Soil Microbiology

October ١٧
٢٠١٤

Soil: is the outer region of earth crust, consist of loose material formed by series of various processes called soil-forming factors.

Soil Microbiology: It is a branch of science microbiology which dealt with the study of soil microorganisms and their activities in soil.

Soil is a microbial environment, soil composition and cycles of elements

Soil is a microbial environment

Soil is an important environment for microbial activities through:

١. It contains many microbes and their spores which are pathogenic to human (*Clostridium tetani, C. perfringens, Bacillus anthracis*), animals and plants.
٢. It contains microorganisms that responsible of essential elements cycles and decompose humans, animals and plant residues to primary elements.
٣. It contain microorganisms that remove chemical pollutants and used in industrial production like antibiotics, enzymes, organic acids,….etc.

The main contributors of this field:

١- **Muntz and Schloesing ١٨٧٧:**
Pointed out that soil nitrate is formed due to activity of microorganisms
٢- **Frank ١٨٨٩:** isolated the root nodes causing rods.
٣- **Winogradsky:** father of soil microbiology.
١٨٩٠: isolated *Nitrosomonas*.
١٨٩٢: isolated *Nitrobacter*
١٨٩٥: isolated anaerobic spore former rods *Clostridium* that can fix N٢
٤- **Fleming and Waksman:** isolated antibiotic producing microorganisms *Pencillium* and *Streptomyces*.

Soil profile:

It refers to the layers of soil develops over a long period of time as a result of weathering and biochemical processes on earth curst, it consists of a mixture of organic and inorganic compounds, water, gases and biota.

Soil profile consists of horizons which are:

١) **Horizon A (top soil):**
Surface layer about ٢٥ cm of soil profile, contains highest percentage of organic matter and microorganisms (aerobic and anaerobic). The most biological activities occur in this horizon which was likely formed from decomposing plants and mineral materials.

٢) **Horizon B (subsoil):**
Found from ٢٥-٤٠ cm with increase in clay and mineral salts such as deposits of silicates or aluminum. It contains less organic compounds and microbial population from the previous horizon.

٣) **Horizon C (parent soil):**
It's thickness of ٤٥ cm or more. It is indicated with the absence of organic matter and microbial activities but some bacterial and fungal spores may found in it.

٤) **Horizon D (rocky bed):**
This layer found from ٧٥ cm and beyond, represent the parent material that form soil profile due to biogeological factors, this layer lack microorganisms.

Soil texture:

It refers to the proportion and distribution of mineral particles, sand, silt and clay present, the texture of soil can be determined when the percentage of these three soil constituents are known in the following table:

Type of soil particles	**Size range**
Clay	Less than ٠.٠٠٢ mm
Silt	٠.٠٠٢-٠.٠٥ mm
Sand	٠.٠٥-٢ mm

Physiochemical features of soil depend mainly on its texture because it influences plants growth by its direct effects on soil, aeration, water infiltration and cations exchange capacity.

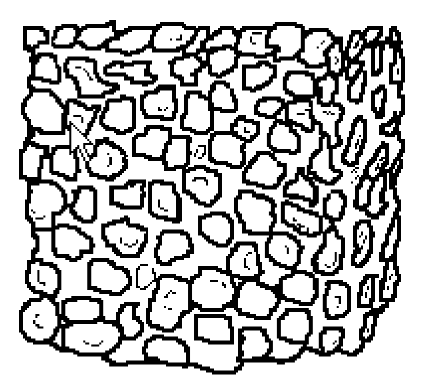
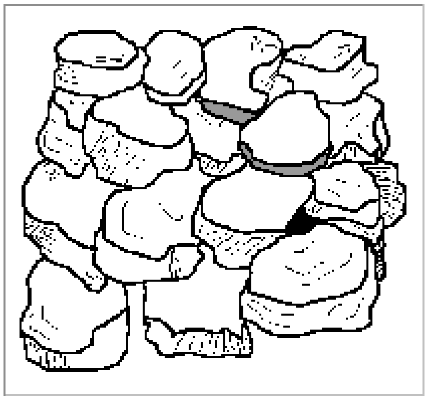
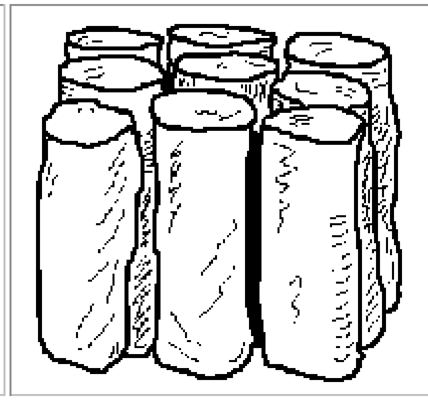
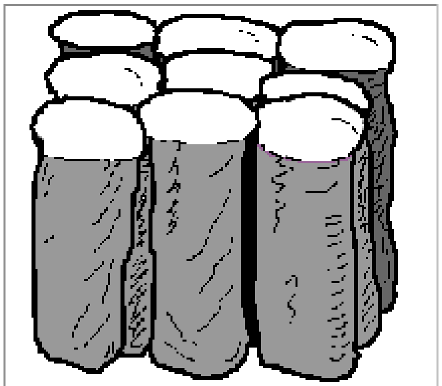
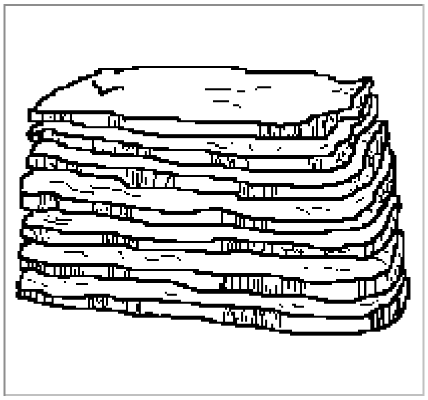
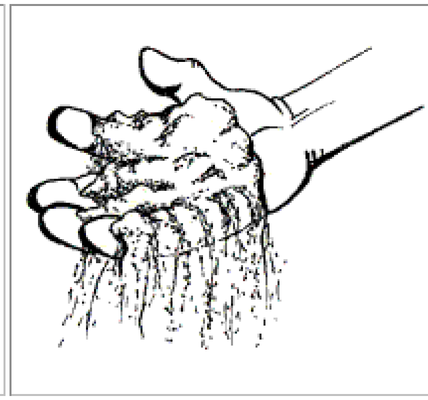
Sand has a large particle size with small surface area in comparison with same mass of silt and clay, in accordance to this little surface area the sand proportion of any soil has an importance as organizing skeleton for other constituents, in case of reasonable sand proportion the soil has enough pores which facilitate aeration and water drainage. Sandy soils are less productive than other.

Silt, produced from fragmentation of rocks, also has a small surface area but larger than those of sand particles, with smooth appearance look like cosmetic powders and with low adherence capacity.

Clay is made up of tiny particles, despite their small size the particles have a very large surface area relative to their volume, this feature allows clay particles to absorb water and retain nutrients. Clay soils are the most productive.

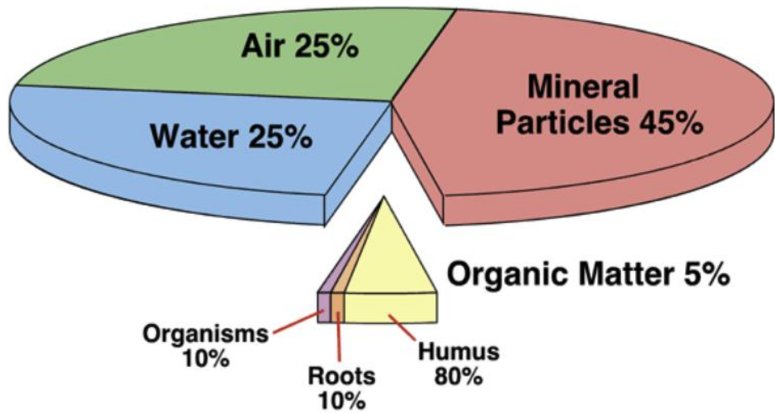
Soil structure:

It is the arrangement of soil particles into groups called aggregates, which often form distinctive shapes found within certain horizons. For example, granular soil particles are characteristic of surface horizon. The various types of soil structures are provided in the following figure:

		
Granular: Resembles cookie crumbs and is usually less than 0.5 cm in diameter. Commonly found in surface horizons where roots have been growing.	**Blocky**: Irregular blocks that are usually 1.5 - 5.0 cm in diameter.	**Prismatic**: Vertical columns of soil that might be a number of cm long. Usually found in lower horizons.
		
Columnar: Vertical columns of soil that have a salt "cap" at the top. Found in soils of arid climates.	**Platy**: Thin, flat plates of soil that lie horizontally. Usually found in compacted soil.	**Single Grained**: Soil is broken into individual particles that do not stick together. Always accompanies a loose consistence. Commonly found in sandy soils.

Soil composition

Soils are made up of four basic components: mineral particles, air, water and organic matter.



١- Mineral particles (Inorganic material):

In most soil types inorganic materials represent about ٤٥٪ of total soil volume. The mineral portion formed from rock bed by weathering and biogeochemical factors, mineral portion consist of ٣ particles: sand, silt and clay.

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

a) **Non-silicate group:** includes; oxides, hydroxides, sulfates, chlorides, carbonates and phosphates.

b) **Silicate group:**

Very complex in structure, vary in its stability and resistance to decomposition, among this group $SiO_{٢}$ is the most abundant one, it may composes about ٧٠٪ of total inorganic soil mass.

Most soil influential particle are clay, it plays a significant role in determining the availability of nutrients and water to different life forms.

Clay particles are negatively charged due to exchange of $Si^{٤+}$ and $Al^{٣+}$

$$Al^{٣+} + SiO_{٢} \longrightarrow AlO_{٢}^{-} + Si^{٤+}$$

٢- 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 soil types is about ٥٠٪ of total soil volume. After raining the soil pores space will have a high percentage of water in relation to air.

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 require water for their metabolic activities. Soil water thus indirectly affects plant growth through its effect on soil and microorganisms. The percentage of soil-water is about ٢٥٪ of total soil volume.

Soil water amount affected by many factors:

a) **Porosity:**

Refer to the spaces between soil particles, which consist of various amounts of water and air.Porosity depends on both soil texture and structure, for example, a fine soil has a small but numerous pores than coarse soil. A coarse soil has a bigger particles and less porosity, water can be held tighter in small pores than in large one.

b) **Infiltration:**

Refers to the movement of water from soil surface to the soil profile. Soil texture, structure, slope and gravitation have the large impact on filtration rate. Water move by gravity into the open pores in the soil;the size of soil particles and their spacing determine how much water can flow in.

c) **Permeability:**

Refers to the movement of air and water through the soil. Water holding capacity controls permeability by the combination effects of soil texture and organic matter. Soil with smaller particles (silt and clay) have a large surface area than those with large sand particles, so the first one has a high water holding capacity and allow soil to hold more water than the second type.

Soil air:

A part of soil pores which is not occupied with water are filled with air. Compared with atmospheric air, soil is lower in $O_٢$ and higher in $CO_٢$, because $CO_٢$ is continuous recycling by microorganisms during the process of decomposition of organic matter. Soil air comes from external atmosphere and contains $N_٢$, $O_٢$, $CO_٢$ and water vapor.

$CO_٢$ in soil air (٠.٣-١٪) is more than atmosphere air (٠.٠٣٪). Soil aeration plays an important role in plant growth, microbial population and microbial activities in soil. A good aeration soil type leads to a complete oxidation of organic matter, and characterized with high redox potential capacity results in thrive of aerobic and facultative anaerobic microorganisms, but poor aerated soil types (saturated soils), which featured by low redox potential capacity and accumulation some harmfully intermediates like $CH_٤$ and $H_٢S$ that effect soil fertility and increase of anaerobic microorganism population.

٣- Organic matter:

Soil organic matter is one of the most important components of soil ecosystem. It comprises all living soil organisms and all the remains in their various degree of decomposition. The proportion of organic matter in the soil ranging from ٣-٥٪ of total soil volume.

Non-living organic matter can be considered to exist in two distinct forms:

a. **Non-humic substances:**

It's a particulate matter represents microbial metabolites products with identifiable structure like polysaccharide, amino acids, organic phosphorous. This organic matter constitute up to ٢٥٪ of total organic matter in soil.

b. **Humicsubstances:**

Carbon decomposition, successive decomposition of dead material and modified organic matter results in the formation of more complex organic matter called **humic substances** or **humus** by a process called **humification**. Humus comprises both of identifiable structure like proteins and cellulose and molecules with unidentifiable structure like plant residues such as lignin, remains of animal carcasses (waxes, hair, nail, wool and feather). Humus effects soil properties as it slowly decomposes, it colors the soil darker and spongy appearance, increases soil aggregation and stability and increase the ability to attract and retain nutrients.

Soil living organic matter (soil biota):

It includes a wide variety of organisms such as plants, insects, earthworms, animals and microorganisms. Soil is excellent culture media for the growth and development of various microorganisms which are: bacteria, fungi, actinomycetes, algae, protozoa and viruses. Microorganisms form a very small fraction of soil mass and occupy a volume less than ١٪. In the top soil (١٠-٢٥ cm depth) the microbial population is very high and decrease with depth of soil. Each organism or groups of organisms are responsible for a specific change in the soil. The final effect of various activities of soil microorganisms is to make the soil fit for growth and development of higher plants.

> Formerly, any forms of life that reside in a specific region are referred as ‘Flora’. This term is changed to biota as the previous one is restricted to plant forms of life.

Living organisms present in soil are grouped into two categories:

١. Soil flora (microflora): e.g. bacteria, fungi, actinomycetes and algae.
٢. Soil fauna: animals like protozoa, nematodes, earthworms, moles, ants and rodents.

Soil microflora:

١- Bacteria:

Is the most abundant group and usually more numerous than others. Soil bacterial numbers vary between $١٠^{٨}$-$١٠^{٩}$ cell/gm of soil, while in agricultural field the No. is about ٣×١٠^{٩} cell/gm.

Based on its regular presence soil bacteria are divided into two groups:

١. *Soil indigenous (true resident) or autochthonous.*
٢. *Soil invaders or allochthonous.*

Bacterial No. and variety influenced by soil type, microenvironment, organic matter and cultivation processes. They are found in a high No. in cultivated soil than virgin one, in a maximum in 'rhizospheric region' than non-rhizosheric one, possibly due to aeration and availability of nutrients. The inner region contain high level of G-ve bacteria while the outer region with G+ve bacteria, thus may be due to polymer formation, motility, surface charge andlife cycle of bacteria.

The major roles of bacteria in soil are:

١. Mineralization of elements.
٢. $N_٢$ fixation from atmosphere.
٣. Stabilization of mineral ions.
٤. Biotransformation of chemicals.
٥. Biogas formation.

Rhizosphere: It is a zone of increased microbial growth and activity in soil around of plants, it may be extended several inches into soil around root system of growing plants. The m.o. growing in this zone is under the influence of roots quantitatively and qualitatively.

Examples of soil bacteria: *Bacillus, Erwinia, Clostridium, Nitrobacter, Nitrosomonas, Rhizobium.*

٢- Fungi:

In most aerated and cultivated soil, fungi share a major part of total microbial biomass because of their large diameter and extensive network of mycelia. However, the population of soil fungi ranging from $١٠^{٤} – ١٠^{٦}$ cell/gm of dry soil. Fungi obtain their growth from organic matter, living animals and living plants forming different types of relationships.

The most important relationship between fungi and plants in soil is **Mycorrhiza**, which is symbiotic relationshipbetween fungi and plants that occurs in plant root systems.

Examples of soil fungi: *Aspergillus, Cladosporium, Alternaria, Fusarium, Pythium.*

Some of fungi roles are:

١. Production of antibiotics.

٢. Contributes in soil aggregation that protects soil particles from weathering effects.

٣. Degrade some of tough plant residues like lignin.

٤. Support soil microenvironment biobalance by their feeding practices on protozoa and nematodes.

٥. Mycorrhizal fungi supplies minerals to associated plants, likewise fungi receives benefit from plants exudation, like some carbohydrates.

٣- Actinomycets:

They share characteristics of both bacteria and fungi, they are G+ve and release antibiotic substances; however, The No. of actinomycetes ranging from ١٠^٥ – ١٠^٨ cell/gram.

> The earthy odor of newly wetted soil has been found to be volatile growth products of actinomycetes which called 'geosmin'.

Some examples of actinomycetes: *Actinomyces, Streptomyces, Nocarida, Micromonospora, Actinoplans.*

Their roles are:

١. Contributes mainly in humus substances formation.

٢. Decompose plant and animal residues that are resisting to bacterial and fungal decomposition.

٣. Production of antibiotics (streptomyces) andlysis enzymes(lysozyme).

٤- Algae:

They grow where adequate amount of moisture and light presence due to their need of photosynthesis process. Most of them prefer growth in neutral to alkaline pH ٧-١٠. Some examples of soil algae: *Anabaena, Nostoc, Calothrix, Oscillotoria, Scytonema.*

Their roles are:

١. Some species used commercially as biofertilizers.
٢. Facilitate agriculturing due to their photosynthetic capacity, that act as a source of carbon and nitrogen.
٣. Used in reclamation of sodas soil and alkaline soil types.

Cycles of Elements

All elements that are essential component of protoplasm undergo cyclical alteration between an inorganic state, free in nature, and combined state in living organisms. This repeated transformation of elements from living protoplasm to free state in nature constitutes the cycle of elements in nature. Among these essential elements are: carbon, hydrogen, nitrogen, sulfur and phosphorus.

The carbon cycle:

The most important recycling process since it is the basic building block of life. The carbon atoms are recycled over and over, take place between living things and non-living environment. Carbon occurs in nature into two main states: complex carbonated organic compounds and inorganic carbon, most of inorganic carbon is in form of **CO٢**. So CO٢ is the source of all carbonated compounds both in living organisms and fossil deposits.

Carbon dioxide constitutes about ٠.٠٤٥٪ of atmosphere gases, in this rate is less than the requirements of photosynthetic organisms, so CO٢ release continuously from respiration and decomposition to maintain carbon balance in nature.

Biology plays an important role in the movement of carbon through three types of participation in recycling:

Producers:

They represent the beginning of all food chains in nature, the producers fix atmospheric CO_2 through photosynthesis process.

Consumers:

Primary → secondary → tertiary → ……. They subsequently utilize organic matter thus formed by producers either to become their cellular materials or to release to atmosphere through respiration, in which carbohydrates are oxidized to yield **CO_2, H_2O** and **energy** as follows:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{Respiration}} 6CO_2 + 6H_2O + \text{energy}$$

Decomposers:

These are m.o. those contribute to carbon pool by their feeding and processing of rotting remains of other organisms. It is their job to consume both waste products and dead organic matter. Decomposers also breakdown, remove and recycle nature’s garbage.

<u>Microorganisms are main decomposers in soil, play a key role in carbon recycling, they contribute to the system by</u>:

Fixation of CO_2 (Photosynthesis):

An important step in carbon cycle is the fixation of atmospheric CO_2 and its subsequent assimilation into organic molecule. Autotrophic organisms are able to convert CO_2 into organic molecule via photosynthesis process, include plants, algae, some bacteria, and some archaea. The Cyanobacteria and specific soil bacteria have the ability to conduct photosynthesis.

Decomposition:

<u>A biological process that includes physical breakdown and biochemical transformation of complex organic molecules of dead materials into simple organic and inorganic molecules</u>. The major function of soil m.o. in the carbon cycle is as **decomposers-degraders** of complex organic matter that

would otherwise permanently lock carbon, keeping it from being useful to organisms. Bacteria and fungi are particularly effective in breaking down of organic matter, while actinomycetes are responsible in degradation of tough remains, like lignin and chitin.

The rate of decomposition is affected by; soil temperature, moisture, aeration and food availability. Mainly decomposition is either aerobic or anaerobic:

Aerobic decomposition (mineralization) —complete oxidation→ $CO_2 + H_2O$ + energy

- assimilation → Immobilized in form of living matter
- respiration → again recycled to atmosphere

Mineralization: this is the process by which organic matter is decomposed to release simpler inorganic compound (e.g. CO_2, H_2).

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

Anaerobic decomposition → $H_2 + CH_4$ + alcohol + organic acid

Decomposition of organic matter under anoxic conditions differs from oxygen availability, the end products accumulated including **organic acids** and **CH_4**.

Some soil bacteria are capable of producing $CH_{٤}$, termed **methanogenes**, these bacteria are biologically <u>very primitive, strict anaerobic and sensitive to pH</u>.

Methanogenic bacteria are a part of carbon cycle, anaerobically, they **either** combine with acetic acid made by acetogenes bacteria to $CH_{٤}$, $CO_{٢}$ and water

$$HC_{٢}H_{٣}O_{٢} + ٤H_{٢} \xrightarrow{\text{methanogenic m.o.}} CO_{٢} + H_{٢}O + CH_{٤}$$

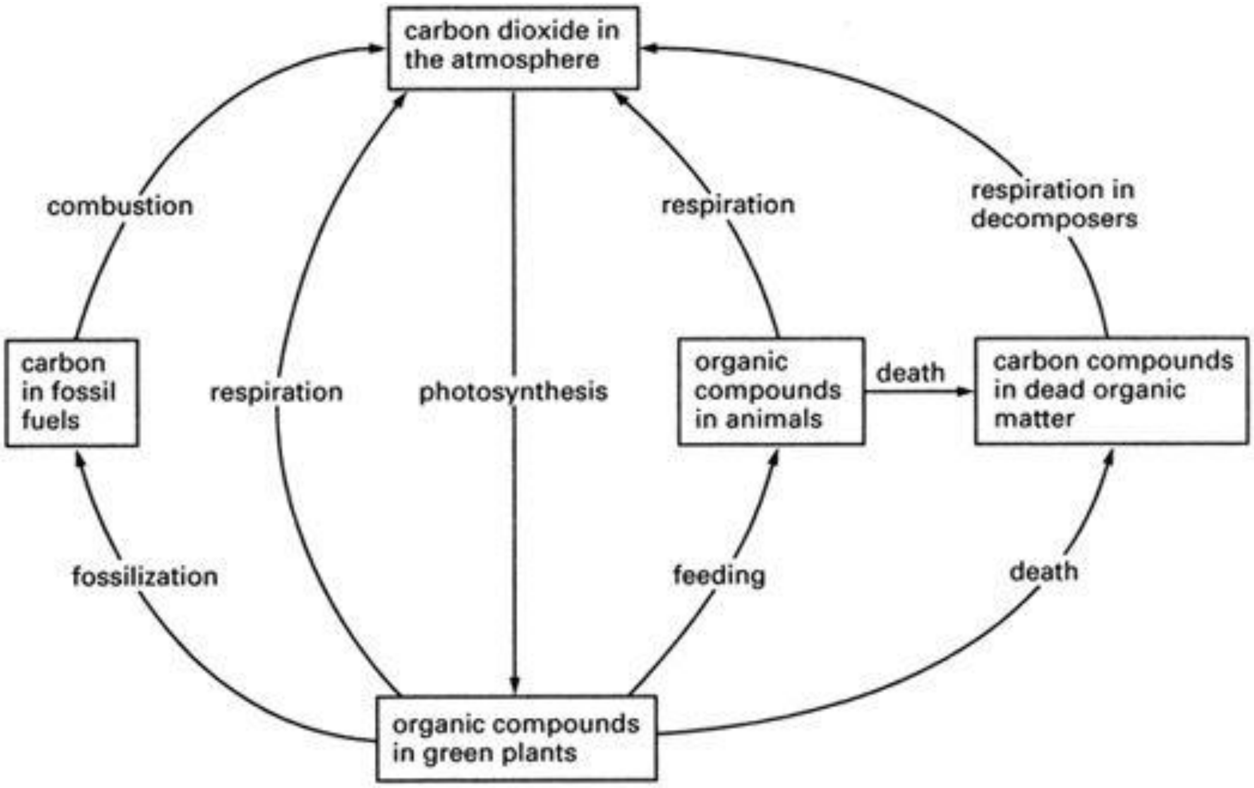
Or derived $CH_{٤}$ from oxidation of hydrogen and reduction $CO_{٢}$

$$CO_{٢} + H_{٢} \longrightarrow CH_{٤} + ٢H_{٢}O$$

<u>Soil methanogenes are</u>: *Methanococcus, Methanobacteria*, *Methanosarcina*.

Some soil bacteria are able to reoxidize released $CH_{٤}$ again to $CO_{٢}$ like *Pseudomonas* and *Methylomonas*, they termed as **methanotrophes**.

Carbon monoxide as a part of carbon cycle, is relatively rare gas under ordinary condition, also released from some activities, it results commonly from partial combustion. Exceedingly poisonous for most aerobic organisms, including man, its relished as a source of energy and carbon by at least one autotrophic bacterial genus *Carboxydothermus.*



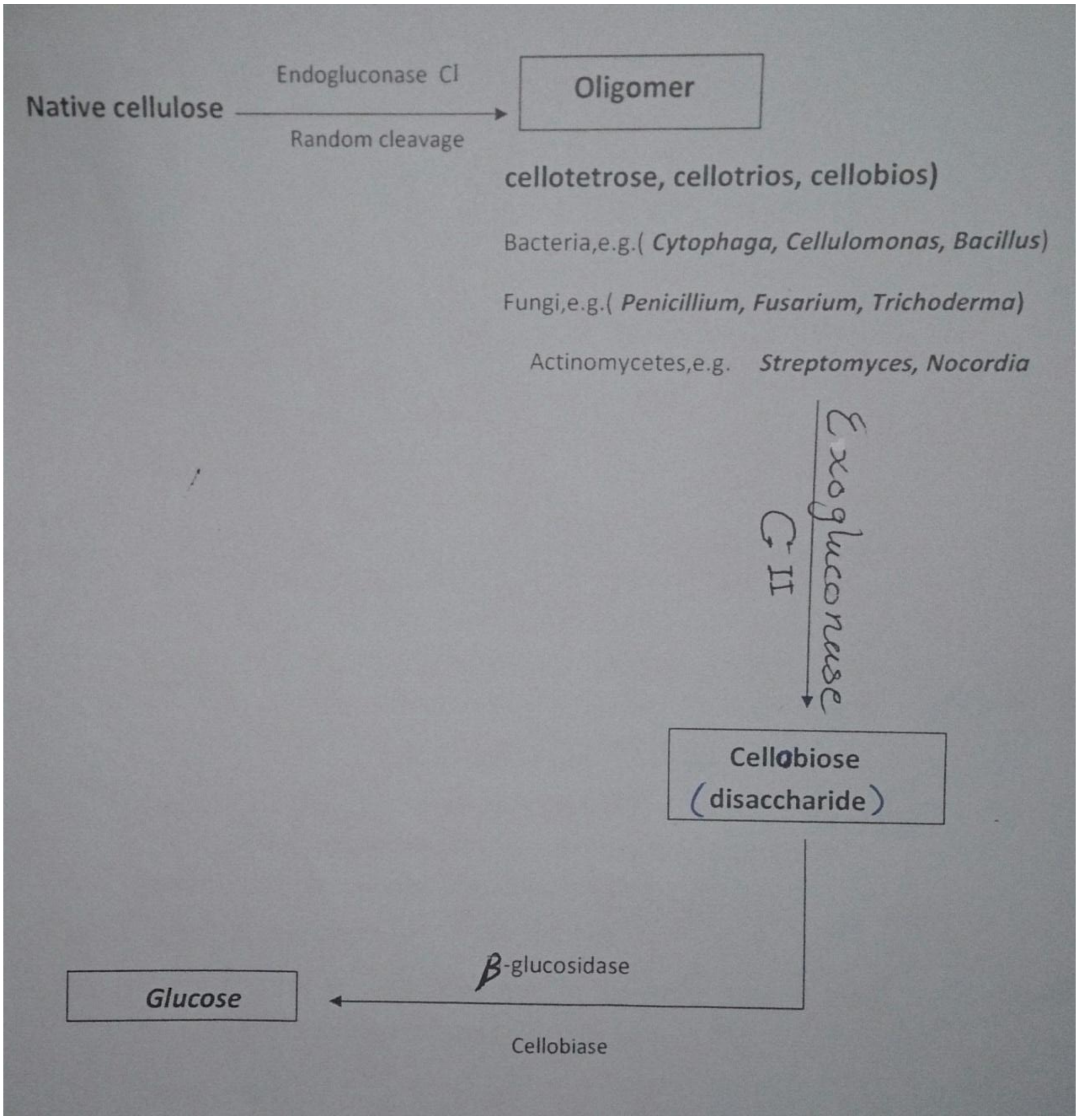
Carbon cycle

Cellulose decomposition:

Cellulose is the most abundant chemical constituent of plant cells, its polysaccharides contain glucose units linked by β-١,٤-linkgae, the total number of glucose units in cellulose molecule vary from ٢ x $١٠^{٣}$ to ١ x $١٠^{٤}$. The total amount of cellulose in plants tissue varies from ١٥ to ٦٠٪.

Cellulose are not tough for decomposition, variety microbes present in millions per ١ millimeter of soil are capable to breakdown cellulose under different circumstances, oxic and anoxic; in availability of oxygen, cellulose decomposes into $CO_٢$, while in anoxic condition cellulose incompletely decomposes with release of many intermediates like organic acids and alcohols.

Three different enzymes involve in cellulose breakage, they collectively termed cellulases, each enzyme participates in certain stage of cellulose decomposition and produced by different microorganisms. Microorganisms that are able to biosynthesize all three enzymes called true cellulytic microorganism. Initial stages of cellulose decomposition take place by cleavage of cellulose by extracellular enzymes then the cleaved pieces are transported into the decomposer cells for energy generation (catabolism) or production of biomass (anabolism) and manipulated by the two rest intracellular lytic enzymes.


Native cellulose
Endogluconase Cl
Random cleavage
Oligomer
cellotetrose, cellotrios, cellobios)
Bacteria,e.g.(Cytophaga, Cellulomonas, Bacillus)
Fungi,e.g.(Penicillium, Fusarium, Trichoderma)
Actinomycetes,e.g. Streptomyces, Nocordia
Exogluconase
C II
Cellobiose
(disaccharide)
β-glucosidase
Cellobiase
Glucose