorganic sulfur compounds**

Hydrogen sulfide (produced from sulfur-containing amino acids) oxidizes to produce elemental sulfur, which is carried out by photosynthetic bacteria from green sulfur bacteria such as *Chlorobium* and purple sulfur bacteria such as *Chromatium.*

$$CO_2 + 2H_2S \xrightarrow{\text{Light}} \underbrace{(CH_2O)\,n}_{\text{Carbohydrate}} + H_2O + \underbrace{2S^{\circ}}_{\text{Elemental sulfur}}$$

Elemental sulfur present in the soil cannot be utilized directly by the plants. Therefore, it is converted into sulfates by chemolithotrophic bacteria such as *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are the main organisms involved in the oxidation of elemental sulfur to sulfates.

$$2S^{\circ} + 3O_2 + 2H_2O \longrightarrow 2H_2SO_4 \longrightarrow 2H^{+} + SO_4^{2-}$$

Sulfur oxidation improves soil fertility, it results in the formation of sulfate, which can be used by the plants, while the acidity produced in sulfur oxidation helps to solubilize plants nutrients and lowers the pH of alkaline soils.

- **Reduction of Sulfates**

Sulfate produced by sulfur–oxidizing bacteria may be reduced to hydrogen sulfide by Sulfur – reducing bacteria. Sulfate- reducing occurs under anaerobic soil conditions (waterlogged soil). Sulfate reduction can be dissimilatory or assimilatory. In assimilatory sulfate reduction, the sulfate is reduced for the biosynthesis of amino acids and proteins. Sulfate reduction is dissimilatory sulfate reduction if the purpose of reducing the sulfate is to produce energy, in which sulfate in the absence of O_2 serves as terminal electron acceptors for anaerobic respiration.

The predominant sulfate-reducing bacteria in soil are *Desulfovibrio desulfuricans* and *Desulfotomaculum*.

Sulfate can be reduced to H_2S by sulfate-reducing bacteria and may diminish the availability of sulfur for plant nutrition, the dissimilatory sulfate reduction is not at all desirable from soil fertility and agricultural productivity.

Hydrogen sulfide produced by the reduction of sulphate is further oxidized anaerobically by some species of green and purple phototrophic bacteria (*Chlorobium* and *Chromatium*) to release elemental sulfur**.**

- **Immobilization of inorganic sulfate compounds**

In this process, microorganisms absorb inorganic sulfate and convert it into organic form for the synthesis of microbial tissue.

Phosphorus Cycle

Phosphorus is an important element for all living organisms. It forms a significant part of the structural framework of DNA and RNA. They are also an important component of the energy storage molecule ATP. Humans contain 80% of phosphorus in teeth and bones.

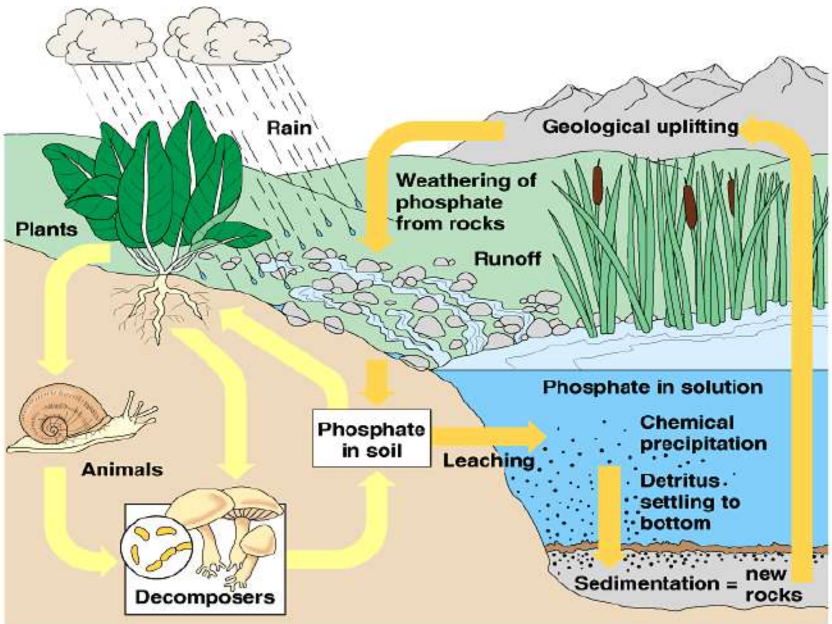
All Phosphorus on the Earth is derived from rock, which is the major reservoir of phosphorus in nature. Phosphorus can be found on earth in water, soil and sediments.

The phosphorus cycle is the process by which phosphorus moves through the lithosphere, hydrosphere, and biosphere.

Since phosphorus and phosphorus-containing compounds are present only on land, the atmosphere plays no significant role in the phosphorus cycle.

Since the main source of phosphorus is found in rocks, the first step of the phosphorus cycle involves the extraction of phosphorus from the rocks by weathering. Weather events, such as rain and other sources of erosion, resulting in phosphorus being washed into the soil, once in the soil, plants take up inorganic phosphate from the soil. The plants may then be consumed by animals. When the plants and animals die, they are decomposed by microorganisms During this process, the organic form of phosphorus is converted into the inorganic form, which is recycled to soil and water.

Phosphorus in the soil can end up in waterways and eventually oceans. Once there, it can be incorporated into sediments over time. As shown in figure-1



(Figure-1): Phosphorus cycle

Microorganisms are known to play a key role in transformations of phosphorus, these include:

- **Solubilization of inorganic phosphates compounds**

Phosphorus (P) is one of the essential elements that are necessary for plant development and growth. The P-content in average soils is about 0.05% (w/w) but only 0.1% of the total P is available to plants because most phosphorus complexes with calcium, iron and aluminium, thus generating insoluble phosphate salts present in the soil.

Fortunately, various kinds of bacteria and fungi have the ability to solubilize unavailable phosphate (P) to available forms. Such transformations increase P availability and promote plant growth. Phosphate Solubilizing Microorganisms (PSM) through various mechanisms of through various mechanisms of solubilization are capable of hydrolyzing organic and inorganic insoluble phosphorus compounds to soluble P form that can easily be assimilated by plants.

The main P solubilization mechanisms employed by PSM include the release of organic or inorganic acids as a side product of microorganisms' activities, these acids act on conversion insoluble salts to more available to plants, such as tricalcium phosphate [$Ca_3\ (PO_4)_2$]. is converted to dicalcium phosphate.

$$Ca_3\,(PO_4)_2 + 2HNO_3 \longrightarrow 2Ca\,(HPO_4)_2 + Ca\,(NO_3)_2$$

Production of H2S by bacteria which may react with iron phosphate and liberate orthophosphate, and the production of chelators, which can complex Ca, Fe, or Al all these mechanisms act on conversion insoluble salts to more available to plants.

The organisms involved in phosphate solubilization include *Pseudomonas, Bacillus, Rhizobium, Streptomyces, Penicillium and Aspergillus.*

- **Mineralization of organic phosphates compounds**

Organic phosphorus compounds (e.g., nucleic acids, phospholipids) are mineralized (decomposed) to inorganic phosphate ($H_2PO_4^-$or HPO_4^{2-}) forms of plant available Phosphate known as orthophosphates by a wide range of microorganisms that include *Bacillus, Arthrobacter, Streptomyces*, *Aspergillus* and *Penicillium*.

Phosphatases and phytases are the enzymes responsible for the degradation of phosphorus compounds.

Mineralization is highly influenced by soil moisture, temperature, pH and availability of organic phosphate.

- **Immobilization of phosphorus**

During immobilization, microorganisms convert inorganic forms to organic phosphate, which are then incorporated into their living cells.

Lec 6

An **aquatic ecosystem** is an ecosystem in a body of water. The two main types of aquatic ecosystems are marine ecosystems and freshwater ecosystems. Communities of organisms that are dependent on each other and on their environment live in aquatic ecosystems.

Aquatic microbiology is the science that deals with microscopic living organisms in fresh or salt water systems. While aquatic microbiology can encompass all **microorganisms** , including bacteria, viruses, and **fungi** and their relation to other organisms in the aquatic **environment** .

Bacteria, viruses, and fungi are widely distributed throughout aquatic environments. They can be found in fresh water rivers, lakes, and streams, in the surface waters and sediments of the world's oceans, and even in hot springs..

Microorganisms living in these diverse environments must deal with a wide range of physical conditions, and each has specific adaptations to live in the particular place it calls home. For example, some have adapted to live in fresh waters with very low salinity , while others live in the saltiest parts of the ocean. Some must deal with the harsh cold of arctic waters, while those in hot springs are subjected to intense heat. In addition, aquatic microorganisms can be found living in environments where there are extremes in other physical parameters such as pressure, sunlight, organic substances, dissolved gases, and water clarity.

Aquatic microorganisms obtain nutrition in a variety of ways. For example, some bacteria living near the surface of either fresh or marine waters, where there is often abundant sunlight, are able to produce their own food through the process of **photosynthesis** . Bacteria living at hydrothermal vents on the ocean floor where there is no sunlight can produce their own food through a process known as **chemosynthesis** , which depends on preformed organic **carbon** as an energy source. Many other microorganisms are not able to produce their own food. Rather, they obtain necessary nutrition from the breakdown of organic matter such as dead organisms.

Importance of aquatic microorganisms

Aquatic microorganisms play a vital role in the cycling of nutrients within their environment, and thus are a crucial part of the **food chain** . Many microorganisms obtain their nutrition by breaking down organic matter in dead plants and animals. As a result of this process of decay, nutrients are released in a form usable by plants. These aquatic microorganisms are especially important in the cycling of the nutrients **nitrogen** , **phosphorus** , and carbon. Without this **recycling** , plants would have few organic nutrients to use for growth.

In addition to breaking down organic matter and recycling it into a form of nutrients that plants can use, many of the microorganisms become food themselves. There are many types of animals that graze on bacteria and fungi. For example, some deposit-feeding marine worms ingest sediments and digest numerous bacteria and fungi found there. Therefore, these microorganisms are intimate members of the food web in at least two ways.

Humans have taken advantage of the role these microorganisms play in **nutrient** cycles. At **sewage treatment** plants, bacteria are cultured and then used to break down human wastes.

However, in addition to the beneficial uses of some aquatic microorganisms, others may cause problems for people because they are pathogens, which can cause serious diseases. For example, bacteria such as *Salmonella typhi*, *S. paratyphi*, and the Norwalk **virus** are found in water contaminated by sewage can cause illness. Fecal coliform (*E. coli*) bacteria and Enterococcus bacteria are two types of microorganisms that are used to indicate the presence of disease causing microorganisms in aquatic environments.

Specific zonations , microbial flora and microbial activity in water Column

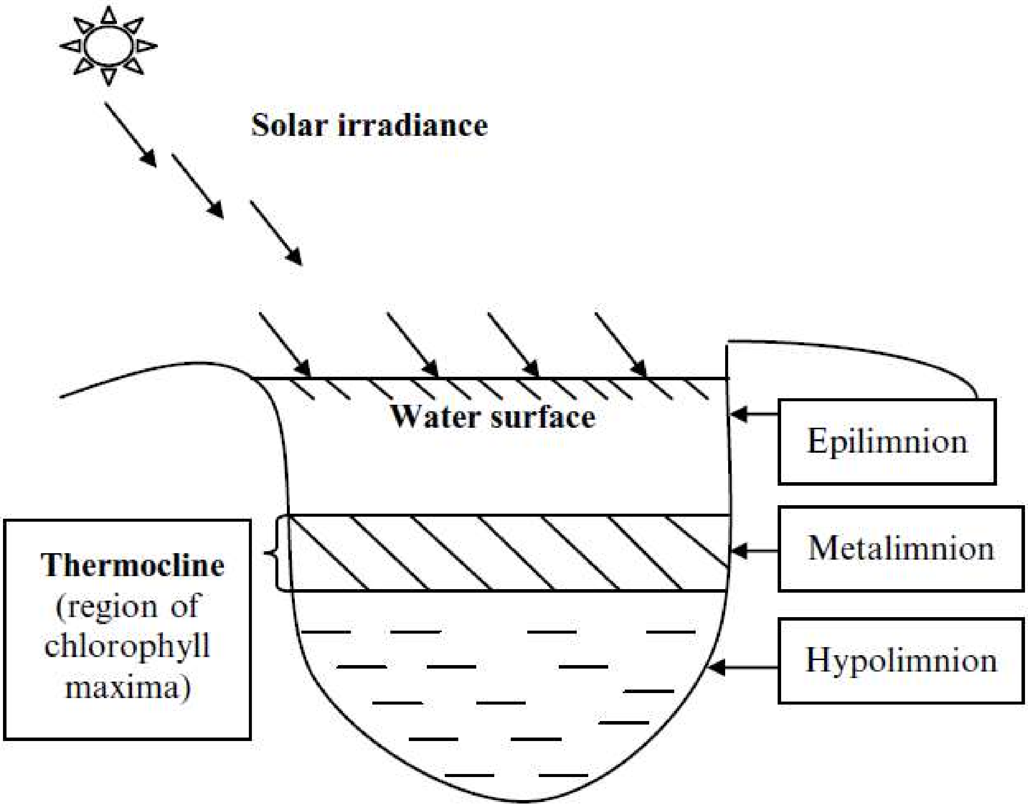


Figure :Specific zonations on the basis of light and temperature variations at different depths of the water column

Water surface :22-25 ° C

Epilimnion : 20-22° C

Metalimnion(Thermocline) : 7 ° C

Hypolimnion : -5° C

Sediments

A-Water surface and Epilimnion : Microbial **flora** consist of 1-photosynthetic bacteria , 2- cyanobacteria ,3- mesophilic contaminating bacteria , 4-psychrotrophic .

Microbial activity of this part of water column are :
1- photosynthesis , 2- Aerobic nitrogen fixation ,3- Aerobic decomposition of organic matter

B- Thermocline : Microbial **flora** consist of Psychrophilic facultative anaerobic bacteria .

Microbial activity of this part of water column aer Aerobic and Anaerobic decomposition .

C-Hypolimnion : Microbial **flora** consist of extrem psychrophilic anaerobic bacteria .

Microbial activity are : 1- Anaerobic nitrogen fixation bacteria (*Clostridium pasteurianum*), 2- Anaerobic decomposition of organic matter , 3- production of CH_4 ,H_2S,NH_3.

D-Sediments: Microbial **flora** consist of 1- Barophilic bacteria ,

2- Anaerobic bacteria ,H_2S ,CH_4 producing bacteria .

Microbial activity is Anaerobic decomposition of sediments

Lec 7

Microbial Water Pollution

Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans and ground waters). Water pollution occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds.

Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels. It has been suggested that it is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than14,000 people daily. An estimated 1,000 Indian children die of diarrheal sickness every day.Some 90 % of China's cities suffer from some degree of water pollution, and nearly 500 million people lack access to safe drinking water.

Main Sources of Water Microbial Pollution:

1-Sewage:

Most treated wastewater goes into rivers, lakes, and oceans. Occasionally, heavy rains overwhelm sewer systems, causing them to overflow increasing the risk of water borne-diseases.. This can put communities at risk from high concentrations of microbial pollutants in raw, untreated sewage. The mian source of water pollution with all microorganisms through dumping untreated sewage into water bodies. This is very dangerous because they contaminate the environment and water bodies and bring many deadly diseases.

2-Agricultural and Animal Wastes

Over the past few decades, the increase in population and advances made in farming technology has increased the demand for crops and livestock from the agricultural industry. This growth in agricultural production has resulted in an increase in contaminants polluting waterways.

Poorly managed animal feeding operations and dropping of dead animals into water bodies also poorly managed and ineffective application of irrigation water, and fertilizer(manure) are important sources of water pollution with microorganisms. Nearly 1.4 billion tons of animal manure are produced annually in the United States. These animal wastes carry concentrations of microbes as high as a billion organisms per gram of feces. Most are part of normal bodily flora, but some are potentially harmful to humans.

3- Industrial pollution: Most of .industrial wastes polluted the water with chemicals ,however some of these industries such as medical and food industries polluted the

water with microorganisms. Unesco classified the water pollution according to the bacterial pollution and organic pollution to seven classes as in the following table :

Pollution level	Bacterial pollution (bacteria per ml water	Organic pollution
Hypersaprobic	10^8	Heavy polluted
Polysaprobic β	$>10^6$	Clearly polluted
α-mesosaprobic	10^5-10^6	polluted
β- mesosaprobic	10^2-10^5	Semi polluted
Oligosaprobic	$<10^2$	Little polluted
Antisaprobic	None	Polluted with toxic chemicals
Katharotic	Very low	Low organic matter Low metals (spring water)

Water-associated diseases

Water-associated diseases can be classified under 4 different categories:

1-Water-borne diseases

Definition: water-borne diseases are diseases caused by the ingestion of water contaminated by human or animal faeces or urine containing pathogens**.**Many bacteria, viruses, protozoa and parasites can cause disease when ingested. The majority of these pathogens derive from human or animal faeces, and are transmitted through the faecal-oral route.

2- Water-washed diseases

Definition: water-washed diseases are diseases caused by inadequate use of water(water scarcity) for domestic and personal hygiene.
Control of water-washed diseases depends more **on the quantity of water than the quality**. Most of the diarrhoeal diseases should be considered to be water-washed as well as water-borne. Four types of water-washed diseases are considered here:**A-** soil-transmitted helminths; **B** -acute respiratory infections (ARI); **C**-skin and eye diseases; and **D-**diseases caused by fleas, lice, mites or ticks. For all of these, washing and improved personal hygiene play an important role in preventing disease transmission. This type of diseases more common in tropical, 3rd world countries where water supplies may be scarce.

3- Water-based diseases

Definition: water-based diseases are infections caused by parasitic pathogens found inaquatic host organisms ,or diseases caused by pathogens that have a complex life-cycle which involves an intermediate aquatic host**.** All of these diseases are caused by worms, e.g. Schistosomiasis caused by the Schistosoma worm which uses aquatic snails as an intermediate host, also the Guinea worm (*Dracunculus medimensis*) which uses a small crustacean as an intermediate host.

4- Water-related diseases

Definition: water-related diseases are caused by insect vectors which either breed in water or bite near water.Very difficult to control and diseases are very severe .

Examples:

1-Yellow fever (viral disease) is transmitted by the mosquito.
1- Dengue (viral) carried by the mosquito (breeds in water).
3-Malaria is caused by a protozo and is also spread by a mosquito .
4-Trypanosomiasis (Gambian sleeping sickness) is also caused by a protozoan transmitted by the riverine Tetse fly .

Classification	Transmission Details	Examples
Waterborne	Fecal-Oral Route	Cholera, Typhoid, Hepatitis A
Water-washed	Water-Hygiene	Diarrhea, Trachoma, Scabies
Water-based	Water-Contact	Guinea Worm
Insect Vector	Insect-Blood	Malaria, River Blindness

Indicators of microbial water quality

To determine if a given water supply is safe, the source needs to be protected and monitored regularly. There are two broad approaches to water quality monitoring for pathogen detection.

The first approach is direct detection of the pathogen itself, for example, the protozoan *Cryptosporidium parvum*. While it will be more accurate and precise if specific disease-causing pathogens are detected directly for the determination of water quality, there are several problems with this approach. **First**, it would be practically impossible to test for each of the wide variety of pathogens that may be present in polluted water. **Second,** even though most of these pathogens can now be directly detected, the methods are often difficult, relatively expensive, and time-consuming . Instead, water monitoring for microbiological quality is primarily based on a second approach, which is to test for indicator organisms

This concept of indicator organisms was introduced in 1892 and is the basis for most microbiological quality standards in water today.

Indicator Microorganism :A nonpathogenic microorganism whose presence suggests the presence of enteric pathogens. Indicator organisms are used because pathogens themselves are frequently difficult to detect in drinking water and wastewater low numbers ,difficult, time consuming, or expensive to culture.

The US Environmental Protection Agency (EPA) lists the following criteria for an organism to be an ideal indicator of fecal contamination.

1- An indicator should always be present when pathogens are present;

2- Indicators and pathogens should have similar persistence and growth characteristics.

3- Indicators and pathogens should occur in a constant ratio so that counts of the indicators give a good estimate of the numbers of pathogens present.
4- Indicator concentrations should far exceed pathogen concentration at the source of pollution.
5 - The indicator should not be pathogenic and should be easy to quantify.
6- Tests for the indicator should be applicable to all types of water.
7- The test should detect only the indicator organisms thus not giving false-positive reactions.
8-Should survive longer in the environment than the toughest enteric pathogen.
9-Should be a member of the normal intestinal microflora of warm-blooded animals.

Lec 8

Types of indicators

1- Coliform Organisms (Total Coliform)

Coliform bacteria are metabolically defined as gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts and able to ferment lactose at an optimum 35ºC. The main reason is because they are easy to detect and enumerate in water and are representative enough for determining microbial contamination of drinking water.

Besides the criteria discussed previously in regard to the choice of indicator organisms, there are numerous reasons for their use. Waterborne pathogens such as *Vibrio cholerae* and *Salmonella*spp. usually die very quickly and are present in very low numbers. These characteristics make their isolation and detection difficult and impractical.

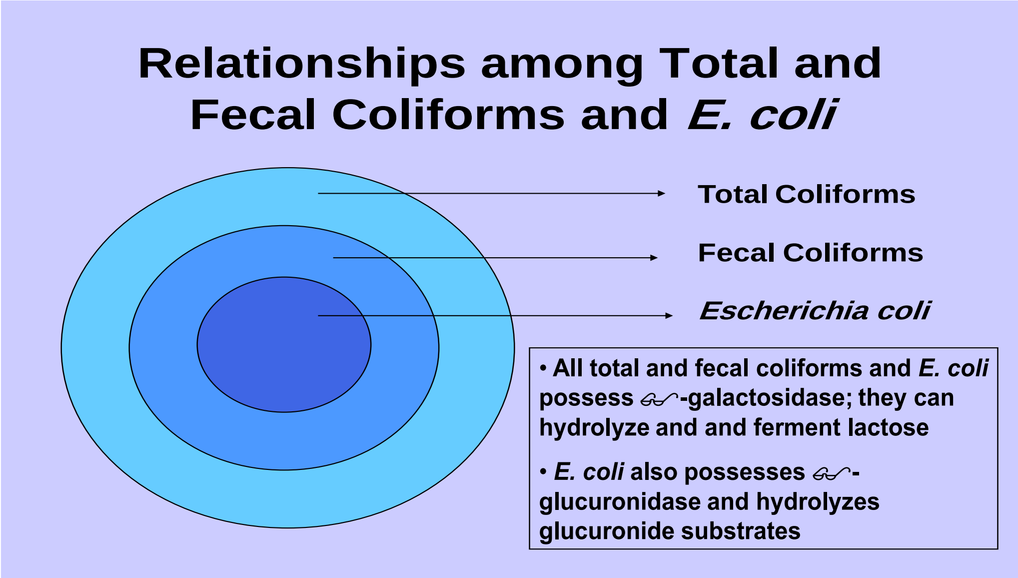
2-Thermotolerant Coliform Bacteria

This group of bacteria comprises the bacteria genus *Escherichia*, and to a lesser extent, *Klebsiella*, *Enterobacter,* and *Citrobacter*. They are defined as a group of coliform organisms that are able to ferment lactose at 44 to 45ºC. Sometimes, this group is also called Fecal Coliform (FC) to specify coliforms of fecal origin.

However, concentrations of thermotolerant coliforms are usually directly related to that of *E.coli* and thus can be used as a surrogate test for *E.coli*.

Escherichia coli (E.coli)

Escherichia coli is a specific subset of the thermotolerant coliform bacteria . They are found abundantly in human feces and warm-blooded animals .Usually, *E.coli* cannot multiply in any natural water environment and **they are, therefore, used as specific indicators for fecal contamination**. Both WHO Guidelines and EPA standards require zero *E.coli* to be found per 100 ml of drinking water sample.



3- Fecal Streptococci

Most of the species under the genus *Streptococcus* are of fecal origin and can be generally regarded as specific indicators of human fecal pollution. However, certain species may be isolated from the feces of animals. Fecal streptococci seldom multiply in polluted water and they are more persistent than coliform and *E.coli* bacteria. Therefore, they are generally useful as additional indicators of treatment efficiency . This indicator organism is commonly tested with *E.coli* for evidence of recent fecal contamination.

Four key points in favour of the faecal streptococci were:

(1) Relatively high numbers in the excreta of humans and other warmblooded animals.

(2) Presence in wastewaters and known polluted waters.

(3) Absence from pure waters, virgin soils and environments having no contact with human and animal life.

(4) Persistence without multiplication in the environment

4- Sulfite-Reducing Clostridia

Sulfite-reducing clostridia are gram-positive, anaerobic, spore-forming bacteria. Clostridial spores can resist treatment and disinfection processes better than most pathogens. One of the members, *Clostridium perfringens*, like *E.coli*, is normally present in feces, but in much smaller numbers. However, they are not exclusively of fecal origin and can be found in other environmental sources , Clostridial spores can survive in water much longer and resist disinfection better than other coliform groups .However, they are not recommended for routine monitoring of distribution systems(**on other word they tend to accumulate and are detected long after pollution has occurred, thus giving rise to false alarms).**

5- Bacteriophages

Bacteriophages (phages) are viruses that infect and replicate in specific bacteria. The ability to identify phages (coliphages) of *E.coli*, also detects fecal contamination. This is because the presence of coliphages also indicates the presence of *E.coli.* The significance of coliphages as indicators of sewage contamination, and their greater persistence compared to bacterial indicators make them useful as additional indicators of treatment efficiency.

6- Heterotrophic Bacteria

Heterotrophic bacteria are members of a large group of bacteria that use organic carbon for energy and growth. Many laboratories measure heterotrophic bacteria(Pseudomonas, *Aeromonas, Klebsiella, Flavobacterium, Enterobacter,)* by the heterotrophic plate count (HPC) Varies from 1 to 10^4 CFU/mL, and depends on temperature, residual chlorine concentration, and availability of organic nutrients .

. The presence of heterotrophic bacteria does not indicate the likelihood of pathogen presence. However, a sudden increase in HPC may suggest a problem with treatment or water disinfection . HPC > 500 CFU/mL indicates poor water quality.

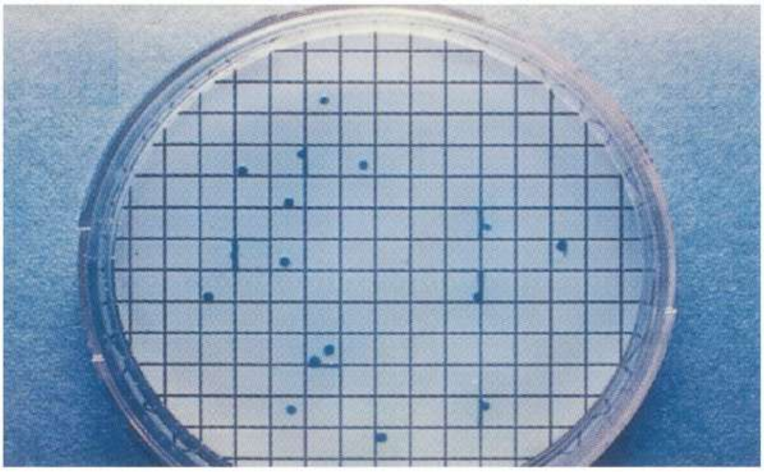
Current methods of detection

1-Most Probable Number (MPN)

2- Heterotrophic Plate Counts (HPC)

3- Membrane filtration and culture on selective media

- Indicator bacteria can be cultured on media which are specifically formulated to allow the growth of the species of interest and inhibit growth of other organisms. Typically, environmental water samples are filtered through membranes with small pore sizes and then the membrane is placed onto a selective agar. It is often necessary to vary the volume of water sample filtered in order to prevent too few or too many colonies from forming on a plate.



Membrane filtration method

Emerging methods of detection

A- Fast detections using chromogenic substances

The time required to perform tests for indicator organisms has stimulated research into more reliable and faster methods. One result is the use of chromogenic compounds, which may be added to the conventional or newly devised media used for the isolation of the indicator bacteria. These chromogenic substances are modified either by enzymes (which are typical for the respective bacteria) or by specific bacterial metabolites. After modification the chromogenic substance changes its colour or its fluorescence, thus enabling easy detection of those colonies displaying the metabolic capacity. **In this way these substances can be used to avoid the need for isolation of pure cultures and confirmatory tests. The time required for the determination of different indicator bacteria can be cut down to between 14 to 18 hours.**

B-Gene sequence-based methods

Advances in molecular biology in the past 20 years have resulted in a number of new detection methods, which depend on the recognition of specific gene sequences. **Such methods are usually rapid and can be tailored to detect specific strains of organisms on the one hand or groups of organisms on the other.** The new methods will influence epidemiology and outbreak investigations more than the routine testing of finished drinking water.

1- PCR (polymerase chain reaction)

With the polymerase chain reaction and two suitable primer sequences (fragments of nucleic acid that specifically bind to the target organism) trace amounts of DNA can be selectively multiplied. In principle, a single copy of the respective sequence in the assay can produce over a million-fold identical copies, which then can be detected and further analysed by different methods. **One problem** with PCR is that the assay volume is in the order of some micro-litres, whereas the water sample volume is in the range of 100–1000 ml. Bej *et al*. (1991) have published a filtration method to concentrate the sample, but **another problem** is that natural water samples often contain inhibitory substances (such as humic acids and iron) that concentrate with the nucleic acids. **Hence, it is critical** to have positive (and negative) controls with each environmental sample PCR to check for inhibition and specificity. It may **also be critical** to find out whether the signal obtained from the PCR is due to naked nucleic acids or living or dead micro-organisms .**One solution** has been established by using a three-hour pre-incubation period in a selective medium so that only growing organisms are detected .

2- FISH (fluorescence in situ hybridisation)

Microbiological standards for water

The microbiological examination of water is used worldwide to monitor and control the quality and safety of various types of water. These include potable waters (water intended for drinking or use in food preparation), treated recreational waters (swimming pools, spa pools, and hydrotherapy pools), and untreated waters used for recreational purposes such as sea, river, and lake water.

Various standard and guideline values have been introduced over the years. **Many of these have become legally enforceable, while others have been recommended by appropriate bodies and trade associations**.

Water quality standards are regulations or rules that protect lakes, rivers, streams and other surface water bodies from pollution.

Drinking Water Standards : Defined as water quality parameters established for public water supplies by regulatory authorities to define the limiting concentrations of various constituents.

- Limiting concentrations are those that can be tolerated for the intended use ,are revised periodically.

Standards Classifications

- **Primary Standards**: are health related and enforceable which includes such parameters as: – Coliforms, turbidity, toxic inorganic and organic chemicals.

- **Secondary Standards**: are non-health related used for aesthetic purposes which includes: – Color, odor, chloride ion, sulfate ion, dissolved solids, manganese, pH, copper, and zinc.

Some Important World Standards

- The World Health Organization (WHO)

– Set up some guidelines for drinking-water quality which are the international reference point for standards setting and drinking-water safety.

- Unesco :united nation education ,science and culture organization
- The European Union (EU)
- US Environmental Protection Agency (EPA)

-Icsqc :Iraqi center of standardization and quality control

Microbiological guidelines and standards for drinking water

Type of water	Parameters	Permitted concentration or value	Guide level	Imperative level
Mains water[9] (continuous sampling recommended)	coliforms / *E. coli* Cryptosporidium oocysts*	0/100mL		< 10/100L
Private supplies[10] Classes A to E and I to 4	coliforms / *E. coli* colony counts at 22°C and 37°C	0/100mL	No significant increase over normal levels	
Class F	coliforms / *E. coli*		No organism detrimental to public health	
Natural mineral waters sampled any time up to sale[14]	coliforms/*E. coli* enterococci *Pseudomonas aeruginosa* sulphite reducing clostridia parasites/pathogens	0/250mL 0/250mL 0/250mL 0/50mL Absent		
within 12 h of bottling[14]	As above plus colony count 22°C/72h colony count 37°C/48h	 100/mL 20/mL		
Drinking water in containers at any time[14]	coliforms/*E.coli* enterococci *Pseudomonas aeruginosa* sulphite reducing clostridia colony counts at 22°C and 37°C	0/100mL 0/100mL 0/100mL 0/20mL Should show no appreciable increase after bottling		
within 12 h of bottling[14]	As above plus colony count 22°C / 72h colony count 37°C / 48h presumptive coliforms	 100/mL 20/mL 0/100mL		
Vending machines[7]	*E. coli* *Pseudomonas aeruginosa* colony count 22°C / 72h colony count 37°C / 24h	0/100mL 0/100mL ≤10000/mL ≤1000/mL†		
Meat premises[18,19] routine sampling	coliforms / *E. coli* colony count 22°C / 72h colony count 37°C / 48h	0/100mL ≤100/mL ≤10/mL		
if coliforms found:	resample as for routine plus test for: enterococci sulphite reducing clostridia	 0/100mL 0/20mL		

* applies to treatment works shown by risk assessment to be potentially contaminated
† provided that colony counts are no more than 10 times greater than those in water entering the machine

For example :DrinkingWater Standards

Fecal coliform counts should be zero per 100 mL of sample (0/100 mL) in all water supplies, piped or unpiped, treated or untreated. Total coliform counts should be 0 per 100 mL in piped, treated water supplies with an occasional occurrence of two organisms per 100 mL being allowed. In unpiped water supplies, total coliform counts of 10 organisms per 100 mL can occur infrequently. Frequent occurrences of high coliform counts signify the need for an alternative water source, or sanitary protection of the current source. A “boil water” order is needed when emergency water supplies fail to meet a criterion of zero (0) fecal and total coliforms.

Lec 9

Water Treatment

-The principal objective of water treatment is to provide potable water that is chemically and biologically safe for human consumption. It should also be free from unpleasant tastes and odors.

Steps of water treatment

1-Flocculation

Flocculation refers to water treatment processes that combine small particles(clay, organic material, metals, microorganisms) into larger particles, which often quite small and so will not settle out from the water column without assistance. To help the settling process along, "coagulating" compounds are added to the water, and suspended particles "stick" to these compounds and create large and heavy clumps of material. Alum and iron salts or synthetic organic polymers (used alone or in combination with metal salts) are generally used to promote coagulation.

2-Sedimentation

The water is left undisturbed to allow the heavy clumps of particles and coagulants to settle out.

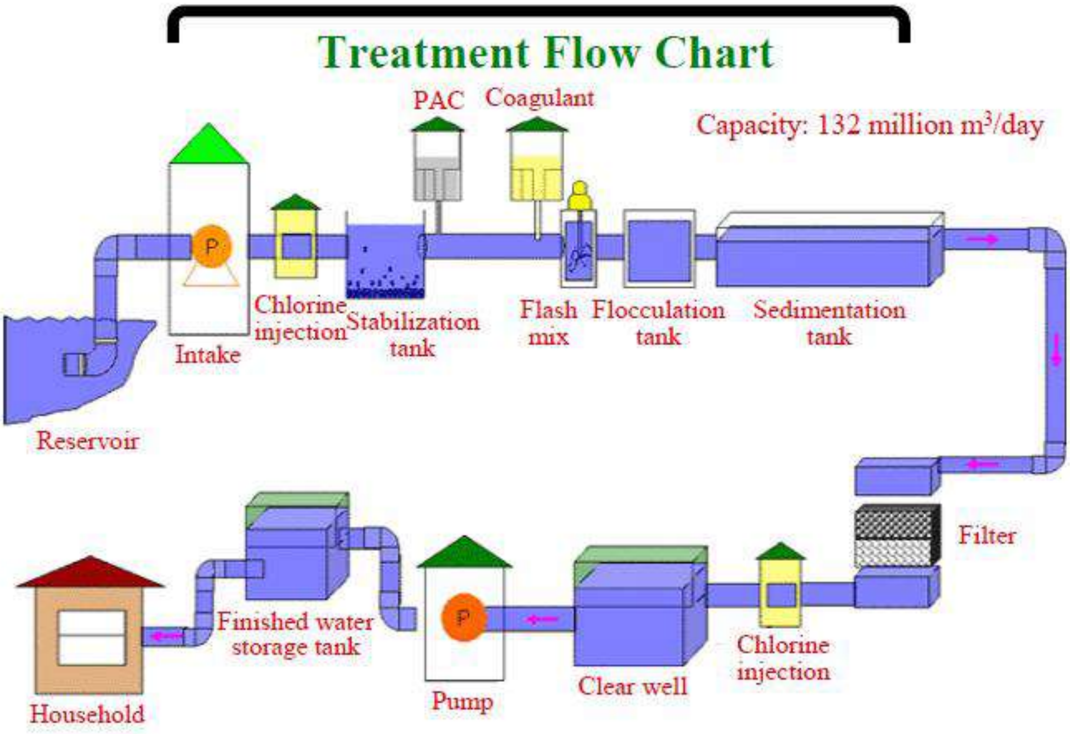
3-Filtration

The water is run through a series of filters which trap and remove particles still remaining in the water column. Typically, beds of sand or charcoal are used to accomplish this task.

Many water treatment facilities use filtration to remove all particles from the water. Those particles include clays and silts, natural organic matter, precipitates from other treatment processes , filtration clarifies water and enhances the effectiveness of disinfection.

5-Disinfection (chlorination/ozonation)

Water is often disinfected before it enters the distribution system to ensure that potentially dangerous microbes are killed. Chlorine, chloramines, or chlorine dioxide are most often used because they are very effective disinfectants, not only at the treatment plant but also in the pipes that distribute water to our homes and businesses. Ozone is a powerful disinfectant, and ultraviolet radiation is an effective disinfectant and treatment for relatively clean source waters, but neither of these are effective in controlling biological contaminants in the distribution pipes



To produce water that is safe to drink, pleasant in taste and could be used for various purposes, treatment processes should be selected to remove specific constituents from raw water. The basis for selecting treatment process alternatives is established by the characteristics of raw water and the finished water quality goals.

In addition to these five steps of water treatment ,there are other steps :

1-Fluoridation

2-remove of iron and manganese.

3-water softening (remove of hardness)

4-remove of dissolved salts ,phosphprus ,nitrogen

5-remove of color ,odor, taste,etc.

Post treatment water contamination

The treated drinking water may be exposed to the contamination in distribution system (pipes) of water due to :

1- Main breaks, repairs and installation in WTP and distribution system, operation and maintenance deficiencies and cross-connections cause entering the pollutants from the surrounding soil .

2-From the sewers through leaks or flooding of sewers.

3-Connection the new pipes from new buildings.
4-Biofilm in the old pipes are reduced the chlorine content in the distribution system which lead to growth of resistant bacteria.

Water chlorination

In water treatment, disinfection is the most important treatment step. The most important oxidant used for disinfection is chlorine Cl_2.
- Chlorine is rather soluble in water: solubility decrease at rising temperature.

-It is very applicable and very effective for the deactivation of pathogenic microorganisms.

-Chlorine can be easily applied, measures and controlled. It is relatively cheap.

Chlorination

Chlorination can be achieved by using liquefied chlorine gas, sodium hypochlorite solution or calcium hypochlorite granules and on-site chlorine generators.
Chlorine, whether in the form of chlorine gas from a cylinder, sodium hypochlorite or calcium hypochlorite, dissolves in water to form hypochlorous acid (HOCl) and hypochlorite ion (OCl^-).
Free "active" chlorine= Hypochloric acid (HOCl) & hypochlorite ion (OCl^-). Hypochloric acid is the most reactive and a stronger disinfectant because it's neutral.

Chlorination is employed primarily for microbial disinfection. However, chlorine also acts as an oxidant and can remove or assist in the removal of some chemicals for example,decomposition of easily oxidized pesticides.
A disadvantage of chlorine is its ability to react with natural organic matter and plant phenolic compounds to produce carcinogenic such as trihalomethane and chlorophenolic compounds respectively.
When chlorine is added to water, some of the chlorine reacts first with organic materials and metals in the water and is not available for disinfection (this is called the chlorine demand of the water). The

remaining chlorine concentration after the **chlorine demand** is accounted for is called total chlorine. Total chlorine is further divided into: 1) the amount of chlorine that has reacted with nitrates and is unavailable for disinfection which is called **combined chlorine** and, 2) the **free chlorine** , which is the chlorine available to inactivate disease-causing organisms, and thus a measure to determine the potability of water.

Breakpoint chlorination

is a point in which the chlorine dose is sufficient to rapidly oxidize all the ammonia nitrogen in the water and to leave a suitable free residual chlorine available to protect the water against reinfection **from the point of chlorination to the point of use.**

$Cl_2 + H_2O$ -------------------- $HOCl + H^+ + Cl^-$

Monochloramine:

$$NH_4 + HOCl \longrightarrow NH_2Cl + H^+ + H_2O \qquad \textit{pH 4.5 to 8}$$

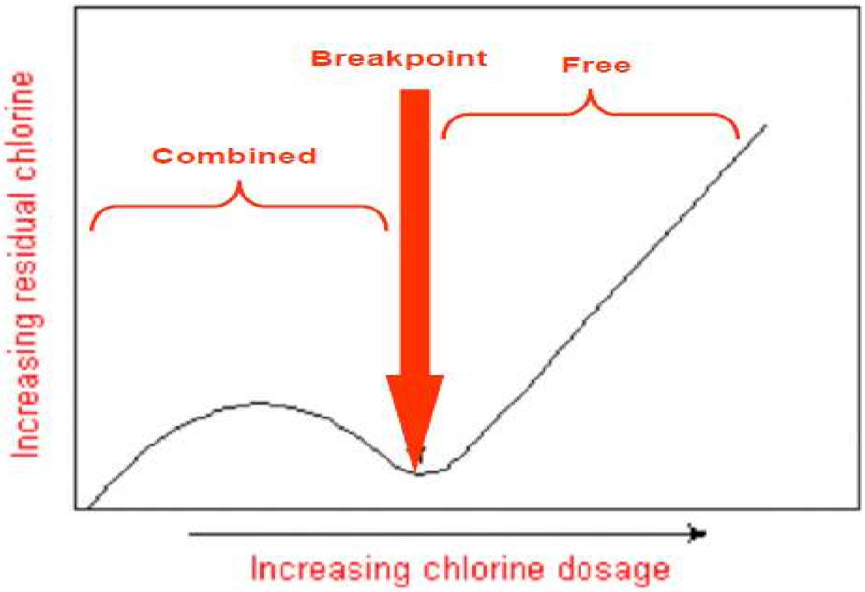
Dichloramine:

$$NH_2Cl + HOCl \longrightarrow NHCl_2 + H_2O \qquad \textit{pH 4.5 to 8}$$

Trichloramine or nitrogentrichloride:

$$NHCl_2 + HOCl \longrightarrow NCl_3 + H_2O \qquad pH < 4.5$$

Whole ammonia removed from water when reaching the breakpoint reaction ,this point is achieved when the ratio of chlorine to ammonia – nitrogen will be ten to one $\frac{10}{1}$



The steps of chlorine action in water

How chlorine kills/deactivates

Chlorine disinfects water through two mechanisms:

–oxidizing power of free oxygen

–chlorine substitution reactions

•Free oxygen is produced by the breakdown of chlorine compounds in water

$HOCl$-------------$HCl + O$

•Oxygen attacks the lipids in cell walls and destroys the enzymes and structures inside the cell which either destroys or inactivates it

Liquid form – $NaOCl$ (bleach)

$NaOCl \rightarrow Na^+ + OCl^-$ ← Hypochlorite ion

$OCl^- + H_2O \leftrightarrow HOCl + OH^-$

Powder form – $Ca(OCl)_2$ (pool tablets/powder)

$CaOCl_2 + H_2O \rightarrow Ca(OH)_2 + Cl_2$

$Cl_2 + H_2O \rightarrow HOCl + HCl$

$HOCl \rightarrow HCl + O$

Hypochloric acid

Gaseous form – Cl_2

$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$

$Cl_2 + 2H_2O \rightarrow HOCl + H_3O + Cl^-$

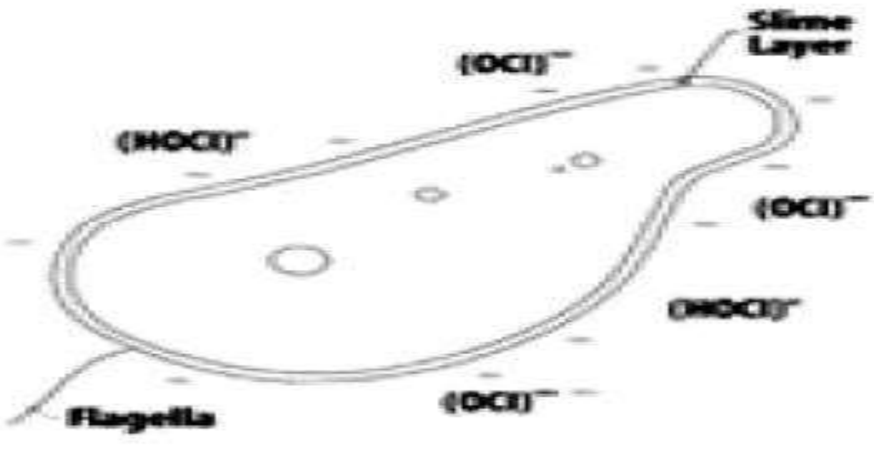
$HOCl + H_2O \rightarrow H_3O^+ + OCl^-$

$OCl^- \rightarrow Cl^- + O$

Chlorine substitution

•Chlorine breaks chemical bonds in enzyme molecules and replace some hydrogen atoms with chlorine.

•This changes the shape or destroys the molecules and makes it difficult for the enzymes to function properly.



Post-chlorination

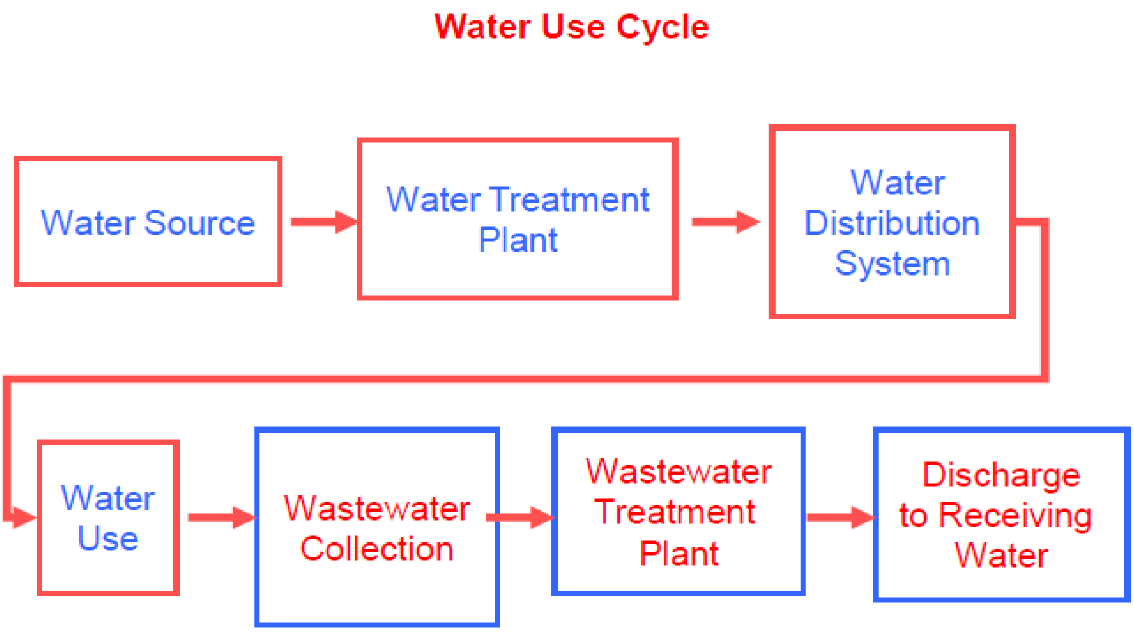
The development of an excessive growth of bacteria can be prevented by the application of a water treatment system proper for taking out the nutrients necessary for bacterial growth and by the maintenance of a free chlorine residual in the water to be distributed.

In **post chlorination** so much chlorine is added to the already disinfected water that drinking water entering the distribution system contains a free chlorine residual of 0.3to 0.5g Cl_2/m^3.

Lec 10

Biological Wastewater Treatment

Wastewater: is simply that part of the water supply to the community or to the industry which has been used for different purposes and has been mixed with solids either suspended or dissolved.Wastewater is 99.9% water and 0.1% solids. The main task in treating the wastewater is simply to remove most or all of this 0.1% of solids People excrete 100-150 grams wet weight of feces .And 1-1.3 liters of urine per person per day.



The amount of organic matter in domestic wastewater determines the degree of biological treatment required. Three tests are used to assess the amount of organic matter:

•Biochemical Oxygen Demand (BOD)

•Chemical oxygen demand (COD)

•Total Organic Carbon (TOC)

Measurements of organic matter

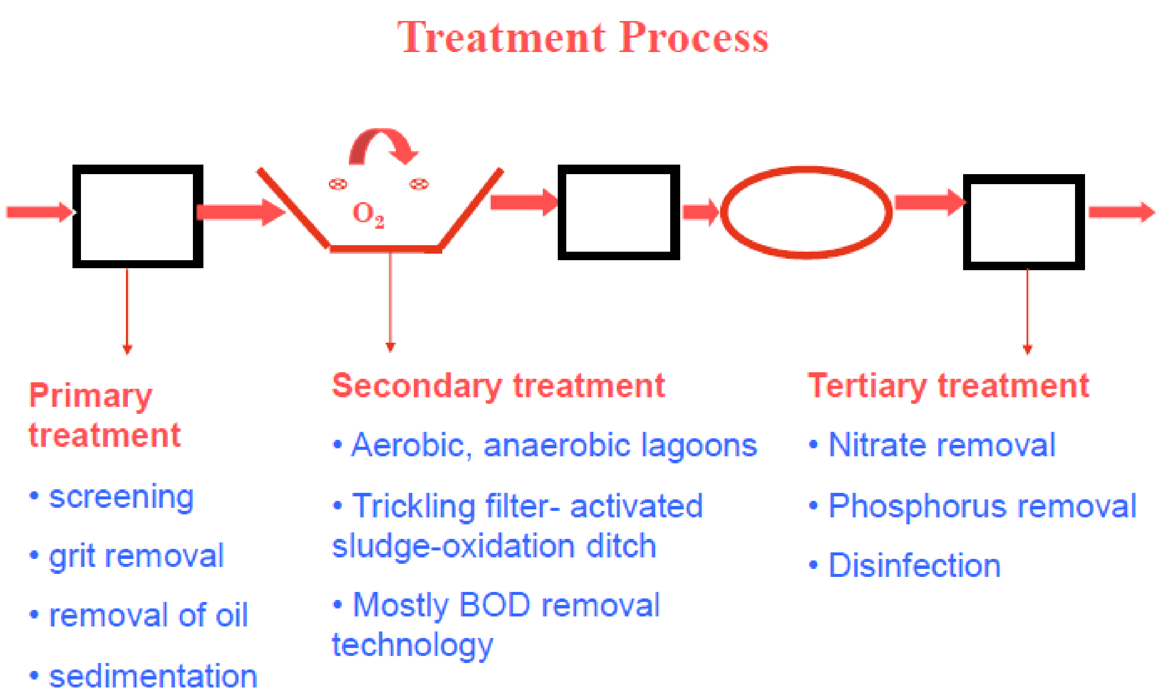
Many parameters have been used to measure the concentration of organic matter in wastewater. The following are the most common used methods:

1. Biochemical oxygen demand (BOD)

BOD: is the oxygen equivalent of organic matter. It is determined by measuring the dissolved oxygen used by microorganisms during the biochemical oxidation of organic matter in 5 days at 20oC.

2. Chemical oxygen demand (COD) It is the amount of oxygen necessary to oxidize all the organic carbon completely to CO2and H2O. Is measured by oxidation with potassium dichromate (K2Cr2O7) in the presence of sulfuric acid and silver and expressed in milligram per liter.

3. Total organic carbon (TOC) This method measures the organic carbon existing in the wastewater by injecting a sample of the wastewater in special device in which the carbon is oxidized to carbon dioxide then carbon dioxide is measured and used to quantify the amount of organic matter in the wastewater . This method is only used for small concentration of organic matter.



Biofilms in Drinking Water Distribution Systems

biofilm is a complex mixture of microbes, organic and inorganic material accumulated amidst a microbially produced organic polymer matrix attached to the inner surface of the distribution system pipe . The inner surface of a water pipe may have a continuous biofilm, but usually biofilms are quite patchy.

Factors causing biofilm growth

1- Presence of microbial nutrients in water.
2- Characteristics of pipe wall such as roughness.
3- Microbial and chemical quality of water entering the system.
4- Water temperature and pH.
5- Low chlorine level in water .
6- Velocity of water .

Biofilms in drinking water pipe networks **can be responsible for a wide range of water quality and operational problems there are :**

1-Biofilms can be responsible for loss of distribution system disinfectant residuals.

2- Increased bacterial levels, bacteria may be sloughed from the biofilm into the water column due to changes in the flow rate,as a result, biofilms can act as a slow-release mechanism for persistent contamination of the water.

3- Reduction of dissolved oxygen.

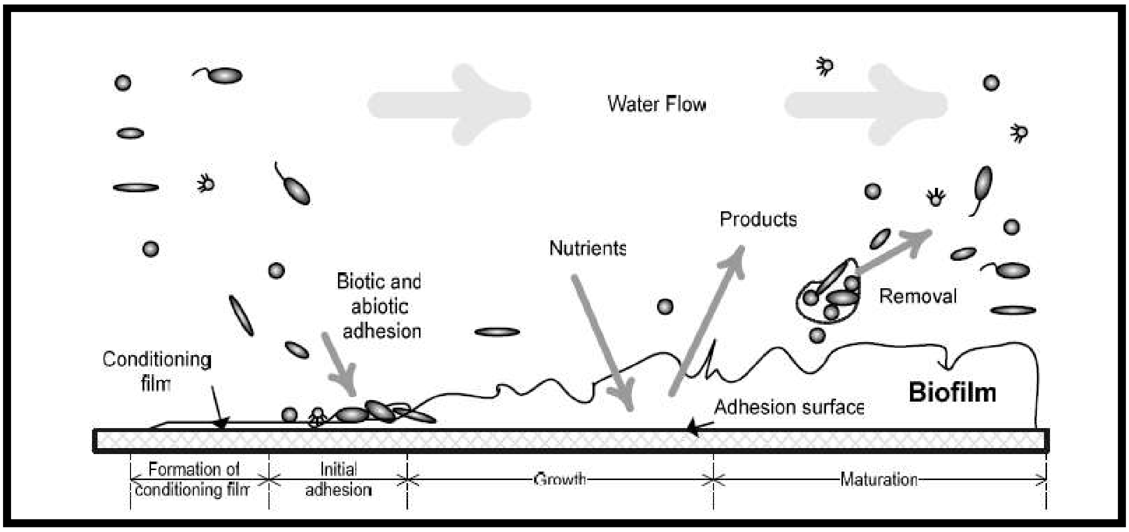
4- Reduce the utility of total coliforms as indicator organisms

5-Taste and odor changes, red or black water problems due to iron or sulfate-reducing bacteria.

6- Microbial-influenced corrosion, hydraulic roughness, and reduced materials life .

Many different microbes have demonstrated the ability to survive in the distribution system, with some possessing the ability to grow and/or produce biofilms. Some of these organisms may be primary pathogens (i.e., those that cause disease in healthy individuals), while others may be opportunistic pathogens (i.e., those that cause disease in individuals with underlying conditions that may facilitate infection).

The biofilm can protect microbes from disinfection and allow microbes injured by environmental stress and disinfectants to
recover and grow.



Processes involved in the biofilm growth

Opportunistic Bacterial Pathogens Detected in the Distribution System and/or Biofilms

Opportunistic pathogens	Health Effects
Acinetobacter calcoaceticus	pneumonia, meningitis, infections of urinary tract, septicemia
Aeromonas hydrophila	sepsis, gastrointestinal illness, respiratory tract infections
Citrobacter spp.[3]	septicemia, pneumonia
Enterobacter spp.[3]	septicemia, pneumonia
Flavobacterium spp.	septicemia, meningitis
Klebsiella pneumoniae [3]	septicemia, pneumonia
Moraxella spp.	pneumonia, conjunctivitis, septicemia, otitis, urethritis, meningitis, bronchitis, sinusitis
M. avium complex	chronic diarrhea, chronic lung disease
Pseudomonas cepacia	foot infections
Pseudomonas aeruginosa	infections when severe burns, cancer patients, lungs when cystic fibrosis, pneumonia, meningitis, others
Serratia marcescens [3]	septicemia, pneumonia