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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – Environmental Biotechnology – SBTA1304

INTRODUCTION TO ECOLOGY

The meaning of the word ecology was given by German Biologist Hackle in 1869.

• The word ecology is derived from Greek words ‘Oikos’ meaning house, habitat or place of living and ‘Logos’ meaning to study.

• Ecology is defined as the study of interrelationship of different organisms with each other and with their environment. It is concerned with the general principles that apply to both animals and plants.

CLASSIFICATION OF ECOLOGY

Autecology : It deals with the study of an individual species of organisms and it’s population. The ecologists study the behavior and adaptations of particular species to the environmental condition at every stage of that individual’s life cycle. It is also called the Species ecology.

2. Synecology : It deals with the study of communities, their composition, their behavior and relation with the environment. It is also called as Ecology of communities.

It is further divided into 3 types :

1) Population Ecology

2) Community Ecology

3) Ecosystem Ecology

ATMOSPHERE

The atmosphere’s high mobility supports transport of airborne pollutants from one area of the Earth to other regions and even their dispersion on a global scale. A large part of the processes taking place in the biosphere depend on the composition of the air that is used for maintaining life processes, especially in the case of organisms which are much more complicated.Even microscopic amounts of toxic substances can cause adverse effects on human health if exposure takes place for longer periods of time. Earth’s surface, while short-wavelength radiation (ultraviolet light, X-rays, γ rays) is absorbed in the upper layers of the atmosphere.

HYDROSPHERE

The hydrosphere is the sum of all water on the Earth's surface and in the Earth's crust near the surface. The World Ocean makes the largest part of it. The presence of water is a precondition for life onthe Earth, and the existing forms of living organisms are to a great extent determined by water. Water is the main substance forming living organisms. Water is not only the main component of the hydrosphere; it also significantly affects the processes in the biosphere, atmosphere and – being a critical factor for many geological processes – the lithosphere.

LITHOSPHERE

The hard and rigid outer layer of the Earth – the lithosphere – is up to 200 km deep, and it comprises the Earth's crust and the outer part of the upper mantle. The lithosphere is underlained by the asthenosphere .The lithosphere is underlain by the asthenosphere and the deeper part of the upper mantle made of partly melted flowing rock and magma that can come to the Earth's surface during volcanic eruptions. The Earth's outer core is liquid, and it makes ~30%from the Earth's mass, whereas the inner core is solid and composed mostly of iron and nickel.

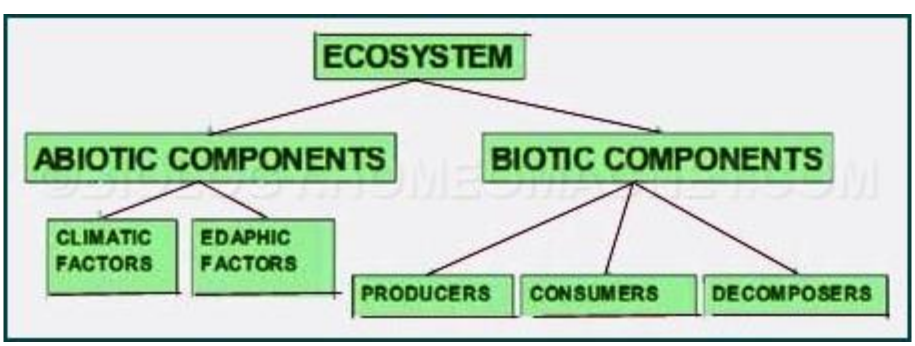
ECOSYSTEM

The term Ecosystem was first proposed by A.G. Tansley in 1935. he defined it as "the system resulting from the interaction of all the living and non living factors of the Environment.

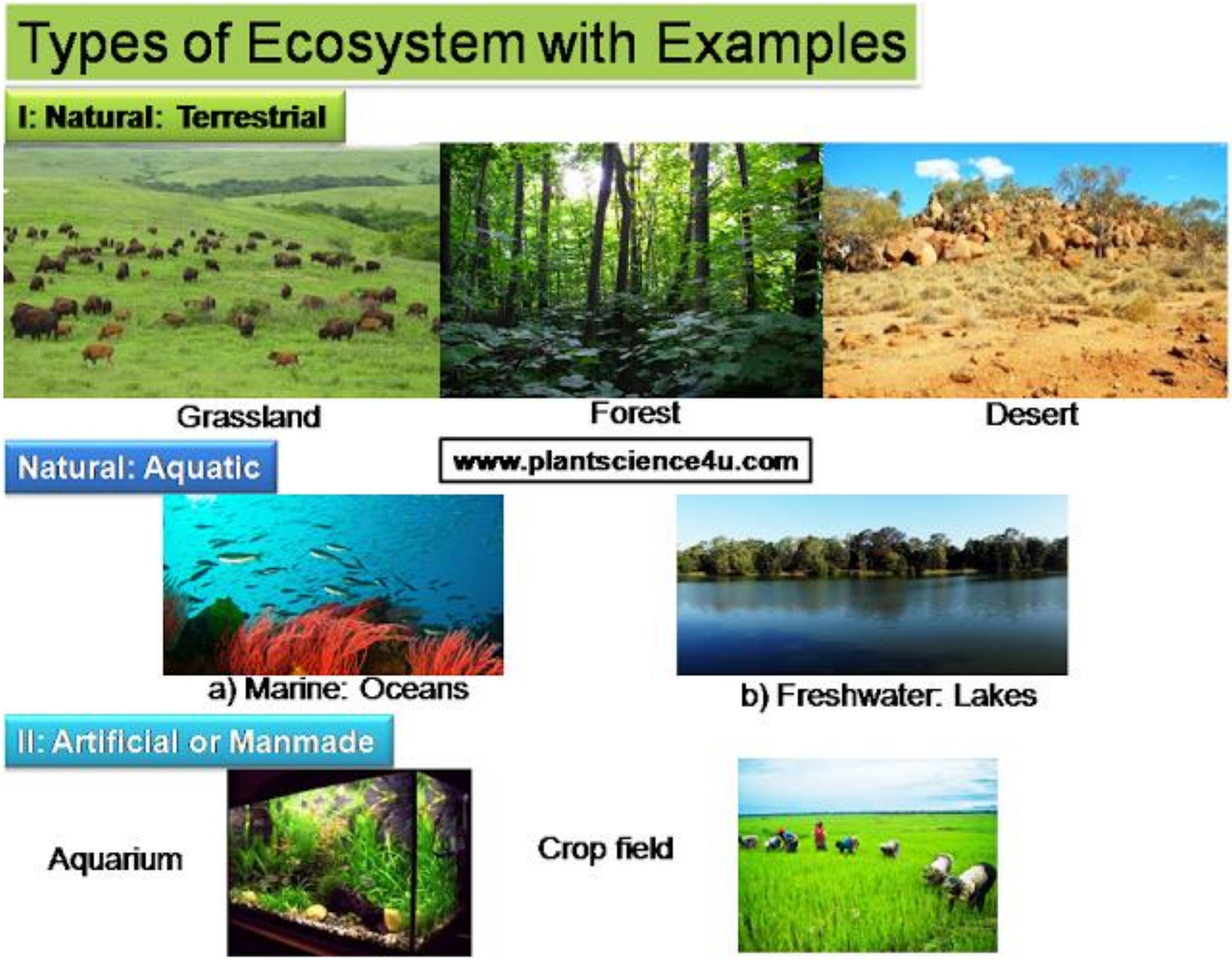
• An ecosystem consists of the biological community that occurs in some locale, and the physical and chemical factors that make up its non-living or abiotic environment. There are many examples of ecosystems

- a pond, a forest, an estuary, a grassland.

STRUCTURE OF ECOSYSTEM



TYPES OF ECOSYSTEM

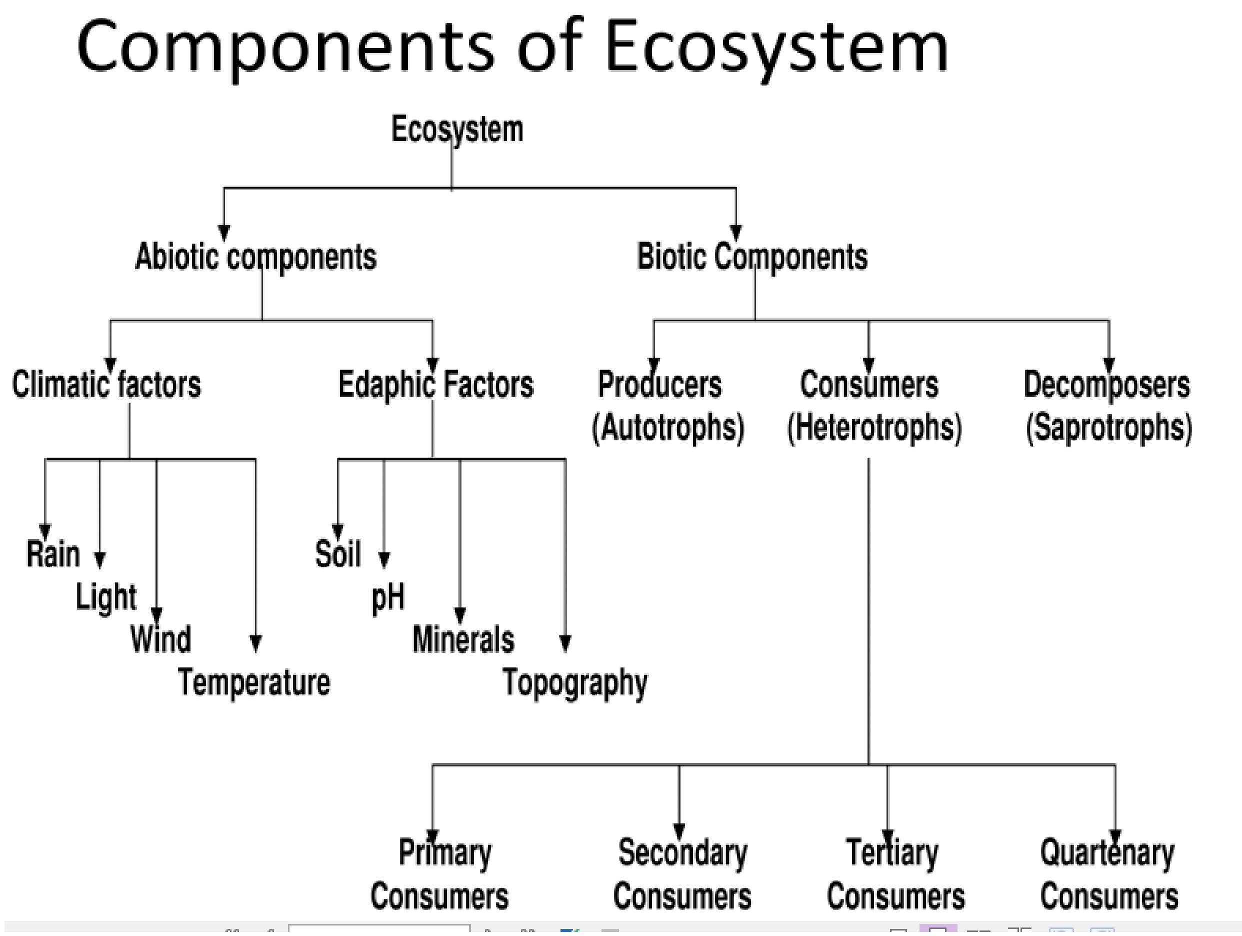


1. Natural Ecosystems : These operate under natural conditions without any major interference by man.

i. Terrestrial Ecosystem : Forest, grassland, desert, etc.

ii. Aquatic Ecosystem : a. Fresh water : Lotic (running water like spring, stream, or rivers) or Lentic (standing water as lake, pond, pools, etc.)

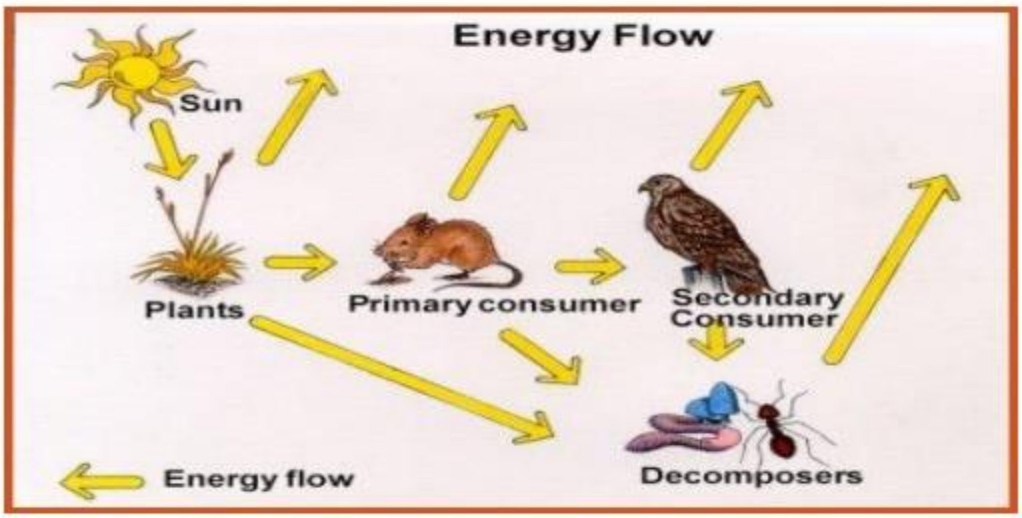
b. Marine water : Such as deep bodies as ocean or shallow ones as Sea or an estuary.



Cybernectics: Cybernetics is a transdisciplinary approach for exploring regulatory systems—their structures, constraints, and possibilities. Norbert Wiener defined cybernetics in 1948 as "the scientific study of control and communication in the animal and the machine"

Homeostatis: homeostasis is the state of steady internal, physical, and chemical conditions maintained by living systems. This is the condition of optimal functioning for the organism and includes many variables, such as body temperature and fluid balance, being kept within certain pre-set limits (homeostatic range). Other variables include the pH of extracellular fluid, the concentrations of sodium, potassium and calcium ions, as well as that of the blood sugar level, and these need to be regulated despite changes in the environment, diet, or level of activity. Each of these variables is controlled by one or more regulators or homeostatic mechanisms, which together maintain life.

Energy transfer in a ecosystem

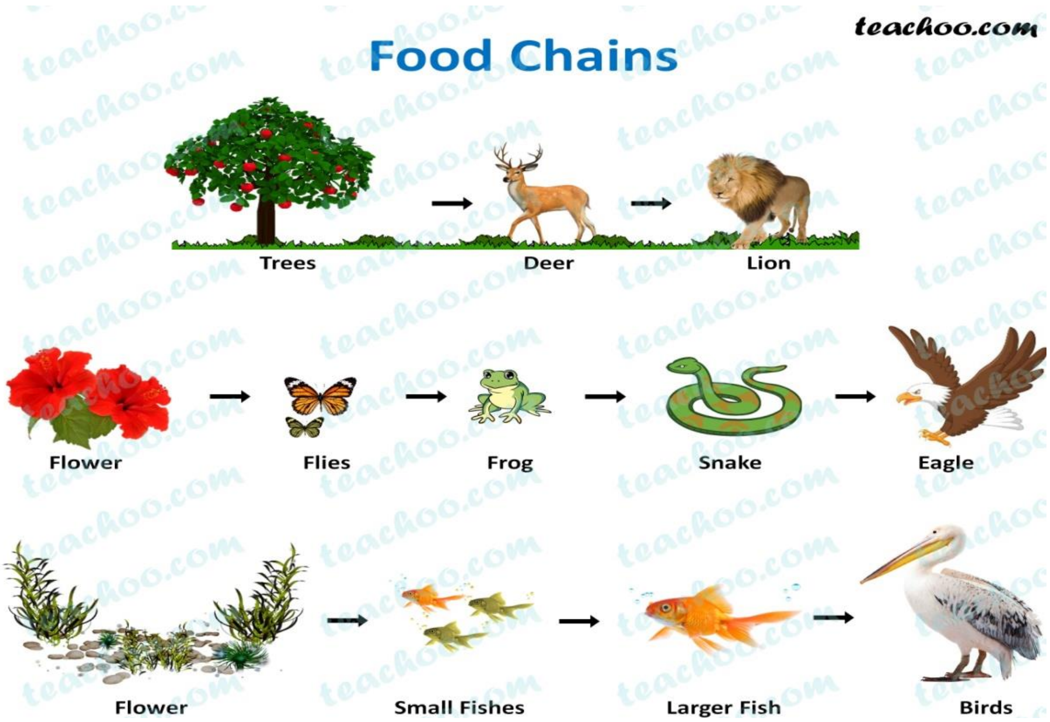


Energy transfer is a one-way process. Once potential energy has been released in some form from its storage in biomass, it cannot all be reused, recycled, or converted to waste heat. This means that if the sun, the ultimate energy source of ecosystems, were to stop shining, life as humans know it would soon end. Fortunately, every day, the sun provides new energy in the form of protons to sustain the food webs of Earth. n 1927, British ecologist Charles Sutherland Elton (1900–1991) wrote that most food webs have a similar pyramidal shape. At the bottom, there are many photosynthetic organisms that collectively have a large biomass and productivity. On each of the following trophic levels, or feeding levels, there are successively fewer heterotrophic organisms, with a smaller productivity. The pyramid of biomass and productivity is now known as the Eltonian pyramid.

Food chain and food web

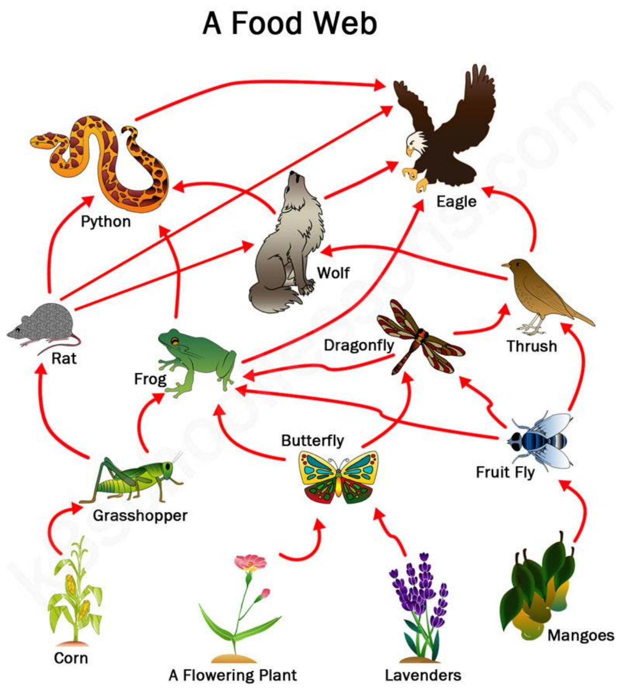
Every organism needs to obtain energy in order to live. For example, plants get energy from the sun, some animals eat plants, and some animals eat other animals.

Food chain



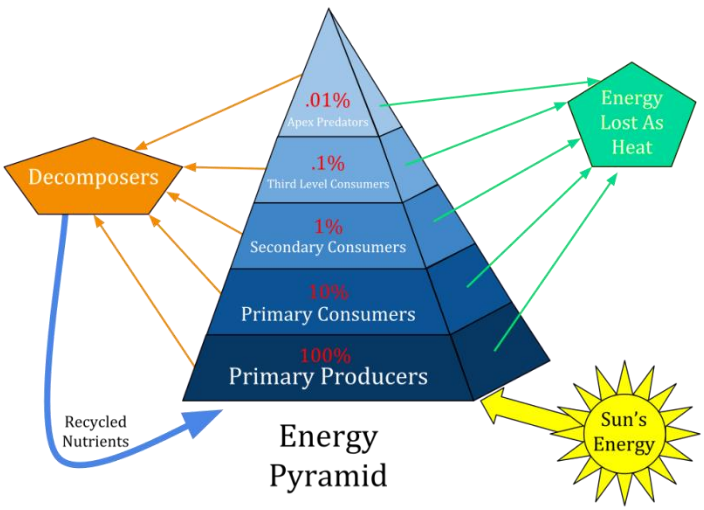
A food chain is the sequence of who eats whom in a biological community (an ecosystem) to obtain nutrition

Food Web



A food web(or food cycle) depicts feeding connections (what-eats-what) in an ecological community and hence is also referred to as a consumerresource system.The food web is a simplified illustration of the various methods of feeding that links an ecosystem into a unified system of exchange.Various food chains are often interlinked at different tropic levels to form a complex interaction between different species from the point of view of food. Food Web provides more than one alternatives of food to most of the organisms in an ecosystem and thus increases their chances of survival.

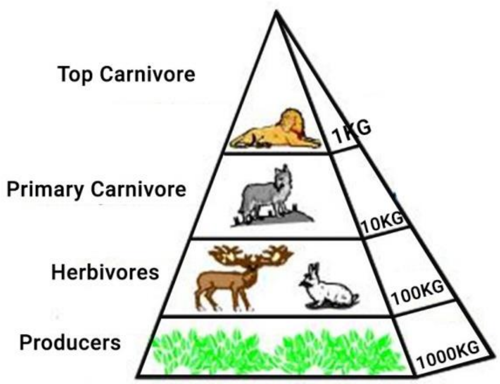
Energy pyramid



Graphic representation of trophic structure & functioning of ecosystem starting with producers at the base & successive trophic levels forming the apex.

All organisms in an ecosystem can be placed in trophic levels depending on what energy source they rely upon and how they provide energy for other organisms in the food web. With the exception of life near hydrothermal vents in the deepbocean, life is always dependent directly or indirectly on the energy from the sun. In everyecosystem, there is an organism at the lowest level that converts energy from the sun into useable energy for other organisms.

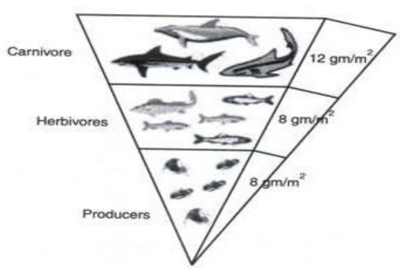
Pyramid Of Numbers



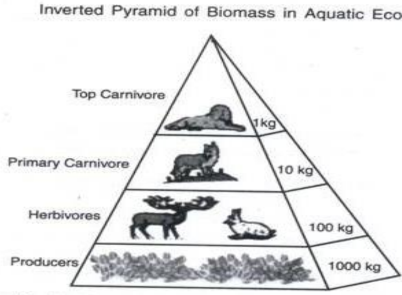
Upright Pyamid of Biomass in aTerrestrial Ecosystem

A pyramid of numbers is a graphical representation of the numbers of individuals in each population in a food chain. Often it is drawn from the autotrophic level up. A pyramid of numbers can be used to examine how the population of a certain species affects another. Often, the autotrophic level in a pyramid of numbers is much larger than any of the higher trophic levels, and the numbers decreases upon ascending the pyramid. There are exceptions, however. For example, in a tree community, a single tree could support many different populations of larger numbers.

Pyramid of Biomass



Inverted Pyramid of Biomass in Aquatic Ecosystem



Upright Pyramid of Biomass for Grassland Ecosystem

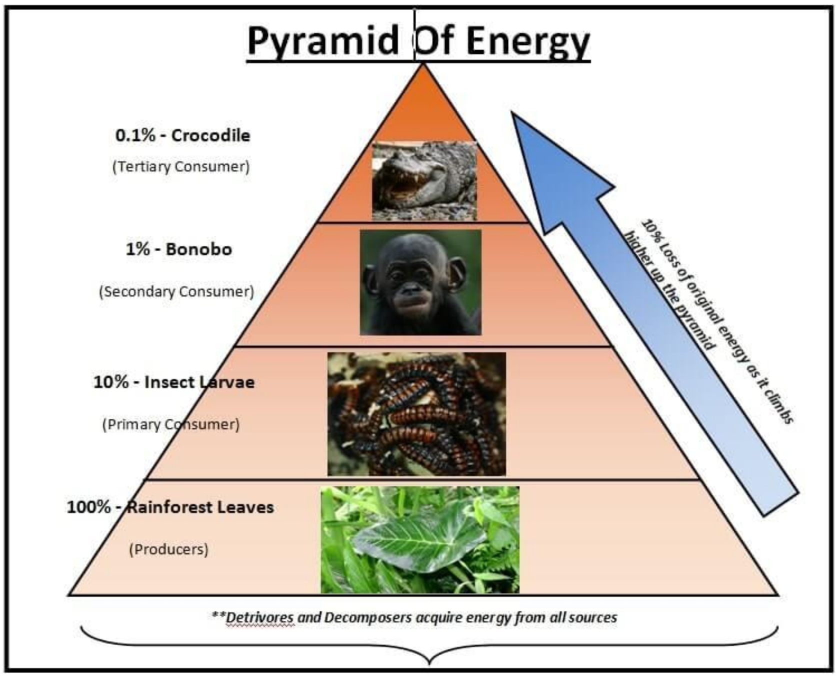
Illustrates the amount of biomass in each trophic level

– Biomass weight is determined after dehydration

• Shows the amount of matterlost between trophic levels.

• Measured in Kg, grams or pounds

Pyramid of Energy



Shows the energy availableat each trophic level.

– The sizeof the blocks represents the proportion of productivity

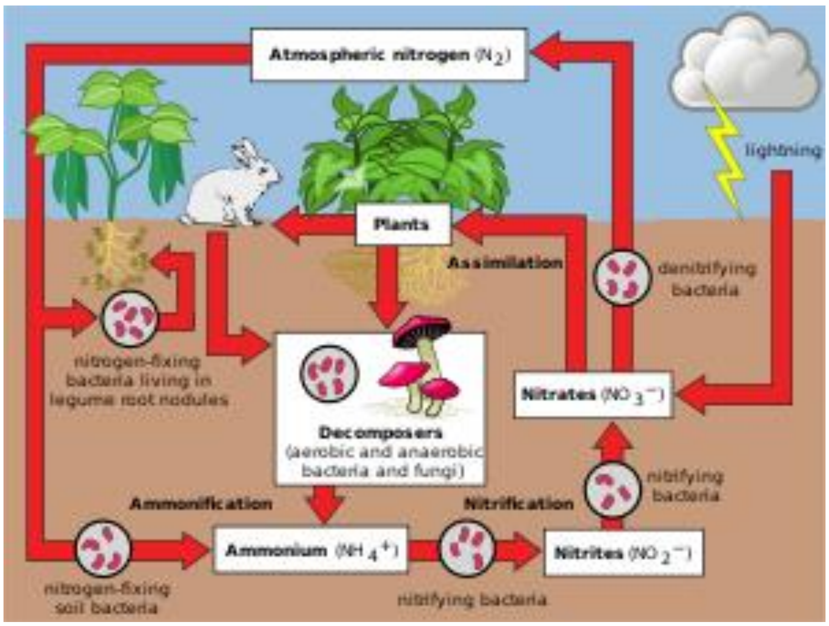
– Measured in Joules or Calories

BIOGEOCHEMICAL CYCLES

Biogeochemical cycle, any of the natural pathways by which essential elements of living matter are circulated. The term biogeochemical is a contraction that refers to the consideration of the biological, geological, and chemical aspects of each cycle.

NITROGEN CYCLE

The nitrogen cycle is the biogeochemical cycle by which nitrogen is converted into multiple chemical forms as it circulates among atmosphere, terrestrial, and marine ecosystems. The conversion of nitrogen can be carried out through both biological and physical processes. The nitrogen cycle involves three major steps: nitrogen fixation, nitrification, and denitrification. It is a cycle within the biosphere which involves the atmosphere, hydrosphere, and lithosphere. Nitrogen is a crucially important component for all life. It is an important part of many cells and processes such as amino acids, proteins and even our DNA. It is also needed to make chlorophyll in plants, which is used in photosynthesis to make their food.

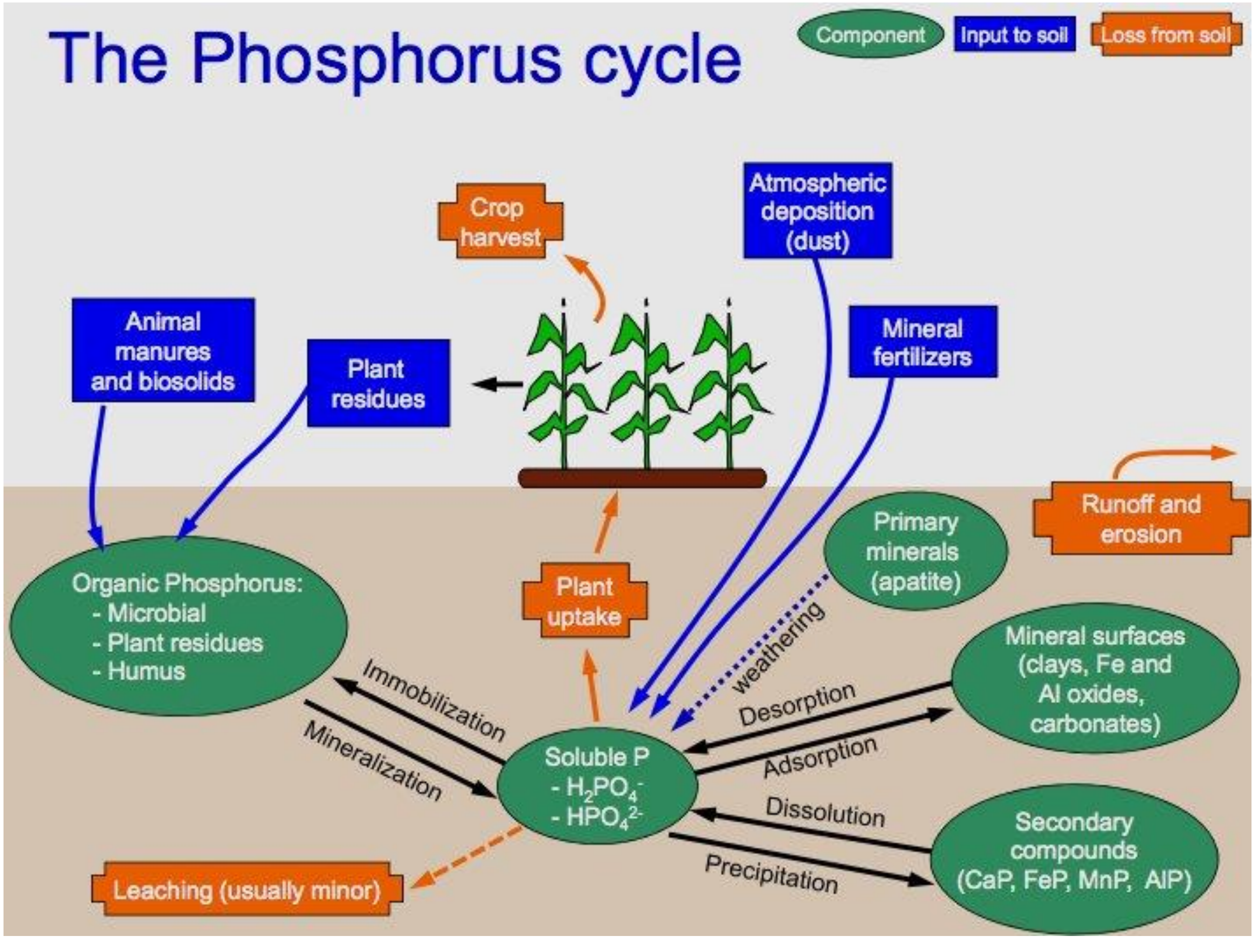


PHOSPHORUS CYCLE

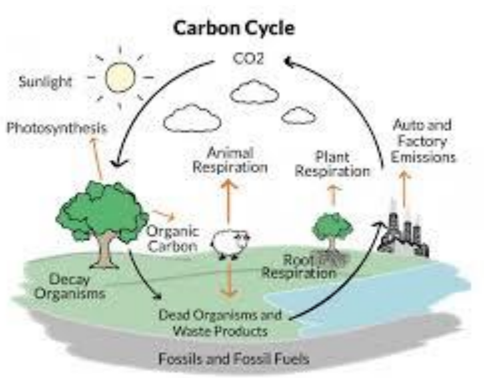
Phosphorus moves in a cycle through rocks, water, soil and sediments and organisms. Over time, rain and weathering cause rocks to release phosphate ions and other minerals. This inorganic phosphate is then distributed in soils and water. Plants take up inorganic phosphate from the soil.

The six steps of phosphorus cycle is

- **Weathering**. Phosphorus is found in the rocks in abundance. ...
- Absorption by Plants. The phosphate salts dissolved in water are absorbed by the plants. ...
- Absorption by Animals. The animals absorb phosphorus from the plants or by consuming plant-eating animals. ...
- Return of Phosphorus Back to the Ecosystem.



CARBON CYCLE



The carbon cycle is the biogeochemical cycle by which carbon is exchanged among the biosphere, pedosphere, geosphere, hydrosphere, and atmosphere of the Earth.

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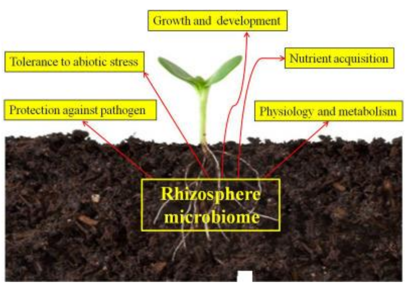
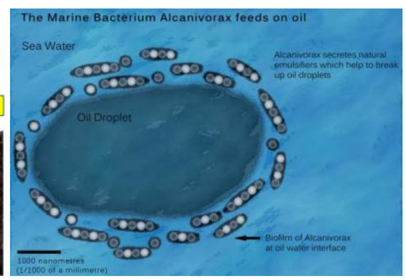
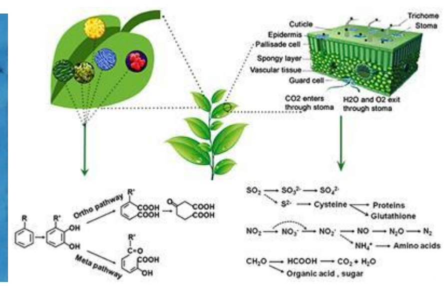
ROLE OF MICROBES IN NATURAL SYSTEM

Microorganisms have special impact on the whole biosphere.

They are the backbone of ecosystems of the zones where light cannot approach.In such zones, chemosynthetic bacteria are present which provide energy and carbon to the other organisms there. Some microbes are decomposers which have ability to recycle the nutrients.So, microbes have a special role in biogeochemical cycles.Microbes, especially bacteria, are of great importance in the sense that their symbiotic relationship (either positive or negative) have special effects on the ecosystem.Microorganisms are cost effective agents for in-situ remediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The ability of each microorganism to degrade toxic waste depends on the nature of each contaminant.







SOIL MICROFLORA

Soil is the outer, loose material of earth’s surface which is distinctly different from the underlying bedrock and the region which support plant life. soil is the region which supports the plant life by providing mechanical support and nutrients required for growth. The soil is one of the most dynamic sites of biological interactions in the nature.It is the region where most of the physical, biological and biochemical reactions related to decomposition of organic weathering of parent rock take place.The population compositions in the activity of microorganism are largely regulated by soil properties and by climate and vegetation.

Microbes in the soil are important to us in maintaining soil fertility / productivity, cycling of nutrient elements in the biosphere and sources of industrial products such as enzymes, antibiotics, vitamins, hormones, organic acids etc. At the same time certain soil microbes are the causal agents of human and plant diseases.

The soil organisms are broadly classified in to two groups viz soil flora and soil fauna, the detailed

Soil Flora

a) Microflora: Bacteria, Fungi, Molds, Yeast, Mushroom, Actinomycetes, Streptomyces, Algae (e.g., BGA,

Yellow Green Algae, Golden Brown Algae).

Bacteria is classified into

I) Heterotrophic e.g. symbiotic & non - symbiotic N2 fixers, Ammonifier, Cellulose Decomposers, Denitrifiers

II) Autotrophic e.g. Nitrosomonas, Nitrobacter, Sulphur oxidizers, etc.

b) Macroflora: Roots of higher plants

Soil Fauna

• a) Microfauna: Protozoa, Nematodes

• b) Macrofauna: Earthworms. moles, ants & others

Ecological impacts of microbes

BACTERIA

Bacteria are the most abundant and predominant organisms. These are primitive, prokaryotic, microscopic and unicellular microorganisms without chlorophyll.Morphologically, soil bacteria are divided into three groups viz Cocci (round/spherical), (rodshaped) and Spirilla/Spirillum (cells with long wavy chains).bacilli are most numerous followed by Cocci and Spirilla in soil.Bacterial population is one-half of the total microbial biomass in the soil ranging from 1,00000 to several hundred millions per gram of soil, depending upon the physical, chemical and biological conditions of the soil.

On the basis of ecological characteristics classified soil microorganisms in general and bacteria in particular into two broad categories:
1. Autochthonous (Indigenous species) : bacterial population is uniform and constant in soil, since their nutrition is derived from native soil organic matter.e.g. Arthrobacter and Nocardia
2. Zymogenous (fermentative): bacterial population in soil is low, as they require an external source of energy.e.g. Pseudomonas & Bacillus.

Bacteria are also classified on the basis of physiological activity or mode of nutrition, especially the manner in which they obtain their carbon, nitrogen, energy and other nutrient requirements. They are broadly divided into two groups
1. AUTOTROPHS:
Autotrophic bacteria are capable synthesizing their food from simple inorganic nutrients, while heterotrophic bacteria depend on pre-formed food for nutrition.

All autotrophic bacteria utilize Co2 (from atmosphere) as carbon source and derive energy either from sunlight (photoautotrophs) e.g. Chromatrum. Chlorobium. Rhadopseudomonas or from the oxidation of simple inorganic substances present in soil (chemoautotrophs) e.g. Nitrobacter, Nitrosomonas, Thiaobacillus.

2. HETEROTROPHS:

Majority of soil bacteria are heterotrophic in nature and derive their carbon and energy from complex organic substances/organic matter, decaying roots and plant residues. They obtain their nitrogen from nitrates and ammonia compounds (proteins) present in soil and other nutrients from soil or from the decomposing organic matter.

FUNCTION OF BACTERIA

1. Decomposition of cellulose and other carbohydrates,
2. Ammonification (proteins ammonia),
3. Nitrification (ammonia-nitrites-nitrates),
4. Denitrification (release of free elemental nitrogen),
5. Biological fixation of atmospheric nitrogen (symbiotic and non-symbiotic)
6. Oxidation and reduction of sulphur & iron compounds.

ACTINOMYCETES

Actinomycetes are clubbed with bacteria, the class of Schizomycetes and confined to the order Actinomycetales.

They are unicellular like bacteria, but produce a mycelium which is non-septate (coenocytic) and more slender, like true bacteria they do not have distinct cell-wall and their cell wall is without chitin and cellulose (commonly found in the cell wall of fungi).Actinomycetes are numerous and widely distributed in soil and are next to bacteria in abundance.They are heterotrophic, aerobic and mesophilic (25-30c) organisms and some species are commonly present in compost and manures are thermophilic growing at 55-65° c temperature (e.g. Thermoatinomycetes, Streptomyces).

Actinomycetes belonging to the order of Actinomycetales are grouped under four families:

1. Mycobacteriaceae
2. Actinomycetaceae
3. Streptomycetaceae
4. Actinoplanaceae

FUNCTIONS OF ACTINOMYCETES

1. Degrade/decompose all sorts of organic substances like cellulose, polysaccharides, protein fats, organic acids etc.
2. Organic residues / substances added soil are first attacked by bacteria and fungi and later by actinomycetes, because they are slow in activity and growth than bacteria and fungi.
3. They decompose / degrade the more resistant and indecomposable organic substance/matter and produce
a number of dark black to brown pigments which contribute to the dark colour of soil humus.
4. They are also responsible for subsequent further decomposition of humus (resistant material) in soil.
5. They are responsible for earthy / musty odour / smell of freshly ploughed soils.
6. Many genera species and strains (e.g. Streptomyces if actinomycetes produce/synthesize number of antibiotics like Streptomycin, Terramycin, Aureomycin etc.

FUNGI

Fungi in soil are present as mycelial bits, rhizomorph or as different spores.

Soil fungi possess filamentous mycelium composed of individual hyphae. The fungal hyphae may be aseptate /coenocytic (Mastigomycotina and Zygomycotina) or septate (Ascomycotina, Basidiomycotina & Deuteromycotina).Most commonly encountered genera of fungi in soil are; Alternaria, Aspergillus,Cladosporium, Cephalosporium Botrytis, Chaetomium, Fusarium, Mucor, Penicillium, Verticillium, Trichoderma, Rhizopus, Gliocladium, Monilia, Pythium, etc. Most of these fungal genera belong to the subdivision Deuteromycotina / Fungi imperfecta which lacks sexual mode of reproduction.As these soil fungi are aerobic and heterotrophic, they require abundant supply of oxygen and organic matter in soil. Fungi are dominant in acid soils, because acidic environment is not conducive / suitable for the existence of either bacteria or actinomycetes. The optimum PH range for fungi lies-between 4.5 to 6.5. They are also present in neutral and alkaline soils and some can even tolerate PH beyond 9.0

FUNCTIONS OF FUNGI

Fungi plays significant role in soils and plant nutrition.

2. They plays important role in the degradation / decomposition of cellulose, hemi cellulose,starch, pectin, lignin in the organic matter added to the soil.
3. Lignin which is resistant to decomposition by bacteria is mainly decomposed by fungi.
4. They also serve as food for bacteria.
5. Certain fungi belonging to sub-division Zygomycotina and Deuteromycotina are predaceous in nature and attack on protozoa & nematodes in soil and thus, maintain biological equilibrium in soil.
6. They also plays important role in soil aggregation and in the formation of humus.
7. Some soil fungi are parasitic and causes number of plant diseases such as wilts, root rots, damping-off and seedling blights e.g. Pythium, Phyiophlhora, Fusarium, Verticillium etc.
8. Number of soil fungi forms mycorrhizal association with the roots of higher plants (symbiotic association of a fungus with the roots of a higher plant) and helps in mobilization of soil phosphorus and nitrogen.e.g. Glomus, Gigaspora, Aculospora, (Endomycorrhiza) and Amanita, Boletus, Entoloma, Lactarius (Ectomycorrhiza).

ALGAE

Algae are present in most of the soils where moisture and sunlight are available.Their number in soil usually ranges from 100 to 10,000 per gram of soil. They are photoautotrophic, aerobic organisms and obtain CO2 from atmosphere and energy from sunlight and synthesize their own food. They are unicellular, filamentous or colonial. Soil algae are divided in to four main classes or phyla as follows:

1. Cyanophyta (Blue-green algae)
2. Chlorophyta (Grass-green algae)
3. Xanthophyta (Yellow-green algae)
4. Bacillariophyta (diatoms or golden-brown algae)

Out of these four classes / phyla, blue-green algae and grass-green algae are more abundant in soil. The green-grass algae and diatoms are dominant in the soils of temperate region while blue green algae predominate in tropical soils.

FUNCTIONS OF ALGAE OR BGA

1. Plays important role in the maintenance of soil fertility especially in tropical soils.
2. Add organic matter to soil when die and thus increase the amount of organic carbon in soil.
3. Most of soil algae (especially BGA) act as cementing agent in binding soil particles and thereby reduce/prevent soil erosion.
4. Mucilage secreted by the BGA is hygroscopic in nature and thus helps in increasing water retention capacity of soil for longer time/period.
5. Soil algae through the process of photosynthesis liberate large quantity of oxygen in the soil environment and thus facilitate the aeration in submerged soils or oxygenate the soil environment.
6. They help in checking the loss of nitrates through leaching and drainage especially in un-cropped soils.
7. They help in weathering of rocks and building up of soil structure

SOIL MICROORGANISM – PROTOZOA

These are unicellular, eukaryotic, colourless, and animal like organisms (Animal kingdom). They are larger than bacteria and size varying from few microns to a few centimetres. Their population in arable soil ranges from l0,000 to 1,00,000 per gram of soil and are abundant in surface soil. They can withstand adverse soil conditions as they are characterized by "cyst stage" in their life cycle.Most of the soil protozoa are motile by flagella or cilia or pseudopodia as locomotors organs.Depending upon the type of appendages provided for locomotion, protozoa are

- Rhizopoda (Sarcondia)
- Mastigophora
- Ciliophora (Ciliata)
- Sporophora (not common Inhabitants of soil

FUNCTION OF PROTOZOA

1. Most of protozoans derive their nutrition by feeding or ingesting soil bacteria belonging to the genera Enterobacter, Agrobacterium, Bacillus, Escherichia, Micrococcus, and Pseudomonas and thus, they play important role in maintaining microbial / bacterial equilibrium in the soil.
2. Some protozoa have been recently used as biological control agents against phytopathogens.
3. Species of the bacterial genera viz. Enterobacter and Aerobacter are commonly used as the food base for isolation and enumeration of soil protozoans.
4. Several soil protozoa cause diseases in human beings which are carried through water and other vectors, e.g. Amoebic dysentery caused by Entamoeba histolytica.

Symbiosis-Nitrogen fixation

Nitrogen is an essential constituent of all biomolecules both in plants and in animals. Nitrogen fixation is the first step of nitrogen cycle where molecular nitrogen is reduced by nitrogen fixing bacteria to yield ammonia. Nitrogen cycle has 3 process

• Ammonification

• Nitrification

• Denitrification

Nitrogen fixation is the process of reducing dinitrogen to ammonia so that plants can absorb nitrogen.

Symbiotic nitrogen fixation :

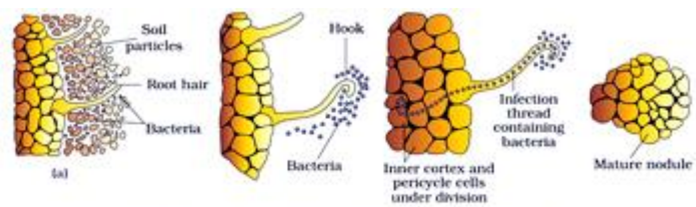
• Symbiotic nitrogen fixation occurs in plants that harbour nitrogenfixing bacteria within their tissues

• The best-studied example is the symbiotic association between roots of legumes and bacteria of the genus Rhizobium .This association results form the root nodules in legumes

• Root nodules – it is a enlarged multicellular structures on roots

• Legume – rhizobium association will fix 25 – 60 kg of molecular nitrogen annually

Root nodule formation

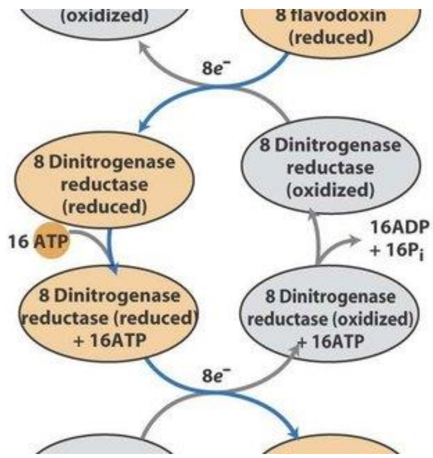


Development of root nodules in soyabean : (a) *Rhizobium* bacteria contact a susceptible root hair, divide near it, (b) Successful infection of the root hair causes it to curl, (c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroids and cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation. (d) A mature nodule is complete with vascular tissues continuous with those of the root

The reaction is as follows:

$$N_2 + 8e^- + 8H^+ + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

Action of nitrogenase enzyme :



• For reducing nitrogen into ammonia nitrogenase requires 8 electrons

• At first dinitrogense is reduced by transferring of electrons to dintrogenase reductase

• Dinitrogenase has a 2 binding sites for reductase

• The 8 electrons are transferred from reductase to dinitrigenase one at a time :

• Reduced reductase binds to dinitrogenase and transfers single electron , oxidized form dissociates in a repeating cycle

• Each cycle requires the hydrolysis of ATP molecule by dinitrogenase reductase

• Immediate source of electrons to reduce reductase is reduced ferredoxin

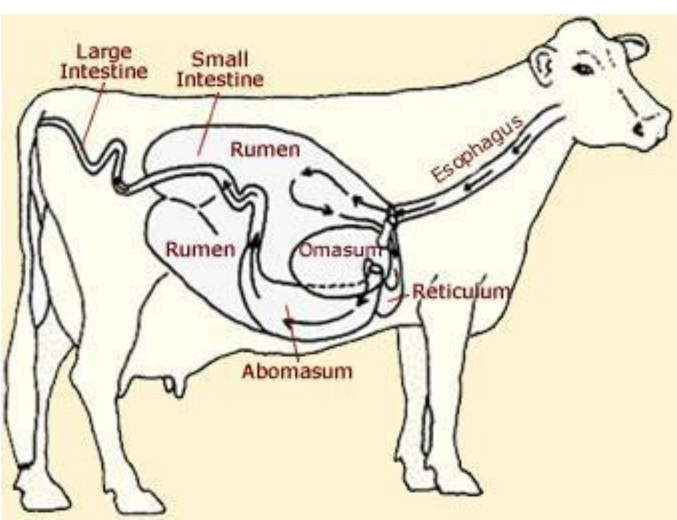
• Ultimate source of electrons to reduce ferredoxin is Pyruvate

Ruminant symbiosis

Ruminant are any of the various cud-chewing mammals having a stomach divided into four compartments.eg., Cows, sheep, moose, goats, antelope, and camels.

Ruminant stomachs have four compartments: the **rumen**, the reticulum, the omasum and the abomasum. **Rumen** microbes ferment feed and produce volatile fatty acids, which is the **cow's** main energy source. Unlike monogastrics such as swine and poultry, ruminants have a digestive system designed to ferment feedstuffs and provide precursors for **energy** for the animal to use.

There are four sections of the stomach **— rumen, reticulum, omasum and abomasum** — each with a particular job to do. These sections store chewed plant material and grain, absorb nutrients and vitamins, break down proteins, aid in beginning **digestion** and dissolve material into processable pieces.



1. Rumen

The four components of a cattle's stomachThe rumen, also known as the “paunch,” is the first area of the cow’s stomach, connected to the cattle’s esophagus. This compartment acts as storage for chewed vegetation and forms balls of cud. Cud consists of large, non-digestible pieces of plant matter that must be regurgitated, chewed a second time and swallowed before continuing through the process. The rumen absorbs nutrients through papillae of the rumen wall and facilitates fermentation, creating the rumen bacteria and rumen microbes necessary to break down and digest the proteins in feed. Microorganisms in the rumen are responsible for digesting cellulose and complex starches, as well as synthesizing protein, B vitamins and vitamin K. As a storage area, it can hold up to 40 gallons of material. The rumen, combined with the reticulum, makes up 84% of the volume of the entire stomach. A few common health issues with the rumen include bloat, which occurs when a cow can’t eradicate a buildup of gas, acidosis and rumenitis, which occur when low pH balance allows for high acid production. These can be prevented by managing and paying attention to cattle’s food and water intake.

2. Reticulum

The reticulum is frequently referred to as the “honeycomb,” because the inner lining appears like and is structured similar to a honeycomb. While it does have its independent functionality, the reticulum is attached to the rumen with only a thin tissue divider. This component holds heavy or dense objects — such as metal pieces and rocks — and trap large feed particles that are not small enough to be digested. The reticulum facilitates regurgitation. Both the rumen and reticulum contain digestive bacteria, so no acid is included in the regurgitation of materials. The reticulum holds about 5 gallons of material. One common health issue involving the reticulum is hardware disease, which occurs when cattle ingest heavy or sharp objects — like nails, screws or wire. They are swept into the reticulum and may puncture the stomach wall. This disease is preventable by putting magnets on feeding equipment to catch any metal, or cured by the placement of an intraruminal magnet that traps already swallowed objects.

3. Omasum

The globe-shaped omasum is nicknamed “manyplies” because of its internal structure. It is lined with large leaves and folds of tissue that resemble the pages of a book. These folds absorb water and nutrients from feed that passes through after its second round of chewing. The omasum is smaller than the rumen and reticulum, making up about 12% of the stomach’s total volume. It can hold up to about 15 gallons of material.

4. Abomasum

The abomasum is the last component of the stomach and is often known as the "true stomach," because it operates the most similar to a non-ruminant stomach. This true stomach is the only compartment of the stomach lined with glands. These glands release hydrochloric acid and digestive enzymes to help the abomasum further break down feed and plant material. In comparison to the other chambers, the abomasum is on the smaller side, representing about 4% of the total stomach volume and only holding about 7 gallons of material.

Each of these components is vital in maintaining a healthy digestive process. They must cooperate quickly and efficiently to turn grain and plant matter into energy for the cattle. If one section becomes incapable of performing or ceases to work correctly, it will affect all of the other functions in the digestive system.

Because the rumen is the largest area of the stomach and the section that focuses on reducing feed to be passed through the digestive process, it is crucial that it is properly developed and remains healthy.

Ruminal Microflora

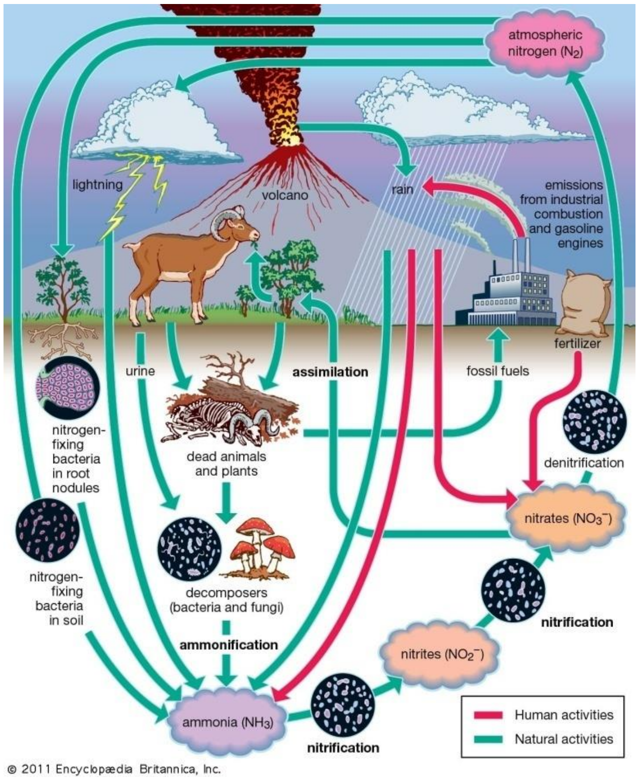
The rumen hosts a very diverse community called the microbiota. A microbiota is the whole of the ecosystem (bacteria, yeast, fungi and viruses) living in a specific environment. Intestinal microbe ota was previously called "intestinal flora."

Bacteria: The Ruminococcus genus, which includes Ruminococcus albus and Ruminococcus flavifaciens, is just one of many microbes living in the rumen. Others include Megasphaera, Fibrobacter, Streptococcus, Escherichia, Chytridiomycetes fungi, and methanogens.Role of protozoa: Digestion and fermentation carbohydrates and proteins, ingest bacteria and feed particles and engulf feed particle, digest CHOH, proteins and fats.

Nitrogen Cycle: It is a biogeochemical process through which **nitrogen** is converted into many forms, consecutively passing from the atmosphere to the soil to organism and back into the atmosphere. It involves several processes such as **nitrogen fixation**, nitrification, denitrification, decay and putrefaction.

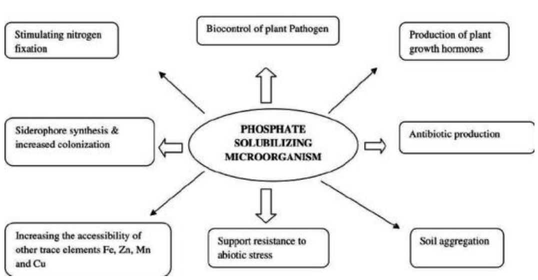
Process of Nitrogen cycle includes

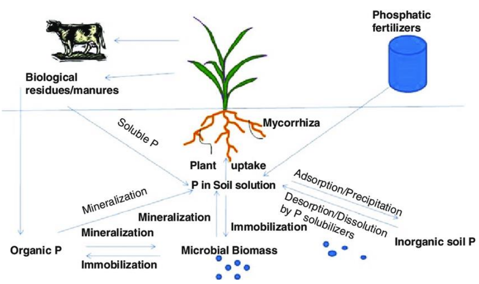
- Ammonification
- Nitrification
- Denitrification
- Nitrogen Fixation



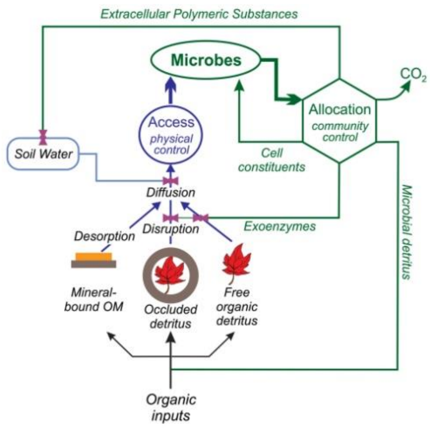
Phosphorus

Phosphorus moves in a **cycle** through rocks, water, soil and sediments and organisms. Over time, rain and weathering cause rocks to release phosphate ions and other minerals. When the plant or animal dies, it decays, and the organic phosphate is returned to the soil.



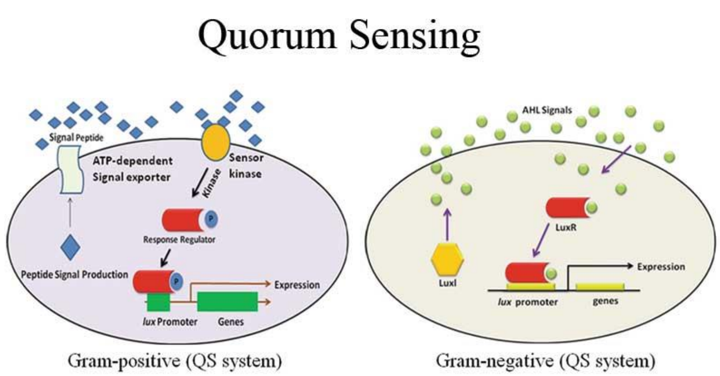


Carbon



- **Carbon Cycle Carbon cycle** explains the movement of **carbon** between the earth's biosphere, geosphere, hydrosphere and atmosphere. ... **Carbon** atoms are then released as **carbon** dioxide when organisms respire. The formation of fossil fuels and sedimentary rocks contribute to the **carbon cycle** for very long periods. Carbon enters the atmosphere as CO2
- CO2 is absorbed by autotrophs such as green plants
- Animals consume plants, thereby, incorporating carbon into their system
- Animals and plants die, their bodies decompose and carbon is reabsorbed back into the atmosphere.
-

QUORUM SENSING



Quorum' is a Latin word. It means the number of members of a grouprequired to be present to transact business or carry out an activity legally. Quorum sensing is a process of bacterial cell-tocell communicationinvolving the production and detection of extracellular signaling molecules (autoinducers). "Autoinducers"contribute to the regulation of the expression of particular genes.

This is the intra communication system that is used by like bacteria to establish presence of their own kind. Each bacteria is able to send out a signals and receive one as well. This allows each individual bacteria to count how many other bacteria there are.Once the bacterium can assess that there is a proper amount of other bacteria present, it can simultaneously, along with the other bacteria emit a response such as virulence or bioluminescence. Fuqua et al. (1994) introduced the term quorum sensing to describe cell-cell signaling in bacteria. Within a single bacterial species as well as between diverse species.Quorum sensing allows both Gramnegative and Grampositive bacteria to sense one another and to regulate a wide variety of physiological activities.

Examples for quorum sensing.

Microrganism	**Major Signal Molecules**	**Regulatory System**	**Group-Derived Benefits**
Bacillus subtlis	ComX CSF (PhrC) PhrA,-E, -F, -K, -H	ComP/ComA Rap proteins	Competence, sporulation, biofilm formation, antibiotic production,
Myxococcus xanthus	A-signal C-signal	SasSRN	Fruiting body formation or sporulation
Pseudomonas aeruginosa	3O-C12-HSL C4-HSL	LasI/LasR RhlI/RhlR OscR (orphan)	Structured biofilm formation, virulence factors
Staphylococcus aureus	AIP-I, AIP-II, AIP-II, AIP-IV	AgrC/AgrA	Biofilm formation, virulence factors
Streptococcus mutans	CSP (ComC) XIP (ComS)	ComD/ComE ComR	Bacteriocins, biofilm formation, competence
Streptococcus pneumoniae	CSPs	ComD/ComE	Competence, fratricide, biofilm formation, virulence

List of organism involved in quorum sensing

Name of the organism	
Aliivibrio fischeri	Gram-negative
Curvibacter sp	Gram-negative
Escherichia coli	Gram-negative
Salmonella enterica	Gram-positive
Pseudomonas aeruginosa	Gram-positive
***Acinetobacter* sp.**	Gram-positive
***Aeromonas* sp.**	Gram-positive
Yersinia	Gram-positive
Archaea ***Methanosaeta harundinacea* 6Ac**	methanogenic archaeon
Social insects	**Ants**
Social insect	**Honey bees**

Process of quorum sensing:

Quorum sensing can be divided into at least 4 steps:

(1) Production of small biochemical signal molecules by the bacterial cell

(2) Release of the signal molecules, either actively or passively, into the surrounding environment

(3) Recognition of the signal molecules by specific receptors once they exceed a threshold concentration, leading to

(4) Changes in gene regulation

Each individual bacterium is capable of producing a signaling molecule (inducer) and each bacterium also has a receptor for the inducer. Signals lead to activation and suppression of certain genes leading to changes in metabolic activity, morphology, mobility, aggregation and association with other cells of same species or different species.

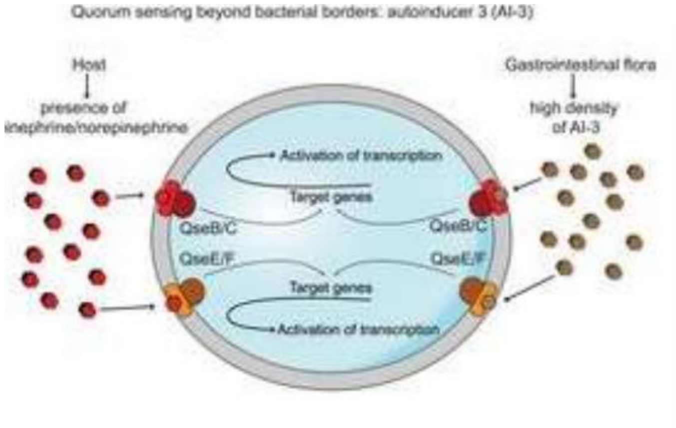
Three main types of inducer molecules :

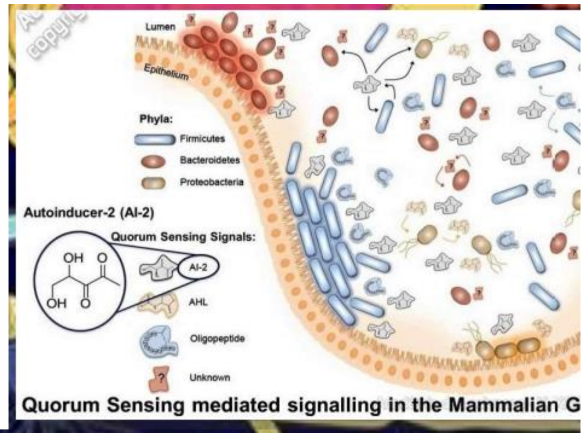
1) Acyl-homoserine lactones (AHLs)

2) Autoinducer peptides (AIPs)

3) Autoinducer-2 (AI-2)

The pathogenic bacteria can interact with eukaryotic host cells, and vice versa, by utilizing each other's autoinducing signals.





MICROBIAL FUEL CELL

Microbial Fuel Cells have the potential to simultaneously treat wastewater for reuse and to generate electricity; thereby producing two increasingly scarce resourcesMicrobial fuel cell technology represents a new form of renewable energy by generating electricity from what would otherwise be considered waste, such as industrial wastes or waste water .

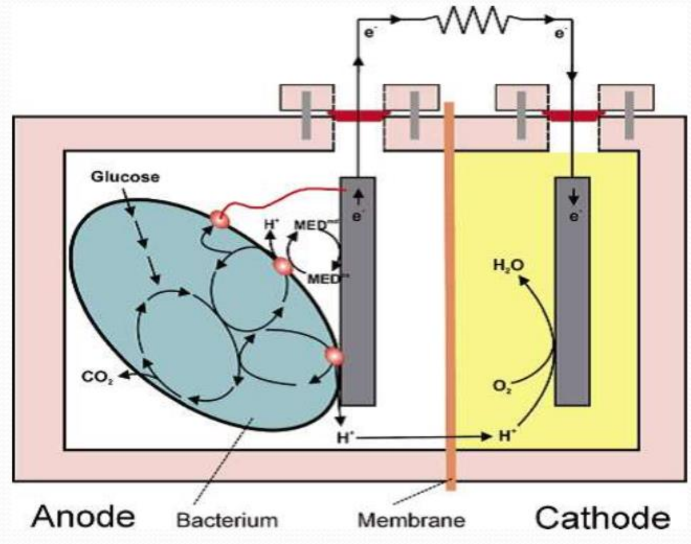
M.C Potter was the first to perform work on the subject in 1911 in E.coli,A professor of botany at the University of Durham. In 1931, however, Barnet Cohen drew more attention to the area when he created a number of microbial half fuel cells that, when connected in series, were capable of producing over 35 volts, though only with a current of 2 milliampsIn 1911 B.H. Kim devoloped mediatorless MFC was a milestone in MFC,Enhanced the commercial viablity,by eliminating costly mediator chemicals.

Fuel cell: Device that converts chemical energy from fuel into electricity through chemical reaction with oxygen or another oxidizing agent.

What are Microbial fuel cells?

- It chemical energy to electrical energy
- Does catalytic reaction of microorganisms
- Has Bio-electrochemical system
- Mimics bacterial interaction

CONSTRUCTION OF MFC:



Components of MFC

- Anode
- Cathode
- Exchange membrane
- Substrate
- Electrical circuit
- Microbes

Microbes used in MFC

- Axenic bacterial culture
- Metal reducing bacteria
- Shewanella putrefaciens
- Geobacter sulfurreducens
- Rhodoferax ferrireducens
- Clostridium beijerinckii
- Mixed bacterial fuel culture
- Desulfuromonas,
- Alcaligenes faecalis,
- Enterococcus faecium,
- Pseudomonas aeruginosa,
- Proteobacteria

Working mechanism:
Anode and cathode separated by cathode specific membrane
- Microbes at anode oxidize organic fuel generates electrons and protons
- Protons move to the cathode compartment through the membrane

 Electrons transferred to the cathode compartment through external circuit to generate current
 Electrons and protons are consumed in cathode chamber, combining with O2 to form water
 Anodic reaction:
$CH_3COO^- + H_2O \rightarrow 2CO_2 + 2H^+ + 8e$acetate
 Cathodic reaction: $O_2 + 4e^- + 4 H^+ \rightarrow 2 H_2O$

Applications of MFC

 Waste water treatment
 Power generation
 Secondary fuel production
 Bio-Sensors
 Desalination
 Educational tool

Advantages of MFC

 Generation of energy out of biowaste / organic matter
 Direct conversion of substrate energy to electricity
 Omission of gas treatment
 Aeration
 Bioremediation of toxic compounds

Limitations of MFC

 Low power density
 High initial cost
 Activation losses
 Ohmic losses
 Bacterial metabolic losses

PREBIOTICS AND PROBIOTICS

- The term probiotics are derived from Greek.

- Literally means “for life”.
- Firstly, observed by Lilly and Stillwell in 1965.
- Currently defined by WHO and FAO
- “live microorganisms which when administered in adequate amounts confer a health benefit to the host.
- Lactic acid bacteria (LAB) and Bifidobacteria are the most common types of microbes used as probiotics but certain yeasts and bacilli may also be used.

CHARACTERISTICS OF PROBIOTICS

- They are microbial organisms.
- probiotics remain viable and stable after culture manipulation and storage before consumption.
- They survive gastric and pancreatic digestion.
- Able to adhere to the mucosal surface.
- Able to induce host response once they enter the intestinal microbial ecosystem and they yield functional and clinical benefits to the host when consumed.
- They should colonize.
- They should colonize the intestine.
- Able to utilize the passage through the digestive system.
- Nonpathogenic and non-toxic.
- Capable of exerting a beneficial effect on the host.

MECHANISMS OF ACTION OF PROBIOTICS

- Although not well documented, some mechanisms probiotic action includes.
- Competition for adhesion sites with pathogens in which:
- Adhere to the epithelium and act “colonization barriers” by preventing the pathogen from adhering to the mucosa.
- Synthesis of antimicrobial compounds:
- Produce bacteriocin and other antimicrobial compounds such as hydrogen peroxide, diacetyl, and short-chain fatty acids.
- Stimulate the immune response:
- Increase the secretion of IgA, enhance the phagocytic activity of macrophages, enhance lymphocyte cytokines response.
- Probiotics may also compete for nutrients that would otherwise be utilized by pathogens:
- Probiotics utilize the monosaccharides, which results in the inhibition of *Clostridium difficile*.
- Promote excretion of toxic substances such as bile acids, nitrosamines, and mutagenic compounds.

COMMERCIAL FORMS OF PROBIOTICS

- There are two main forms in which probiotic organisms can be ingested:
- Fermented foods:
- dairy e.g. yogurt
- vegetable origin e.g. Sauer kraut
- probiotic supplements;
- freeze-dried bacteria in powder, capsule, or tablet form

PREBIOTICS

- Firstly, observed by Gibson and Roberfroid, who define a prebiotic as
- “a non-digestible food ingredient that confers beneficial effects in the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon and thus improves host health and well-being”.

Prebiotics are essentially fiber from plants (fruits, vegetables or whole grains).

- Prebiotics transit through the stomach and small intestines undigested.

- They make their way to intact to the colon, where they will be bacterially fermented together with the unabsorbed nutrients.
- Lactobacilli and Bifidobacterium more likely to benefit from prebiotics.

Examples of prebiotics

- Fructooligosaccharides (such as inulin and its derivatives).
- Other oligosaccharides such as raffinose, stachyose, and verbascose.
- Polysaccharides (such as cellulose, hemicellulose, pectin)
- Soybeans oligosaccharides, etc.

BENEFITS OF PREBIOTICS

The most recognized beneficial effects of prebiotics are:

- Reduce blood glucose.
- reducing energy intake (which results in weight management especially in combination with probiotics.
- having positive effects on the immune system (e.g., less risk for allergy in both infants and adults especially in combination with probiotics).
- Enhancing the feeling of satiety.
- Providing energy-yielding metabolites through colonic fermentation.
- Improving laxation or regularity by increasing stool bulk.

INTESTINAL MICROFLORA

- Most human mucosal surfaces colonized by bacteria.
- Colonization of the gut with healthy normal microflora begins immediately after birth increasing through infanthood.
- At birth, the gut is sterile, but it is rapidly colonized by bacteria from the mother's birth canal and the environment.
- Some bacteria are got from breast milk during breastfeeding.
- With the introduction of solid foods, microflora becomes more complex and resembles that of adults by the second year of life.
- The density of colonization increases from the stomach to that distal colon.

PROBIOTICS IN PREVENTION AND TREATMENT

- Probiotics have a wide range of beneficial effects and numerous indications.
- Acute diarrhea
- Antibiotic associated diarrhea
- Allergy prevention

DIARRHEA

- Probiotics reduce 57% diarrheal diseases especially acute infection diarrhea.
- Antibiotic-associated diarrhea is an acute inflammation of the intestine mucosa by the administration of a broad spectrum of antibiotics.
- Several probiotic bacteria reduce risk from 28.5% to 11.9%.
- Nosocomial diarrhea is reduced by B. bifidum, S. thermophiles.

VAGINAL MICROFLORA

- Lactobacillus normally inhabit the vagina.
- Lactobacilli bacteria produce H_2O_2 which keeps the healthy balance of virginal microorganism.
- H_2O_2 interacts with peroxidase present in vaginal fluid.
- Disruption in microflora balance in vagina results in disease.
- Probiotics cure and reduce the reoccurrence of the vaginal disease.
- It can be administrated orally or vaginally.

DIABETES MELLITUS

- It is a disease in which the body cannot regulate the amount of sugar in the blood.
- Diabetes is increasing alarmingly; it is estimated that 342 million people will be suffering from it by 2030.
- Prebiotics (insulin) controls the glycemic index by reducing the absorption rate of glucose.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – III– Environmental Biotechnology – SBTA1304

Introduction to Water quality parameter

Source and effect of waste water on aquatic environment

Industrial water pollution

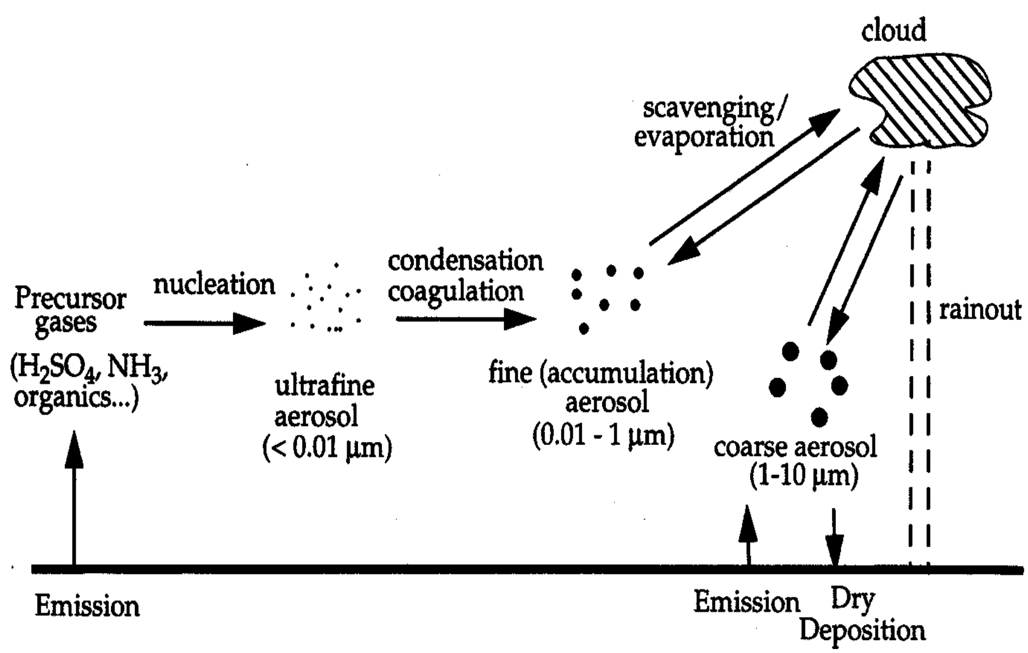
Chemistry of waste water-DO,BOD,COD and Toc

Air pollution-classification and effects on living and non living things

AIR POLLUTION

AEROSOL

Aerosol is a colloid of fine solid particles or liquid droplets in air or another gas. Aerosols can be natural or not.
Examples of natural aerosols are: fog, forest exudates, geyser stream.
Examples of artificial aerosols are haze, dust particulate air pollutants and smoke.
The liquid or solid particles have diameter mostly smaller than 1 μm or so; larger particles with a significant settling speed make the mixture a suspension, but the distinction is not clear-cut.
Aerosols vary in their dispersity. A monodisperse aerosol, producible in the laboratory, contains particles of uniform size. Most aerosols, however, as polydisperse colloidal systems, exhibit a range of particle sizes.
Liquid droplets are almost always nearly spherical, but scientists use an equivalent diameter to characterize the properities of various shapes of solid particles, some very irregular. The equivalent diameter is the diameter of a spherical particle with the same value of some physical property as the irregular particle. The equivalent volume diameter (d_e) is defined as the diameter of a sphere of the same volume as that of the irregular particle. Also commonly used is the aerodynamic diameter.



Origins of Atmospheric Aerosols

1. Condensation and sublimation of of vapors and the formation of smokes in natural and man-made combustion.
2. Reactions between trace gases in the atmosphere through the action of heat, radiation, or humidity.
3. The mechanical disruption and dispersal of matter at the earth's surface, either as sea spray over the oceans, or as mineral dusts over the continents.
4. Coagulation of nuclei which tends to produce larger particles of mixed constitution

Aerosol Makeup

- Typical substances formed in large quantities by condensation following combustion include ashes, soot, tar products, oils as well as sulfuric acid and sulfates. These particles are primarily within the range of Aitken nuclei.
- Mechanical disintegration, by wind and water, of rocks and soil produces particles with diameters > 0.2 µm. These fall primarily in the large nuclei range.
- According to Jaenicke (Science, 308 p. 73, 2005) about 25% of the number of particles with diameter greater than 0.2 µm are biogenic. (remains to be verified).

Generation and Applications

People generate aerosols for various purposes, including:

- as test aerosols for calibrating instruments, performing research, and testing sampling equipment and air filters;
- to deliver deodorants, paints, and other consumer products in sprays;
- for dispersal and agricultural application of pesticides;
- for medical treatment of respiratory disease and
- in fuel injection systems and other combustion technology.
- Chemical reactions between nitrogen, oxygen, water vapor and various trace gases (e.g., sulfur dioxide, chlorine, ammonia, ozone, and oxides of nitrogen) primarily produce particles in the Aitken and Large range.

Examples

- Formation of ammonium chloride from NH_3 and HCl
- Oxidation of SO_2 to H_2SO_4
- Reaction of sulfur dioxide, ammonia, and water to produce ammonium sulfate particles.
- Production of higher oxides of nitrogen through the action of heat, ozone or ultraviolet radiation

Some devices for generating aerosols are:

- Aerosol spray
- Atomizer nozzle or Nebulizer
- Electrospray
- Electronic cigarette
- Vibrating orifice aerosol generator (VOAG)

Detection

Aerosol can either be measured in-situ or with remote sensing techniques.

In situ observations

Some available in situ measurement techniques include:

- Aerosol mass spectrometer (AMS)
- Differential mobility analyzer (DMA)
- Electrical aerosol spectrometer (EAS)
- Aerodynamic particle sizer (APS)
- Aerodynamic aerosol classifier (AAC)
- Wide range particle spectrometer (WPS)
- Micro-Orifice Uniform Deposit Impactor(MOUDI)
- Condensation particle counter (CPC)
- Epiphaniometer
- Electrical low pressure impactor (ELPI)
- Aerosol particle mass-analyser (APM)
- Centrifugal Particle Mass Analyser (CPMA)

Remote sensing approach

Remote sensing approaches include:

- Sun photometer
- Lidar

Size selective sampling

Particles can deposit in the nose, mouth, pharynx and larynx (the head airways region), deeper within the respiratory tract (from the trachea to the terminal bronchioles), or in the alveolar region. The location of deposition of aerosol particles within the in the respiratory system strongly determines the health effects of exposure to such aerosols.

This phenomenon led people to invent aerosol samplers that select a subset of the aerosol particles that reach certain parts of the respiratory system. Examples of these subsets of the particle-size distribution of an aerosol, important in occupational health, include the inhalable, thoracic, and respirable fractions. The fraction that can enter each part of the respiratory system depends on the deposition of particles in the upper parts of the airway. The inhalable fraction of particles, defined as the proportion of particles originally in the air that can enter the nose or mouth, depends on external wind speed and direction and on the particle-size distribution by aerodynamic diameter. The thoracic fraction is the proportion of the particles in ambient aerosol that can reach the thorax or chest region.

The respirable fraction is the proportion of particles in the air that can reach the alveolar region. To measure the respirable fraction of particles in air, a pre-collector is used with a sampling filter. The pre-collector excludes particles as the airways remove particles from inhaled air. The sampling filter collects the particles for measurement. It is common to use cyclonic separation for the pre-collector, but other techniques include impactors, horizontal elutriators, and large pore membrane filters.

Two alternative size selective criteria, often used in atmospheric monitoring are PM10 and PM2.5. PM10 is defined by ISO as particles which pass through a size-selective inlet with a 50 % efficiency cut-off at 10 μm aerodynamic diameter. PM10 corresponds to the "thoracic convention" as defined in ISO 7708:1995, Clause 6.

PM2.5 as particles which pass through a size-selective inlet with a 50 % efficiency cut-off at 2,5 μm aerodynamic diameter. PM2,5 corresponds to the "high-risk respirable convention" as defined in ISO 7708:1995. The United States Environmental Protection Agency replaced the older standards for particulate matter based on Total Suspended Particulate with another standard based on PM10 in 1987 and then introduced standards for PM2.5 (also known as fine particulate matter) in 1997.

Atmospheric

Three types of atmospheric aerosol have a significant effect on Earth's climate: volcanic; desert dust; and human-made.

Volcanic aerosol forms in the stratosphere after an eruption as droplets of sulfuric acid that can last up to two years, and reflect sunlight, lowering temperature.

Desert dust, mineral particles blown to high altitudes, absorb heat and may be responsible for inhibiting storm cloud formation.

Human-made sulfate aerosols, primarily from burning oil and coal, affect the behavior of clouds.

Although all hydrometeors, solid and liquid, can be described as aerosols, a distinction is commonly made between such dispersions (i.e. clouds) containing activated drops and crystals, and aerosol particles. Atmosphere of Earth contains aerosols of various types and concentrations, including quantities of:

- Natural Inorganic Materials: Fine Dust, Sea Salt, Water Droplets.
- Natural Organic Materials: Smoke, Pollen, Spores, Bacteria
- Anthropogenic Products Of Combustion Such As: Smoke, Ashes Or Dusts

Aerosols can be found in urban Ecosystems in various forms for example:

- Dust,
- Cigarette smoke,
- Mist from aerosol spray cans,
- Soot or fumes in car exhaust.

The presence of aerosols in earth's atmosphere can influence Earth's climate, as well as human health.

Effects of Aerosols

- Volcanic eruptions release large amounts of sulphuric acid, hydrogen sulphide and hydrochloric acid into the atmosphere. These gases represent aerosols and eventually return to earth as acid rain, having a number of adverse effects on the environment and human life.
- Aerosols interact with the Earth's energy budget in two ways, directly and indirectly. E.g., a direct effect is that aerosols scatter sunlight directly back into space. This can lead to a significant decrease in the temperature, being an additional element to the greenhouse effect and therefore contributing to the global climate change.
  The indirect effects refer to the aerosols interfering with formations that interact directly with radiation. For example, they are able to modify the size of the cloud particles in the lower atmosphere, thereby changing the way clouds reflect and absorb light and therefore modifying the Earth's energy budget.
- When aerosols absorb or adsorb pollutants, it facilitates the deposition of pollutants to the surface of the earth as well as to bodies of water. This has the potential to be damaging to both the environment and human health.

- Aerosol particles with an effective diameter smaller than 10 µm can enter the bronchi, while the ones with an effective diameter smaller than 2.5µm can enter as far as the gas exchange region in the lungs, which can be hazardous to human health.

Health problems

The effects of inhaling particulate matter that have been widely studied in humans and animals include asthma, lung cancer, cardiovascular disease, respiratory diseases, premature delivery, birth defects, and premature death.

DROPLET NUCLEI

Airborne transmission occurs when bacteria or viruses travel on dust particles or on small respiratory droplets that may become aerosolized when people sneeze, cough, laugh, or exhale. They hang in the air much like invisible smoke. They can travel on air currents over considerable distances. These droplets are loaded with infectious particles.

Airborne droplet nuclei develop when the fluid of pathogenic droplets (1-5 µm in size; micrometre = one-thousandth of a millimetre) evaporates. They are so small and light they may remain suspended in the air for several hours. These organisms can survive outside the body and remain suspended in the air for long periods of time. They infect others via the upper and lower respiratory tracts.

Diseases that are commonly spread by coughing or sneezing include:

- Bacterial Meningitis
- Chickenpox
- Common cold
- Influenza
- Mumps
- Strep throat
- Tuberculosis
- Measles
- Rubella
- Whooping cough
- SARS
- Leprosy

Droplet nuclei (the residuals of droplets) when suspended in the air, dry and produces partilcrdes of 1-5 microns that can remain suspended indefinitely in the air. Diseases spread by this mode include open/active pulmonary tuberculosis (TB), pulmonary plague, chicken pox, measles and hemorrhagic fever with pneumonia.

Following precautions need to be taken:

1. Implementation of standard precautions.
2. Placing patient in a single room that has a monitored negative airflow pressure called as a “negative pressure room”. The air from this room should be either discharged to the outdoors or specially filtered before circulating it to other areas of healthcare facility.
3. Keeping doors of this room closed always.
4. All the persons entering the room must wear a special, high filtration, particulate respirator (eg. N 95) mask. Limiting the movement and transport of the patient from the room for essential purposes only. If transport is necessary minimize dispersal of droplet nuclei by masking the patient.

REMOVAL OF AIRBORNE CONTAMINANTS

Airborne contaminants

Air contaminants are particles, gases or vapours and combinations of these. ‘Particles’ include dusts, fumes, mists and fibres.

Name	Description and size	Visibility	Examples
Dust	Solid particles – can be supplied, eg powder-handling, or process generated, eg crushing and grinding. Inhalable particle size 0.01 to 100 µm. Respirable particle size below 10 µm.	In diffuse light: Inhalable dust clouds are partially visible. Respirable dust clouds are practically invisible at concentrations up to tens of mg/m^3 .	Grain dust, wood dust, silica flour

Fume	Vapourised solid that has condensed. Particle size 0.001 µm to 1 µm	Fume clouds tend to be dense. They are partially visible. Fume and smoke are generally more visible than equivalent concentrations of dust.	Rubber fume, solder fume, welding fume
Mist	Liquid particles – process generated, eg by spraying. Particle size ranges 0.01 to 100 µm but the size distribution may change as volatile liquids evaporate	As for dust.	Electroplating, paint sprays, steam
Fibres	Solid particles – the length is several times the diameter. Particle size – as for dust.	As for dust	Asbestos, glass fibre
Vapour	The gaseous phase of a liquid or solid at room temperature. Behaves as a gas.	Usually invisible. At very high concentrations, a vapour-laden cloud may just be visible.	Styrene, petrol, acetone, mercury, iodine
Gas	A gas at room temperature	Usually invisible – some coloured at high concentrations.	Chlorine, carbon monoxide

Table shows some of the basic characteristics of airborne contaminants some properties of airborne contaminants.

Water is one of the most important commodities required for the survival of any form of life. Today water resources have been the most exploited natural system since man strode the earth. Pollution of water bodies is increasing tremendously due to population explosion, industrial proliferation, urbanisation, increasing living standards and wide spheres of human activities.

Time is, perhaps not too far when pure and clean water, particularly in densely populated and industrialised water scarce areas may be inadequate for maintaining the normal living standards.

Ground water, rivers, seas, lakes, ponds, streams are finding it more and more difficult to escape from pollution. In India, the major 14 rivers receive heavy flux of sewage, industrial effluents, domestic and agricultural wastes. Most of the large rivers of the world are nothing but open sewers fit only to carry urban wastes, poisonous pesticides and industrial toxic effluents etc.

Many of our lakes, including Dal and Nagin of Kashmir have severely polluted with foul odour, silt deposits and get chocked due to excessive algal growths. Now pollution of water bodies has become universal phenomenon in the present day world.

Signs of Water Pollution:
ADVERTISEMENTS:

These are bad taste of drinking water, offensive odour from water bodies, unchecked growth of aquatic weeds in water, decrease in number of fish in fresh water, oil and grease floating on water surface. These factors disturb the normal uses of water for public water supply, aquatic organisms, agriculture and industry.

WATER POLLUTION

Water gets polluted when its normal functions and properties are altered. Water pollution actually represents the state of deviation from the quality and purity of water sample.

1. Water pollution shows the addition of foreign substances, either from natural or anthropogenic sources, may be harmful to life because of their toxicity, reduction of normal oxygen level of water, aesthetically unsuitable and spread epidemic diseases.

2. It is the natural or induced change in the quality of water which renders it unsuitable and toxic as regards food, man and animal health, industry, agriculture, fishing or leisure pursuits.

3. Water pollution is the by product of rapid and unplanned industrial progress and over population.

4. Any shift in the naturally dynamic equilibrium existing among environmental segments, i.e., hydrosphere, lithosphere, atmosphere or sediments give rise to the state of water pollution.

Water pollution may be in ground water, surface water, lake water, river or ocean water.

Sources of Water Pollution:

Today clean water has become a precious natural resource but its quality is threatened by numerous sources of pollutants which are as follows:

1. Inorganic Pollutants:

This category of water pollutants consists of acids, alkalies, soluble and insoluble salts, metallic complexes, trace elements, organometallic compounds, polyphosphatic detergents from chemical industries, metallurgical processes, coal mines and numerous natural processes causing pollution in water.

2. Toxic Metals:

Toxic metals are added to water from industrial activities, domestic sewage discharges, land run off and fossil fuel burning. Traces of heavy metals such as Hg, Cd, Pb, As, Co, Mn and Cr have been identified deleterious to aquatic ecosystem and human health. In fish mercury is present as $(CH_3)_2Hg$ which is known to concentrate in food chain. Manganese also enters the water system through industrial effluents and dry cell batteries. Selenium content of most drinking water is found as 10 ppb.

3. Organic Pollutants:

Organic pollutants enter into water system through domestic sewage, industrial wastes from paper mills and tanneries, waste from slaughter houses, meat packing plants, plant nutrients, detergents, biocides etc. The addition of carbohydrates, fatty acids, proteins, aldehydes, polychlorinated biphenyls (PCBs), phenolic compounds and polycyclic aromatic hydrocarbons deteriorate water quality.

4. Sewage and Domestic Wastes:

Sewage is a cloudy dilute aqueous solution containing mineral and organic matter. About 75% of water pollution is caused by sewage, domestic wastes, food processing plants, garden wastes and sewage sludge from cess pools etc. Sewage contains decomposable organic matter and exerts oxygen demand on the receiving waters. Domestic sewage contains trace quantities of toxic metals also. Sewage treatment deposits sludge at the bottom while liquid waste consists of ions such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, NO^{-}_2, SO_4^{2-}, PO_4^{3-}, HCO^{-}_3 etc.

5. Sediments:

The natural process of soil erosion gives rise to sediments in water. Sediments include soil, sand and mineral particles washed into aquatic environment by flood waters. In addition, large deposits of sewage sludge, pulverized coal ash and various industrial solids are disposed off into water.

6. Synthetic Detergents:

Detergents include ingradients like surfactants, builders, additives, stabilizers and soil suspending carboxymethyl cellulose etc. The surfactant is a surface active agent. Alkyl Benzene Sulphonates (ABS) are considered as surfactants. ABS showed remarkable resistance to biodegradation (hard detergents) and has been subsequently replaced by Linear Alkyl Sulphonate (LAS).

LAS undergo rapid biodegradation. Builder is usually a sodium polyphosphate of the type $Na_5P_3O_{10}$ or $Na_4P_2O_7$ acting as a sequestering agent. Both surfactants and builders cause serious pollution in water. Additives consist of anticorrosive sodium silicate, enzymes, perfumes and bleaching agents. Phosphates released into streams act as plant nutrient, thus supporting eutrophic conditions. Currently, celluzyme obtained from hermicola insolents, is added in detergents. The high percentage of sodium tripolyphosphate (STPP) in detergents may be partly replaced by enzymes.

7. Oxygen Demanding Wastes Causing Pollution:

Decrease in dissolved oxygen (DO) level is an indication of pollution due to organic matter, e.g., sewage, industrial wastes from food processing plants, run off from agricultural lands etc. All these materials undergo degradation by microbial activities in presence of DO. It causes deoxygenation process and quick depletion of DO.

Biological Oxygen Demand (BOD):

The degree of microbially mediated oxygen consumption in water is known as BOD. It is a measure of oxygen utilized by micro-organisms during the oxidation of organic material in a five day period. The demand for O_2 is directly proportional to the amount of organic waste which has to be broken down. Hence BOD is a direct measure of biodegradable organic matter. Drinking water has a BOD of less than 1 mg/L. When BOD level reaches 5 mg/L, the water is said to be polluted.

8. Plant Nutrients as Pollutant:

Plant nutrients constitute an important limiting factor for plant growth. Nitrogen and phosphorus are the main nutrient species which enter fresh and marine systems changing oligotrophic water to intensely productive eutrophic conditions. According to Wetzel each phosphorus molecule promotes the incorporation of 7 molecules of nitrogen and 40 molecules of carbon in aquatic algae. These nutrients ultimately tend to accumulate in ground water.

Eutrophication:

Eutrophication is a natural process, derived from the Greek word eutrophos meaning well nourished or enriched. This enrichment leads to other slow processes referred to as natural aging of lakes. It is a phenomenon through which a nutrient rich bog in a shallow depression changes to leached bog deficient in nutrients.

Sources of Nutrients:

Eutrophication escalates rapidly, however when abnormally high amounts of nutrients from fertilizers, domestic and industrial wastes, urban drainage, detergents, animal wastes and sediments enter water streams.

9. Thermal Pollutants in Water:

Thermal pollution of water may be defined as the warming up of an aquatic ecosystem to the point where desirable organisms are adversely affected. Chemical industries, electric power plants, atomic energy plants discharge their heated effluents into nearby lakes or rivers.

A coal-fired power plant at 40% efficiency generates 16.7 joule of waste heat for every 41.8 joules of fuel burnt. A single 100 MW power plant may use one half million gallons of cooling water per minute. This process raises the temperature of water by 10°C to 15°C. The heated waters have reduced amount of dissolved oxygen content which results into killing of marine life.

10. Pesticide and Fertilizer Pollutants in Water:

Pesticides like insecticides, fungicides, herbicides, rodenticides and molluscicides enter in water through rain water, spray drift, run off from agricultural fields, domestic sewage, accidental spillage and industrial effluents etc. The annual world production of pesticides (organochlorines, organophosphates, carbamates, chlorophenoxy acids) grew from 6000 million pounds to 24000 million pound. Pesticides hit the aquatic ecosystem and terrestrial organisms ranging from acute toxicity to invisible chronic effects in man, animals and plants.

Control Measures of Water Pollution:

We are now near the stage when water pollution has become a global problem partly because of population explosion and partly due to phenomenal advance in industrialization. In India, 70% of the pollutant load of rivers, lakes and streams is from domestic waste. So obviously it is of no use to apply strict laws only to industries, if municipalities are given free reign to discharge their domestic wastes into water without any treatment.

Minimising Water Pollution:

Following methods can be adopted:

1. Stabilisation of the Ecosystem:

The principles involved in this technique include reduction of the waste at source, harvesting and removal of biomass, trapping of the nutrients, fish management and aeration.

2. Using Water Hyacinth to Remove Water Pollutants:

Water hyacinth is extremely efficient in absorbing and concentrating dissolved nutrients from water in which it lives. Introduction of this weed in the lagoon enhances even 1000 times purifying capacity of water.

Experimental studies have shown that in a lagoon of 0.5 hectare having dense growth of hyacinth, with sewage retention time of 15 days, the daily wastes of 1000 people can be effectively treated. Water hyacinth is capable to absorb phenolic compounds commonly found in domestic and industrial sewage. The phenols so absorbed are broken down and can be utilized rapidly.

3. Chemical Methods:

Generally, chemical precipitation, solvent extraction, electro-deposition, ion-exchange, ultra-filtration, and activated carbon adsorption systems are applied to remove heavy metals. All these methods are extremely expensive.

4. Cooling Methods:

In some developed countries, thermal pollution abatement schemes are used to control water pollution. These methods include once-through cooling, cooling ponds, wet cooling towers, evaporative towers and dry cooling towers.

5. Solar Power:

Solar energy is used for purifying the polluted waste water at low cost. Experiments concluded that a combination of sunlight and a catalyst such as titanium dioxide can dissociate chemical toxicants.

6. Removal of Phosphorus by Electrolysis:

In Norway, organic sewage is mixed with 10% of sea water and subjected to electrolysis to remove phosphorus from sewage water. Phosphorus compounds in sewage get precipitated as Ca or Mg phosphate.

During electrolysis, these salts along with sludge and suspended particles adhere to magnesium hydroxide at the negative pole. H_2 gas liberated during electrolysis makes the phosphate and sludge to float on the surface as scum which can be scrapped off from the top layer. Chlorine gas produced at the positive pole can be used for disinfection of the outlet.

7. Removal of Salts by Reverse Osmosis to Purify Water:

Various salts can be removed by reverse osmosis by forcing the waste water through a semipermeable membrane under a pressure exceeding the osmotic pressure. During the process, flow occurs in the reverse direction. The solvent is attracted while the solute is repelled. The method is mostly applied to salinate the brackish water and to purify water from sewage.

8. Removal of Chlorophenols:

Chlorophenols, used as wood preservatives, pollute surface and ground water. Scientists at Tampere University of Technology, Finland used sand, vulcanite mineral, silica based material, called celite R-633 and pumice to clean up chlorophenol contaminated ground water. The method can remove 99.9% of chlorophenol from ground water at 5°C temperature.

9. Recycling, Renovation, Recharge and Reuse (4R Concept) of Waste Water:

In developed countries the waste water consisting of domestic sewage, industrial effluents, thermal and radioactive pollutants, sullage of municipal waste receives some sort of treatment before it gets mixed into water bodies. For example, urban sewage, sullage etc. may be recycled and reused to generate cheaper fuel, gas and electricity.

NEERI, Nagpur has developed technology for reuse of waste water to provide cheap piped gas and generate electricity by recycling waste water. Recently, one distillery in Gujarat is effective in treating 450,000 litre of water daily and generating energy equivalent to that produced by 10 tonne of coal.

Purification of Water for Municipal Purposes:

The processes used in the purification of water are as follows:

1. Aeration:

The raw water is first aerated by bubbling compressed air. This removes bad odours and CO_2 while Fe and Mn salts get precipitated as their hydroxides.

2. Sedimentation:

The water is then allowed to stand in large settling tanks. Some of the heavier impurities present in water agglomerate and settle down by gravity.

3. Coagulation:

The suspended impurities are removed by coagulation using alum, $FeCl_3$, lime or soda ash. The coagulant sodium aluminate removes HCO^{-}_3, Cl^{-}, SO^{2-}_4 responsible for temporary and permanent hardness of water. By coagulation, turbidity is reduced to 20 ppm and bacterial load by 5%, thereby bringing about partial clarification of water.

4. Flocculation:

The process of coagulation can be intensified by adding flocculants such as polyacrylamide, starch and activated silica.

5. Filtration:

The partially clarified water is then filtered through sand gravity filter.

6. Disinfection:

The elimination of offensive odour caused by dissolved organic substances in water is done by ozonization, chlorination, aeration, coagulation and ultra violet light treatment.

7. Ozonization:

The water is treated with ozonised oxygen. Ozone sterilizes, bleaches, decolourises and deodourises water. Highly palatable water is sterilised with ozone but the cost involved is very high.

8. Chlorination:

Chlorination is the best and the cheapest method of sterilization of water. For chlorination, chlorine may be used directly in the liquid form or hypochlorates of calcium and sodium, e.g., bleaching powder. It kills viruses and bacteria. The purified water is then supplied by municipalities through pipes for domestic purposes.

Pollution Type # 3. Soil Pollution:

With rapidly advancing technology, man's impact upon the world of natural resources is beginning to prove overwhelming. Rapid urbanization, with the consequent increase in population and building construction, has resulted in the reduction of land for the wastes to be disposed.

Every year solid wastes are increasing tremendously all over the world. Several hazardous chemicals and the mountains of wastes are ultimately dumped on the land.

Dumping of industrial and municipal wastes causes toxic substances to be leached and seep into the soil and affect the ground water course (Table 1). Modern agricultural practices introduce numerous pesticides, resulting in severe biological and chemical contamination of soil.

Indiscriminate deforestation, digging for minerals, destruction of grazing land for human habitation have done irreparable damage to the land and even led to harsh climatic changes.

Some of the dangers leading to soil pollution are due to the fact that while number of earth's inhabitants is increasing, the earth's natural resources are by and large fixed as well as limited.

Table 1. Nature of pollutants in soil.

Source	Gases	Colloids	Suspended particles	Dissolved cations	Dissolved anions
Soil	CO_2	Clay Fe_2O_3 Al_2O_3 MnO_2	Clay sand silt	Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Fe^{3+}	CO_3^{2-}, HCO_3^-, OH^-, Cl^-, SO_4^{2-}, F^-
Decomposed organic matter	SO_2, H_2, NH_3, CH_4, CO_2	Organic waste materials	Humus, organic wastes	H^+, Na^+, NH_4^+	Organic, NO_3^-, SO_4^{2-}, Cl^-

The crux of the waste problems in land lies in the leachates which ooze out of the garbage heap, move slowly through the layers of the soil beneath and contaminate the water resources deep down the land. However, the problem of soil pollution differs from air and water pollution in the respect that the pollutants remain in direct contact with the soil for relatively longer periods.

Thus the soil is getting heavily polluted day by day by toxic materials and dangerous micro-organisms which enter the air, water and the food chain. For all this, man is the original and basic pollutant responsible for pollution hazards.

Solid waste, often called the third pollution after air and water pollution, is that discarded material which arises from human activities. The annual solid waste production is: domestic and trade 8.5%, industries 15.2%, thermal power stations 7.3%, mining 67% and construction 2%.

Sources of Soil Pollution:

1. Industrial wastes
2. Urban wastes
3. Radioactive pollutants
4. Agricultural practices
5. Biological agents
6. Soil sediments

1. Soil Pollution by Industrial Wastes:

Industrial effluents are mainly discharged from pulp and paper mills, chemical industries, oil refineries, sugar factories, tanneries, textiles, steel, distilleries, fertilizer factories, pesticide industries, coal and mineral mining industries, metal processing industries, drugs, glass, cement, petroleum and engineering industries etc. It has been estimated that about 50% of the raw materials ultimately become waste products in industry and about 20% of these wastes are extremely deleterious.

2. Soil Pollution by Urban Wastes:

Urban wastes comprise both commercial and domestic wastes consisting of dried sludge of sewage. All the urban solid wastes are commonly referred to as refuse. This refuse contains garbage and rubbish materials like plastics, glasses, metallic cans, fibres, paper, rubbles, street sweepings, fuel residues, leaves, containers, abandoned vehicles and other discarded manufactured products.

It is estimated that in India alone, about 450 million of urban population produces nearly 45 million tonnes of solid wastes causing chronic pollution of land and water. In critically polluted cities like Mumbai, Kolkata, Kanpur and Madras, about 8000 tonnes of waste material collects in a day.

Delhi, which is the third most polluted city, collects about 9000 tonnes of garbage from its streets every day, to be thrown into its five land-fills, thereby polluting the land areas. Connaught place alone generates 50,000 plastic bags every day.

3. Radioactive Pollutants:

Radioactive substances resulting from explosions of nuclear devices, atmospheric fall out from nuclear dust soil radioactive wastes penetrate the soil and accumulate there creating soil pollution. Radionuclides of radium, thorium, uranium, isotopes of potassium (K-40) and carbon (C-14) are very common in soil, rock, water and air.

Explosion of hydrogen weapons and cosmic radiations induce neutron-proton reactions by which nitrogen (N-15) produces C-14. This C^{14} participates in the carbon metabolism of plants which is then introduced into animals and man. Radioactive wastes contain several radionuclides such as strontium-90, iodine-129, cesium-137 and isotopes of iron which are most injurious. Sr-90 gets deposited in bones and tissues instead of calcium.

4. Agricultural Practices:

Today with the advancing agro-technology, huge quantities of fertilizers, pesticides, herbicides, weedicides and soil conditioning agents are employed to increase the crop yield. Apart from these farm wastes, manure slurry, debris, soil erosion containing mostly inorganic chemicals are reported to cause soil pollution. USA alone produces about 18 million tonnes of agricultural wastes every year.

5. Biological Agents:
Soil gets large quantities of human, animals and birds excreta which constitute the major source of soil pollution by biological agents.

The pathogenic organisms that pollute the soil may be classified into three categories as follows:

(i) Pathogenic organisms occurring naturally in contaminated soil such as bacteria, fungi, algae, protozoans.

(ii) Pathogenic organisms excreted by man.

(iii) Pathogenic organisms excreted by animals.

6. Soil Sediments as Land Pollutant:
Soil sediments refer to the deposition of trace metals such as Hg, As, Sb, Pb, Cd, Ni, Co, Mo, Cu and Cr. The process of sedimentation is a comprehensive natural geomorphological process which operates through the chain of erosion of soils, transportation of sediments (eroded material) and deposition of these eroded materials in different paths of water bodies. Sediments thus consist of soil and mineral particles washed from the land by storms and flood waters from crop lands and over grazed pastures.

Unscientific agricultural and forestry practices, uncontrolled dumping of terrestrial effluents, mismanagement of water sheds, ship mining and dredging, construction of dams, roads, reservoirs, overgrazing, infra structural projects and other practices contribute to sedimentation.

Control of Soil Pollution:
With the rapid pace of industrialization and increasing population density, numerous pollutants have posed a serious threat to living organisms. Extensive solid wastes and the use of biocides etc. have put the interest of agriculture and aqua-culture at cross purposes.

The various approaches to control soil pollution are as follows:
1. Production of Natural Fertilizers. Organic wastes contained in animal dung can be used for preparing compost manure and biogas rather than throwing them wastefully polluting the soil. The use of bio-pesticides should be encouraged in place of toxic pesticides.

2. Ban on Toxic Chemicals. Ban should be imposed on chemicals, biocides, pesticides which are fatal to plants and animals. Thus banning the use of highly toxic and resistant pesticides or regulating their use only for special purposes under thorough monitoring can control soil pollution.

3. Using biological weapons to control soil pollution.

4. Plantation and recycling of trees to prevent soil erosion. Other control measures are:
5. Launching of extensive afforestation and community forestry programmes.

ADVERTISEMENTS:

6. Implementing deterrent measures against deforestation.

7. Conservation of soil to prevent the loss of precious top soil by erosion and to maintain it in a fertile state for agricultural purposes.

8. Sponsoring more intensive R and D efforts on bio-fertilizers, microbial degradation of wastes, utilization of wastes by recovery, reusing and .recycling solid wastes, safer treatment and disposal of hazardous wastes.

9. Security land-fills have to be constructed for permanent disposal of hazardous and recalcitrant industrial wastes.

10. Transforming intensive agriculture into a sustainable system by measures such as

(i) Maintaining a healthy soil community in order to regenerate soil fertility by providing organic manures, increasing fallow periods, avoiding excessive use of chemical fertilizers.

(ii) Infusing bio-diversity in agriculture by sowing mixed crops and adopting crop rotation etc.

11. Effective treatment of domestic sewage by suitable biological and chemical methods and adopting modern techniques of sludge disposal.

12. Municipal wastes have to be properly collected by segregation, treated and disposed scientifically. Recycling of glass, paper, plastics should be done carefully.

13. Industrial wastes have to be properly treated at source, by segregation of wastes or adopting integrated waste treatment methods. Basic slag, the major byproduct of steel industries, can be used for making nitrophosphatic fertilizer.

14. Enforcing environment audit for industries and promoting eco labelled products.

15. Implementing stringent and pro-active population control programmes.

16. Formulation of stringent pollution control legislation and effective implementation with powerful administrative machinery.

17. Imparting informal and formal public awareness programmes to educate people at large regarding health hazards and undesirable effects due to environmental pollution. Mass media, educational institutions and voluntary agencies should be involved to achieve these objectives.

18. Extending market support for recoverable products through fiscal concessions.

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV– Environmental Biotechnology – SBTA1304

Air pollution control techniques

Air Sampling Filters

I. Bioaerosol Sampling Filters

1. **<u>Gelatin (presterilized) Filters</u>** ; SKC Gelatin Filters are suitable for the collection and analysis of airborne microbes. Gelatin filters not only reliably retain bacteria but are also highly effective for the collection of viruses. Routinely used to quantitatively collect airborne microorganisms, gelatin filters have an inherent high moisture content that helps to maintain viability of stress-sensitive microorganisms during short sampling periods.
2. **<u>PTFE for *Anthrax* and SARS</u>** ; SKC PTFE filters are strong and completely resistant to acids, bases, and all solvents. Use PTFE filters when sampling in aggressive environments. SKC PTFE filters are temperature-stable to 527 F (275 C).
3. **<u>Cellulose Filters</u>:** Autoclavable, 100% pure, ashless cellulose fiber ideal for gravimetric sampling, collection of organic/inorganic precipitates, and AA and HPLC methods requiring an ashless filter
4. **<u>Coated Filters</u> ;** For your convenience, SKC manufactures coated filters for a wide variety of air sampling methods that require chemically treated filters or pads. SKC Coated Filters are shipped inside the cassettes with end plugs and sealing bands in place. No assembly is required; they are ready to use.
5. **<u>Gelatin Filters</u>** : SKC Gelatin Filters are suitable for the collection and analysis of airborne microbes. Gelatin filters not only reliably retain bacteria but are also highly effective for the collection of viruses. Routinely used to quantitatively collect airborne microorganisms, gelatin filters have an inherent high moisture content that helps to maintain viability of stress-sensitive microorganisms during short sampling periods.
6. **<u>Glass Fiber Depth Filters</u>**; Available with or without binder, high- temperature tolerant, autoclavable, liquid nominal pore size of 1.0 µm, and high particle retention

7. **<u>Marple Impactor Filters</u>** ; SKC offers a selection of specialty glass fiber, MCE, and quartz filters sized and cut to fit the Marple series 290 Personal Cascade Impactor.
8. **<u>Mixed Cellulose Ester (MCE) Filters</u>;** The standard filter for most aerosol sampling in industrial hygiene applications, mixed cellulose ester (MCE or MEC) membrane filters are comprised of pure, biologically inert mixtures of cellulose acetate and cellulose nitrate.
9. **<u>Polycarbonate Filters</u> ;** Hydrophobic; autoclavable; glass-like surface and straight-through pores for true surface capture of contaminants; strong, thin, and transparent; suitable for gravimetric or microscopic analyses
10. **Polyvinyl Chloride (PVC) Filters** ; GLA-5000 PVC, hydrophobic; pure homopolymer; low-ash, silica-free; low tare weight for gravimetric analysis
11. **<u>Quartz Filters</u> ;** Autoclavable, binder-free, heat-treated to remove trace organic impurities, high-purity microfibers for collecting diesel particulates and trace-level environmental pollutants
12. **Respicon Sampler Filters** ; Specialty filters for the Respicon Sampler
13. **<u>Silver Membrane Filters</u> SKC** silver membrane filters are manufactured entirely of pure metallic silver in a controlled environment. The many desirable properties of silver membranes make them suitable for a wide range of applications.

REMOVAL OF AIR BORNE CONTAMINANTS

Source Control Technology

- Air quality management sets the tools to control air pollutant emissions.
- Control measurements describes the equipment, processes or actions used to reduce air pollution.
- The extent of pollution reduction varies among technologies and measures.
- The selection of control technologies depends on environmental, engineering, economic factors and pollutant type.

Settling Chambers

- Settling chambers use the force of gravity to remove solid particles.
- The gas stream enters a chamber where the velocity of the gas is reduced. Large particles drop out of the gas and are recollected in hoppers. Because settling chambers are effective in removing only larger particles, they are used in conjunction with a more

efficient control device.

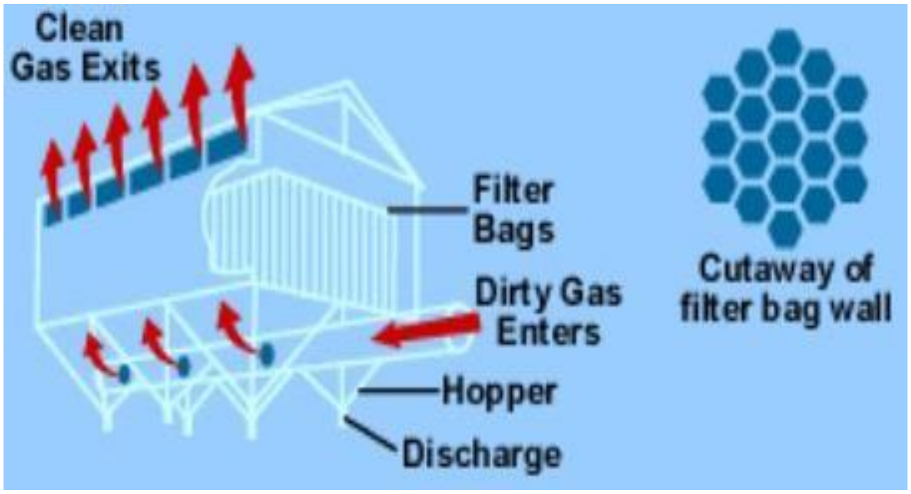


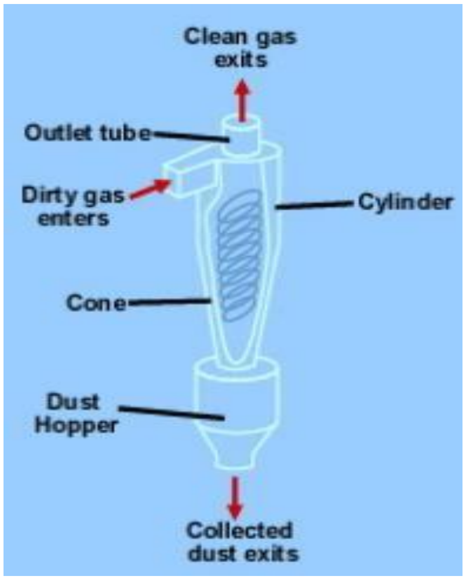
Figure: Settling chambers

2. **Cyclones**

- The general principle of inertia separation is that the particulate-laden gas is forced to change direction. As gas changes direction, the inertia of the particles causes them to continue in the original direction and be separated from the gas stream.
- The walls of the cyclone narrow toward the bottom of the unit, allowing the particles to be collected in a hopper.

The cleaner air leaves the cyclone through the top of the chamber, flowing upward in a spiral vortex, formed within a downward moving spiral.

Cyclones are efficient in removing large particles but are not as efficient with smaller particles. For this reason, they are used with other particulate control devices.



Venturi Scrubbers

- Venturi scrubbers use a liquid stream to remove solid particles.
- In the venturi scrubber, gas laden with particulate matter passes through a short tube with flared ends and a constricted middle.
- This constriction causes the gas stream to speed up when the pressure is increased.
- The difference in velocity and pressure resulting from the constriction causes the particles and water to mix and combine.
- The reduced velocity at the expanded section of the throat allows the droplets of water containing the particles to drop out of the gas stream.
- Venturi scrubbers are effective in removing small particles, with removal efficiencies of up to 99 percent.
- One drawback of this device, however, is the production of wastewater.
- Fabric filters, or baghouses, remove dust from a gas stream by passing the stream through a porous fabric. The fabric filter is efficient at removing fine particles and can exceed efficiencies of 99 percent in most applications.

- The selection of the fiber material and fabric construction is important to baghouse performance.
- The fiber material from which the fabric is made must have adequate strength characteristics at the maximum gas temperature expected and adequate chemical compatibility with both the gas and the collected dust.
- One disadvantage of the fabric filter is that high-temperature gases often have to be cooled before contacting the filter medium.

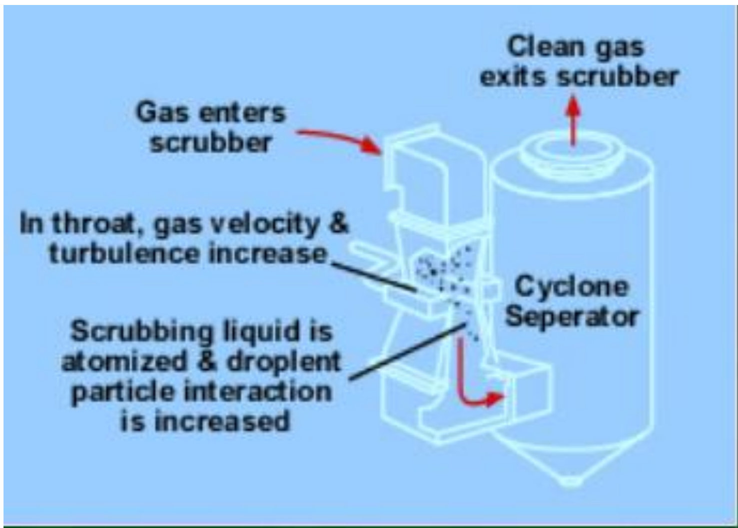
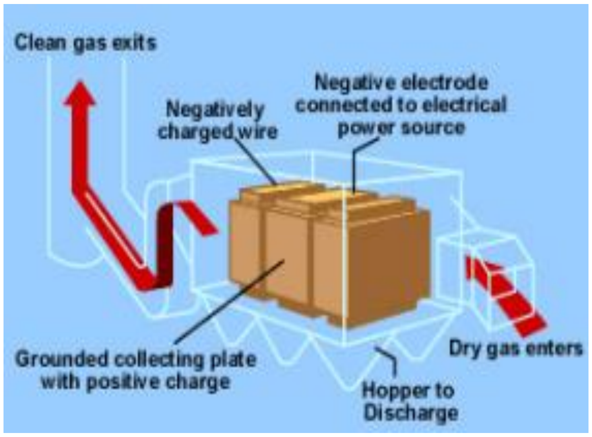


Figure: Fabric filter (baghouse) components

3. Electrostatic Precipitators (ESPs)

- An ESP is a particle control device that uses electrical forces to move the particles out of the flowing gas stream and onto collector plates.
- The ESP places electrical charges on the particles, causing them to be attracted to oppositely charged metal plates located in the precipitator.
- The particles are removed from the plates by "rapping" and collected in a hopper located below the unit.
- The removal efficiencies for ESPs are highly variable; however, for very small particles alone, the removal efficiency is about 99 percent.
- Electrostatic precipitators are not only used in utility applications but also other industries (for other exhaust gas particles) such as cement (dust), pulp & paper (salt cake & lime dust), petrochemicals (sulfuric acid mist), and steel (dust & fumes).



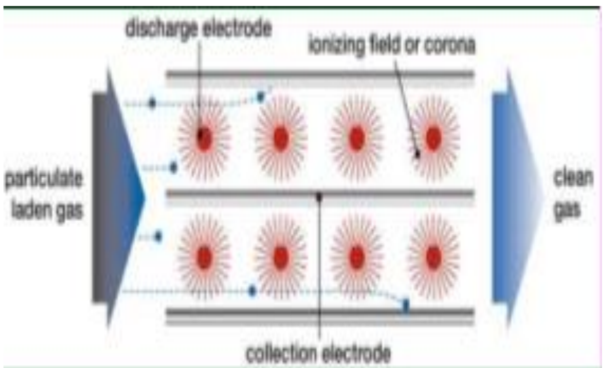


Figure: Electrostatic precipitator components

Control of gaseous pollutants from stationary sources

- The most common method for controlling gaseous pollutants is the addition of add-on control devices to recover or destroy a pollutant.
- There are four commonly used control technologies for gaseous pollutants:

  - Absorption,

  - Adsorption,

  - Condensation, and

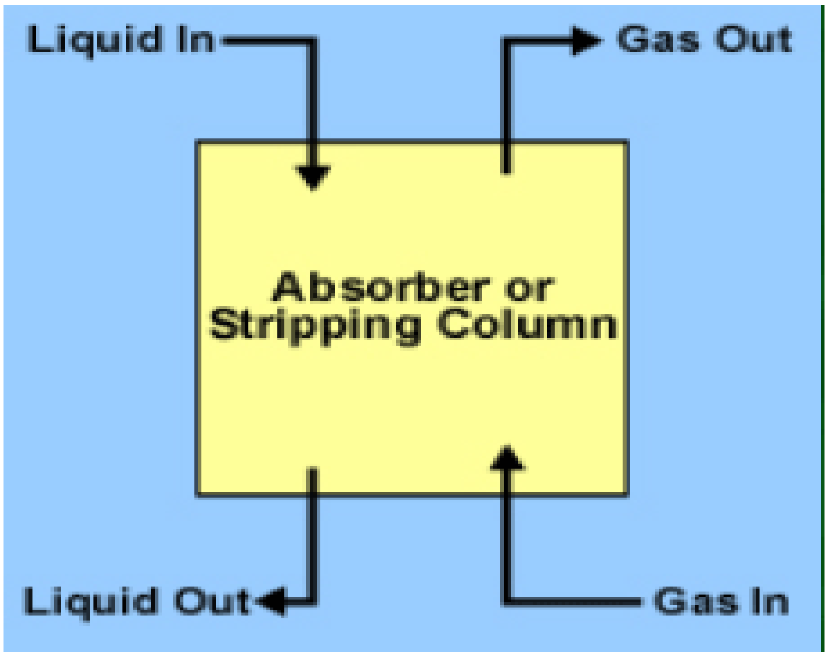
  - Incineration (combustion)

Absorption

The removal of one or more selected components from a gas mixture by absorption is probably the most important operation in the control of gaseous pollutant emissions.

- Absorption is a process in which a gaseous pollutant is dissolved in a liquid.
- As the gas stream passes through the liquid, the liquid absorbs the gas, in much the same way that sugar is absorbed in a glass of water when stirred.

- Absorbers are often referred to as scrubbers, and there are various types of absorption equipment.
- The principal types of gas absorption equipment include spray towers, packed columns, spray chambers, and venture scrubbers.
- In general, absorbers can achieve removal efficiencies grater than 95 percent. One potential problem with absorption is the generation of waste-water, which converts an air pollution problem to a water pollution problem.

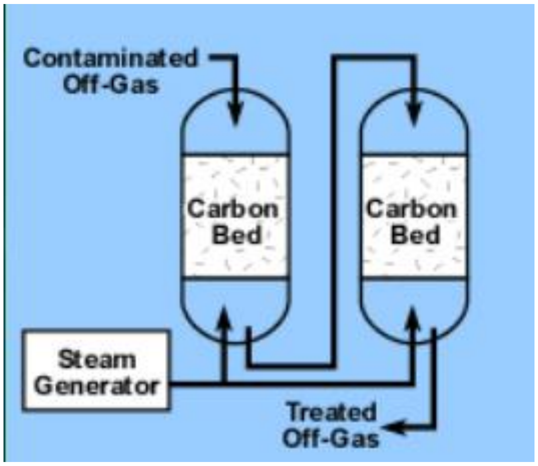


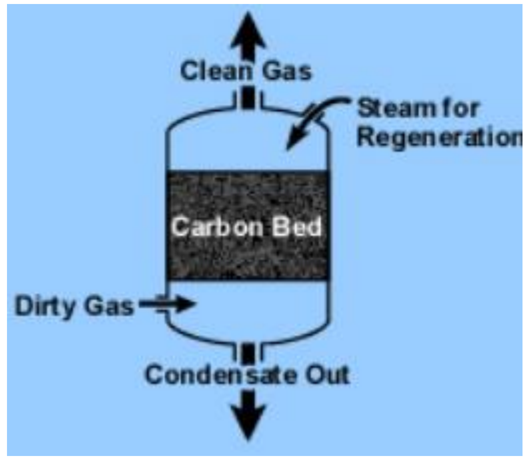
Adsorption

- When a gas or vapor is brought into contact with a solid, part of it is taken up by the solid. The molecules that disappear from the gas either enter the inside of the solid, or remain on the outside attached to the surface. The former phenomenon is termed absorption (or dissolution) and the latter adsorption.
- The most common industrial adsorbents are activated carbon, silica gel, and alumina, because they have enormous surface areas per unit weight.
- Activated carbon is the universal standard for purification and removal of trace organic contaminants from liquid and vapor streams. Carbon adsorption systems are either regenerative or non-regenerative.

  - **Regenerative system** usually contains more than one carbon bed. As one bed actively removes pollutants, another bed is being regenerated for future use.

- Non-regenerative systems have thinner beds of activated carbon. In a non-regenerative adsorber, the spent carbon is disposed of when it becomes saturated with the pollutant.





Condensation

- Condensation is the process of converting a gas or vapor to liquid. Any gas can be reduced to a liquid by lowering its temperature and/or increasing its pressure.
- Condensers are typically used as pretreatment devices. They can be used ahead of absorbers, absorbers, and incinerators to reduce the total gas volume to be treated by more expensive control equipment. Condensers used for pollution control are contact condensers and surface condensers.
- In a **contact condenser**, the gas comes into contact with cold liquid.
- In a **surface condenser**, the gas contacts a cooled surface in which cooled liquid or gas is circulated, such as the outside of the tube.
- Removal efficiencies of condensers typically range from 50 percent to more than 95 percent, depending on design and applications.

Incineration

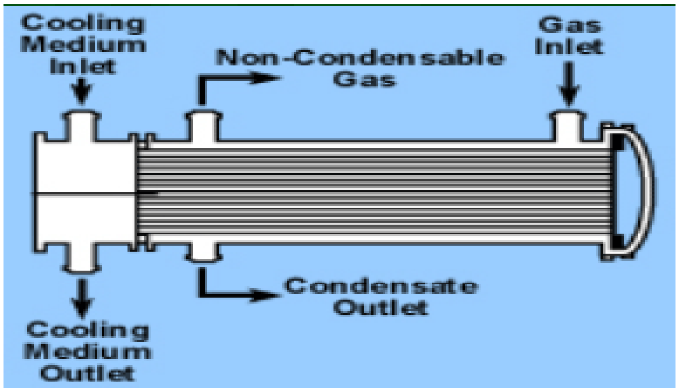
- Incineration, also known as combustion, is most used to control the emissions of organic compounds from process industries.
- This control technique refers to the rapid oxidation of a substance through the combination of oxygen with a combustible material in the presence of heat. When combustion is complete, the gaseous stream is converted to carbon dioxide and water vapor.

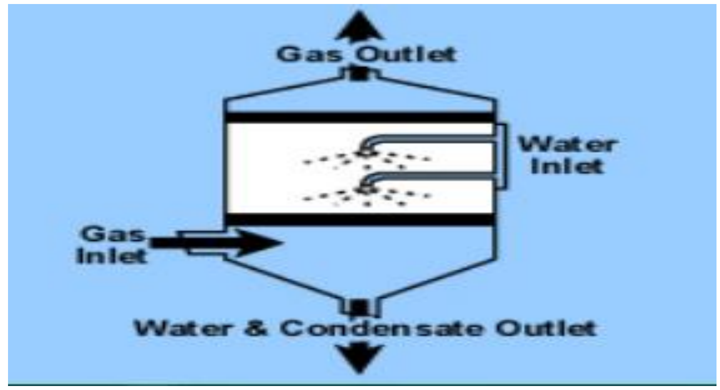
- Equipment used to control waste gases by combustion can be divided in three categories:

  - Direct combustion or flaring,

  - Thermal incineration and

  - Catalytic incineration.

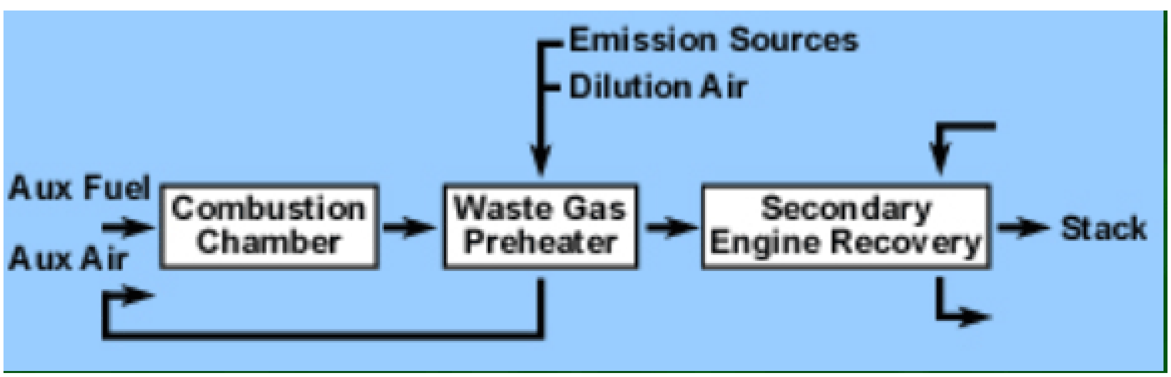




Direct combustor

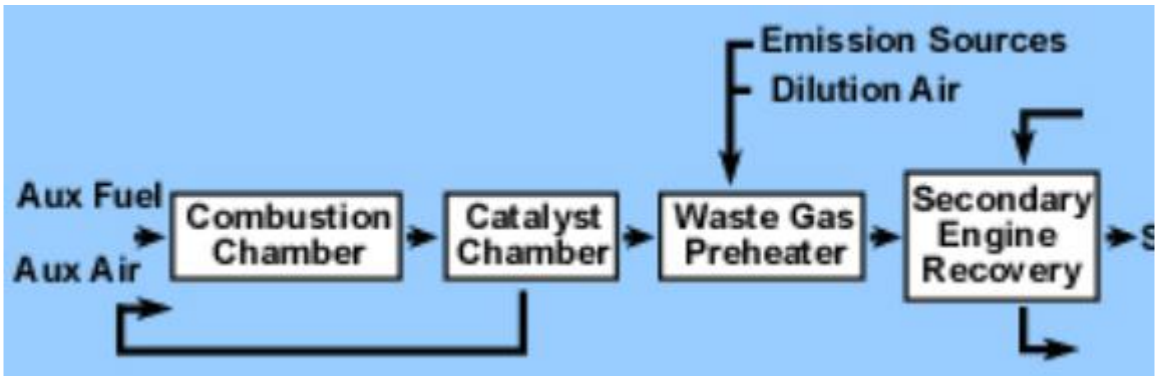
- Direct combustor is a device in which air and all the combustible waste gases react at the burner. Complete combustion must occur instantaneously since there is no residence chamber. A flare can be used to control almost any emission stream containing volatile organic compounds. Studies conducted by EPA have shown that the destruction efficiency of a flare is about 98 percent.

  In **thermal incinerators** the combustible waste gases pass over or around a burner flame into a residence chamber where oxidation of the waste gases is completed. Thermal incinerators can destroy gaseous pollutants at efficiencies of greater than 99 percent when operated correctly.



- ## Thermal incinerator general case

Catalytic incinerators are very similar to thermal incinerators. The main difference is that after passing through the flame area, the gases pass over a catalyst bed. A catalyst promotes oxidation at lower temperatures, thereby reducing fuel costs. Destruction efficiencies greater than 95 percent are possible using a catalytic incinerator.



Catalytic incinerator

QUALITY CONTROL TECHNIQUES OF AIR SAMPLERS BY RADIATION TECHNIQUES

Continuous particulate air monitors (CPAMs) have been used for years in nuclear facilities to assess airborne particulate radioactivity (APR). In more recent times they may also be used to monitor people in their homes for the presence of manmade radioactivity. These monitors can be used to trigger alarms, indicating to personnel that they should evacuate an area. The CPAM use in nuclear power plants, as opposed to other nuclear fuel-cycle facilities, or laboratories, or public-safety applications are following.

In nuclear power plants, CPAMs are used for measuring releases of APR from the facility, monitoring levels of APR for protection of plant personnel, monitoring the air in the reactor containment structure to detect leakage from the reactor systems, and to control ventilation fans, when the APR level has exceeded a defined threshold in the ventilation system.

CPAMs use a pump to draw air through a filter medium to collect airborne particulate matter that carries very small particles of radioactive material; the air itself is not radioactive. The particulate radioactive material might be natural, e.g., radon decay products ("progeny", e.g., ^{212}Pb), or manmade, usually fission or activation products (e.g., ^{137}Cs), or a combination of both. There are also "gas monitors" which pass the air through a sample chamber volume which is viewed continuously by a radiation detector.

Radionuclides that occur in the gaseous form (e.g., ^{85}Kr) are not collected on the CPAM filter to any appreciable extent, so that a separate monitoring system is needed to assess these nuclide concentrations in the sampled air. These gas monitors are often placed downstream of a CPAM so that any particulate matter in the sampled air is collected by the CPAM and thus will not contaminate the gas monitor's sample chamber.

Monitoring vs. sampling

In **monitoring**, the region of deposition of this material onto the filter medium is *continuously* viewed by a radiation detector, concurrent with the collection. This is as opposed to a **sampling** system, in which the airborne material is collected by pumping air, usually at a much higher volumetric flowrate than a CPAM, through a collection medium for some period of time, but there is no continuous radiation detection; the filter medium is removed *periodically* from the sampler and taken to a separate radiation detection system for analysis.

In general, sampling has better detection sensitivity for low levels of airborne radioactivity, due to the much larger total volume of air passing through the filter medium over the sampling interval (which may be on the order of hours), and also due to the more sophisticated forms of quantitative analysis available once the filter medium is removed from the sampler. On the other hand, monitoring with CPAMs provides nearly real-time airborne radioactivity level indication. It is common practice to refer to "sampled" air even when discussing a CPAM, i.e., as opposed to "monitored" air, which would, strictly, be more correct.

CPAM types

There are two major types of CPAMs, fixed-filter and moving-filter. In the former, the filter medium does not move while the airborne material is collected. The latter type has two main variants, the rectangular deposition area (“window”) and the circular window. In both types of CPAM the sampled air is pulled (not pushed) by a pump through the piping of the monitor up to the structure that holds the filter medium. It is important to note that CPAM pumps are specially designed to maintain a constant volumetric flowrate.

As the air passes through the collection medium (usually a form of filter paper), particulate matter is deposited onto the filter in either a rectangular or circular pattern, depending on the instrument's design, and then the air continues on its way out of the monitor. The *entire* deposition area, regardless of its geometric shape, is assumed to be viewed by a radiation detector of a type appropriate for the nuclide in question.

Moving-filter monitors are often used in applications where loading of the filter medium with dust is an issue; this dust loading reduces the air flow over time. The moving-filter collection medium ("tape") is assumed to move across the deposition area at a constant, known rate. This rate is often established in such a way that a roll of the filter tape will last about one month; a typical filter movement rate is about one inch per hour.

CPAM applications: effluent monitoring

CPAMs are used to monitor the air effluents from nuclear facilities, notably power reactors. Here the objective is to assess the amount of certain radionuclides released from the facility. Real-time measurement of the very low concentrations released by these facilities is difficult; a more-reliable measurement of the *total* radioactivity released over some time interval (days, perhaps weeks) may in some cases be an acceptable approach. In effluent monitoring, a sample of the air in the plant stack is withdrawn and pumped (pulled) down to the CPAM location. This sampled air in many cases must travel a considerable distance through piping. Extracting and transporting the particulates for the CPAM to measure in such a way that the measurement is representative of what is being released from the facility is challenging.

CPAM applications: occupational exposure assessment

For occupational exposure (inhalation) assessment, CPAMs may be used to monitor the air in some volume, such as a compartment in a nuclear facility where personnel are working. A difficulty with this is that, unless the air in the compartment is uniformly mixed, the measurement made at the monitor location may not be representative of the concentration of radioactive material in the air that the workers are breathing. For this application the CPAM may be physically placed directly in the occupied compartment, or it may extract sampled air from the HVAC system that serves that compartment. The following portions of 10CFR20 are relevant to the requirement for occupational exposure CPAM applications: 10CFR20.1003 (definition of Airborne Radioactivity Area), 1201, 1204, 1501, 1502, 2103.

CPAM applications: process monitoring and control

Radiation monitors in general have a number of process-control applications in nuclear power plants; a major CPAM application in this area is the monitoring of the air intake for the plant control room. In the event of an accident, high levels of airborne radioactivity could be brought into the control room by its HVAC system; the CPAM monitors this air and is intended to detect high concentrations of radioactivity and shut down the HVAC flow when necessary.

CPAM applications: reactor leak detection

Leakage from the so-called "reactor coolant pressure boundary" is required to be monitored in nuclear power plants. Monitoring the airborne particulate radioactivity in the reactor containment structure is an acceptable method to meet this requirement, and so CPAMs are used. It is the case that when primary coolant escapes into the containment structure, certain noble gas nuclides become airborne, and subsequently decay into particulate nuclides. One of the most common of these pairs is ^{88}Kr and ^{88}Rb; the latter is detected by the CPAM. Relating the observed CPAM response to the ^{88}Rb back to a leakage rate from the primary system is far from trivial.

Industrial waste water treatment-primary,secondary and tertiary

Wastewater treatment consists of applying known technology to improve or upgrade the quality of a wastewater. Usually wastewater treatment will involve collecting the wastewater in a central, segregated location (the Wastewater Treatment Plant) and subjecting the wastewater to various treatment processes. Most often, since large volumes of wastewater are involved, treatment processes are carried out on continuously flowing wastewaters (continuous flow or "open" systems) rather than as "batch" or a series of periodic treatment processes in which treatment is carried out on parcels or "batches" of wastewaters. While most wastewater treatment processes are continuous flow, certain operations, such as vacuum filtration, involving as it does, storage of sludge, the addition of chemicals, filtration and removal or disposal of the treated sludge, are routinely handled as periodic batch operations.

Wastewater treatment, however, can also be organized or categorized by the nature of the treatment process operation being used; for example, physical, chemical or biological. Examples of these treatment steps are shown below. A complete treatment system may consist of the application of a number of physical, chemical and biological processes to the wastewater.

Some Physical, Chemical and Biological Wastewater Treatment Methods

Physical

- Sedimentation (Clarification)
- Screening

- Aeration
- Filtration
- Flotation and Skimming
- Degassification
- Equalization

Chemical

- Chlorination
- Ozonation
- Neutralization
- Coagulation
- Adsorption
- Ion Exchange

Biological

- **Aerobic**

  Activated Sludge Treatment Methods

  Trickling Filtration

  Oxidation Ponds

  Lagoons

  Aerobic Digestion

- *Anaerobic*

  Anaerobic Digestion

  Septic Tanks

  Lagoons

Physical methods

Include processes where no gross chemical or biological changes are carried out and strictly physical phenomena are used to improve or treat the wastewater.

sedimentation (or clarification): In the process of sedimentation, physical phenomena relating to the settling of solids by gravity are allowed to operate. Usually this consists of simply holding a wastewater for a short period of time in a tank under quiescent conditions, allowing the heavier solids to settle, and removing the "clarified" effluent. Sedimentation for solids separation is a very common process operation and is routinely employed at the beginning and end of wastewater treatment operations.

Aeration: Physically adding air, usually to provide oxygen to the wastewater.

Filtration: Here wastewater is passed through a filter medium to separate solids. An example would be the use of sand filters to further remove entrained solids from a treated wastewater. Certain phenomena will occur during the sedimentation process and can be advantageously used to further improve water quality. Permitting greases or oils, for example, to float to the surface and skimming or physically removing them from the wastewaters is often carried out as part of the overall treatment process.

In certain industrial wastewater treatment processes strong or undesirable wastes are sometimes produced over short periods of time. Since such "slugs" or periodic inputs of such wastes would damage a biological treatment process, these wastes are sometimes held, mixed with other wastewaters, and gradually released, thus eliminating "shocks" to the treatment plant. This is call equalization. Another type of "equalization" can be used to even out wide variations in flow rates. For example, the wet well of a pump station can receive widely varying amounts of wastewater and, in turn, pump the wastes onward at more uniform rates.

Chemical treatment

Consists of using some chemical reaction or reactions to improve the water quality. Probably the most commonly used chemical process is

Chlorination: Chlorine, a strong oxidizing chemical, is used to kill bacteria and to slow down the rate of decomposition of the wastewater. Bacterial kill is achieved when vital biological processes are affected by the chlorine. Another strong oxidizing agent that has also been used as an oxidizing disinfectant is ozone.

Neutralization: Neutralization consists of the addition of acid or base to adjust pH levels back to neutrality. Since lime is a base it is sometimes used in the neutralization of acid wastes.

Coagulation consists of the addition of a chemical that, through a chemical reaction, forms an insoluble end product that serves to remove substances from the wastewater. Polyvalent metals are commonly used as coagulating chemicals in wastewater treatment and typical coagulants

would include lime (that can also be used in neutralization), certain iron containing compounds (such as ferric chloride or ferric sulfate) and alum (aluminum sulfate).
Certain processes may actually be physical and chemical in nature. The use of activated carbon to "adsorb" or remove organics, for example, involves both chemical and physical processes. Processes such as ion exchange, which involves exchanging certain ions for others, are not used to any great extent in wastewater treatment.

Biological treatment methods

Use microorganisms, mostly bacteria, in the biochemical decomposition of wastewaters to stable end products. More microorganisms, or sludges, are formed and a portion of the waste is converted to carbon dioxide, water and other end products. Generally, biological treatment methods can be divided into aerobic and anaerobic methods, based on availability of dissolved oxygen.
The purpose of wastewater treatment is generally to remove from the wastewater enough solids to permit the remainder to be discharged to a receiving water without interfering with its best or proper use. The solids which are removed are primarily organic but may also include inorganic solids. Treatment must also be provided for the solids and liquids which are removed as sludge. Finally, treatment to control odors, to retard biological activity, or destroy pathogenic organisms may also be needed.

While the devices used in wastewater treatment are numerous and will probably combine physical, chemical and biological methods, they may all be generally grouped under six methods:

- Preliminary Treatment
- Primary Treatment
- Secondary Treatment
- Disinfection
- Sludge Treatment
- Tertiary Treatment

Degrees of treatment are sometimes indicated by use of the terms primary, secondary and tertiary treatment. Tertiary treatment, properly, would be any treatment added onto or following secondary treatment.

Preliminary Treatment

At most plants preliminary treatment is used to protect pumping equipment and facilitate subsequent treatment processes. Preliminary devices are designed to remove or cut up the larger suspended and floating solids, to remove the heavy inorganic solids, and to remove excessive amounts of oils or greases.

To effect the objectives of preliminary treatment, the following devices are commonly used:

1. Screens -- rack, bar or fine
2. Comminuting devices -- grinders, cutters, shredders
3. Grit chambers
4. Pre-aeration tanks

In addition to the above, chlorination may be used in preliminary treatment. Since chlorination may be used at all stages in treatment, it is considered to be a method by itself. Preliminary treatment devices require careful design and operation.

Primary Treatment

In this treatment, most of the settleable solids are separated or removed from the wastewater by the physical process of sedimentation. When certain chemicals are used with primary sedimentation tanks, some of the colloidal solids are also removed. Biological activity of the wastewater in primary treatment is of negligible importance.

The purpose of primary treatment is to reduce the velocity of the wastewater sufficiently to permit solids to settle and floatable material to surface. Therefore, primary devices may consist of settling tanks, clarifiers or sedimentation tanks. Because of variations in design, operation, and application, settling tanks can be divided into four general groups:

- Septic tanks

- Two story tanks -- Imhoff and several proprietary or patented units
- Plain sedimentation tank with mechanical sludge removal

Upward flow clarifiers with mechanical sludge removal When chemicals are used, other auxiliary units are employed. These are:

1. Chemical feed units
2. Mixing devices
3. Flocculators

The results obtained by primary treatment, together with anaerobic sludge digestion as described later, are such that they can be compared with the zone of degradation in stream self-purification. The use of chlorine with primary treatment is discussed under the section on Preliminary Treatment.

Secondary Treatment

Secondary treatment depends primarily upon aerobic organisms which biochemically decompose the organic solids to inorganic or stable organic solids. It is comparable to the zone of recovery in the self-purification of a stream.

The devices used in secondary treatment may be divided into four groups:

1. Trickling filters with secondary settling tanks
2. Activated sludge and modifications with final settling tanks
3. Intermittent sand filters
4. Stabilization ponds

The use of chlorine with secondary treatment is discussed under the section on Secondary Treatment

Chlorination

This is a method of treatment which has been employed for many purposes in all stages in wastewater treatment, and even prior to preliminary treatment. It involves the application of chlorine to the wastewater for the following purposes:

1. Disinfection or destruction of pathogenic organisms
2. Prevention of wastewater decomposition -- Odor Control, And Protection Of Plant Structures

While chlorination has been commonly used over the years, especially for disinfection, other methods to achieve disinfection as well as to achieve similar treatment ends are also used. Among the most common is the use of ozone. In view of the toxicity of chlorine and chlorinated compounds for fish as well as other living forms, ozonation may be more commonly used in the future. This process will be more fully discussed in the section on disinfection.

Sludge Treatment

The solids removed from wastewater in both primary and secondary treatment units, together with the water removed with them, constitute wastewater sludge. It is generally necessary to subject sludge to some treatment to prepare or condition it for ultimate disposal. Such treatment has two objectives -- the removal of part or all of the water in the sludge to reduce its volume, and the decomposition of the putrescible organic solids to mineral solids or to relatively stable organic solids. This is accomplished by a combination of two or more of the following methods:

1. Thickening
2. Digestion with or without heat
3. Drying on sand bed -- open or covered
4. Conditioning with chemicals
5. Elutriation
6. Vacuum filtration
7. Heat drying
8. Incineration
9. Wet oxidation
10. Centrifuging

Package Units

The term "package units" is used in the field to describe equipment which has been put on the market by a number of manufacturers that is intended to provide wastewater treatment by the use of prefabricated or modular units. Package units can also refer to a complete installation, including both mechanisms and prefabricated containers. This term is also applied to installations where only the mechanisms are purchased and the containers constructed by the purchaser in accordance with plans and specifications prepared by the manufacturer.

Though specific limitations have not been established, individual package units have, in general, been small installations serving a limited population.

Package units have been adapted to practically all the treatment devices, either singly or in various combinations that have been mentioned.

Tertiary and Advanced Wastewater Treatment

The terms "primary" and "secondary" treatment have been used to generally describe a degree of treatment; for example, settling and biological wastewater treatment. Since the early 1970's "tertiary" treatment has come into use to describe additional treatment following secondary treatment. Quite often this merely indicates the use of intermittent sand filters for increased removal of suspended solids from the wastewater. In other cases, tertiary treatment has been used to describe processes which remove plant nutrients, primarily nitrogen and phosphorous, from wastewater.

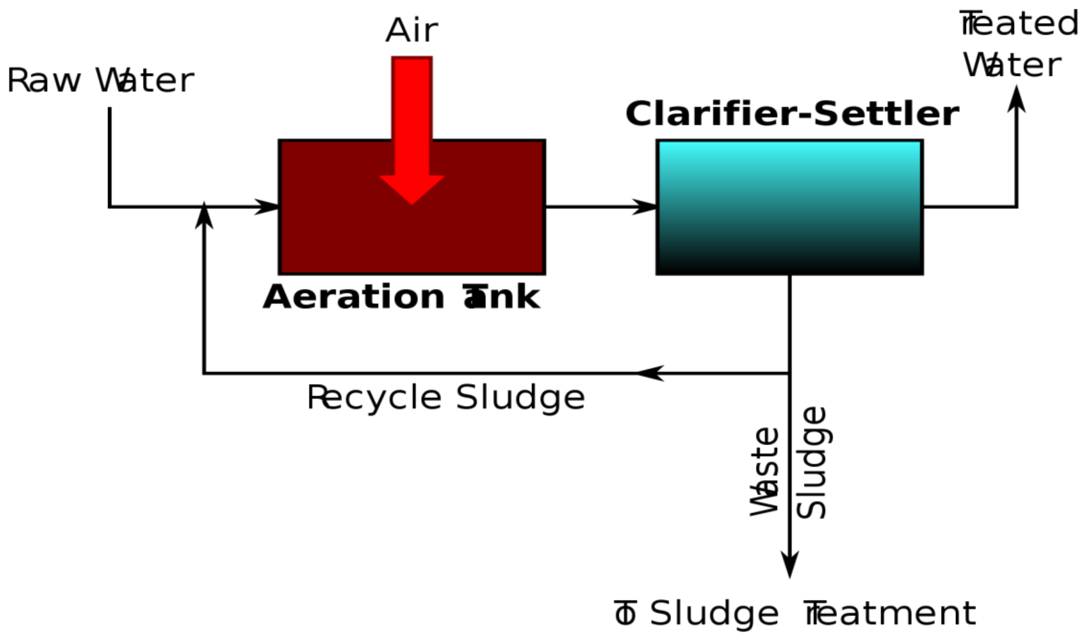
Improvement and upgrading of wastewater treatment units as well as the need to minimize environmental effects has led to the increased use of tertiary treatment.

A term that is also sometimes used to indicate treatment of a wastewater by methods other than primary or biological (secondary) treatment is advanced treatment. This degree of treatment is usually achieved by chemical (for example coagulation) methods as well as physical methods (flocculation, settling and activated carbon adsorption) to produce a high quality effluent water.

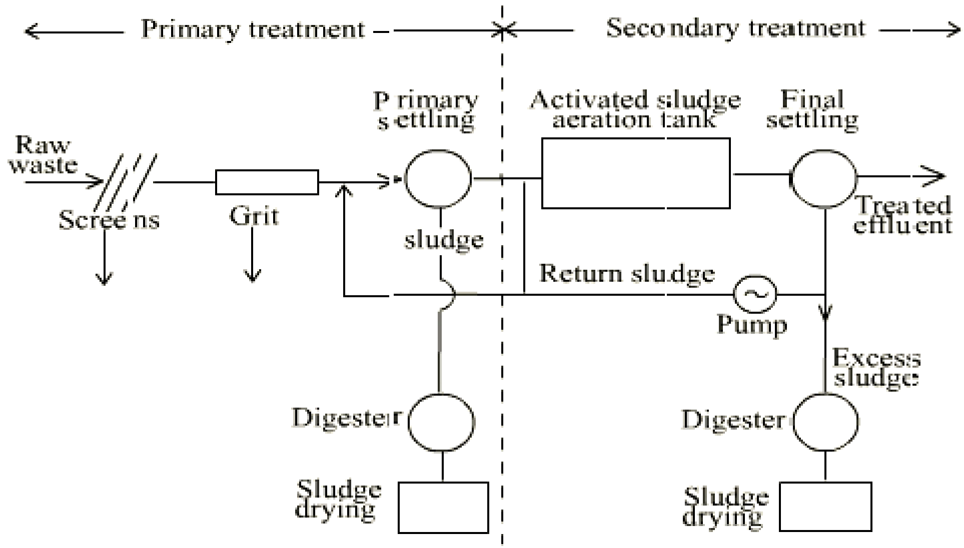
AEROBIC TREATMENT SYSTEMS

1. ACTIVATED SLUDGE PROCESS

The most common suspended growth process used for municipal wastewater treatment is the activated sludge process as shown in figure:



Flow sheet of an activated sludge system



Activated sludge plant involves:

1. wastewater aeration in the presence of a microbial suspension,
2. solid-liquid separation following aeration,
3. discharge of clarified effluent,
4. wasting of excess biomass, and
5. return of remaining biomass to the aeration tank.

In activated sludge process wastewater containing organic matter is aerated in an aeration basin in which micro-organisms metabolize the suspended and soluble organic matter. Part of organic matter is synthesized into new cells and part is oxidized to CO_2 and water to derive energy. In activated sludge systems the new cells formed in the reaction are removed from the liquid stream in the form of a flocculent sludge in settling tanks. A part of this settled biomass, described as activated sludge is returned to the aeration tank and the remaining forms waste or excess sludge. **Activated Sludge Process Variables**

The main variables of activated sludge process are the mixing regime, loading rate, and the flow scheme.

Mixing Regime

Generally two types of mixing regimes are of major interest in activated sludge process: *plug flow* and *complete mixing*. In the first one, the regime is characterized by orderly flow of mixed liquor through the aeration tank with no element of mixed liquor overtaking or mixing with any other element. There may be lateral mixing of mixed liquor but there must be no mixing along the path of flow.

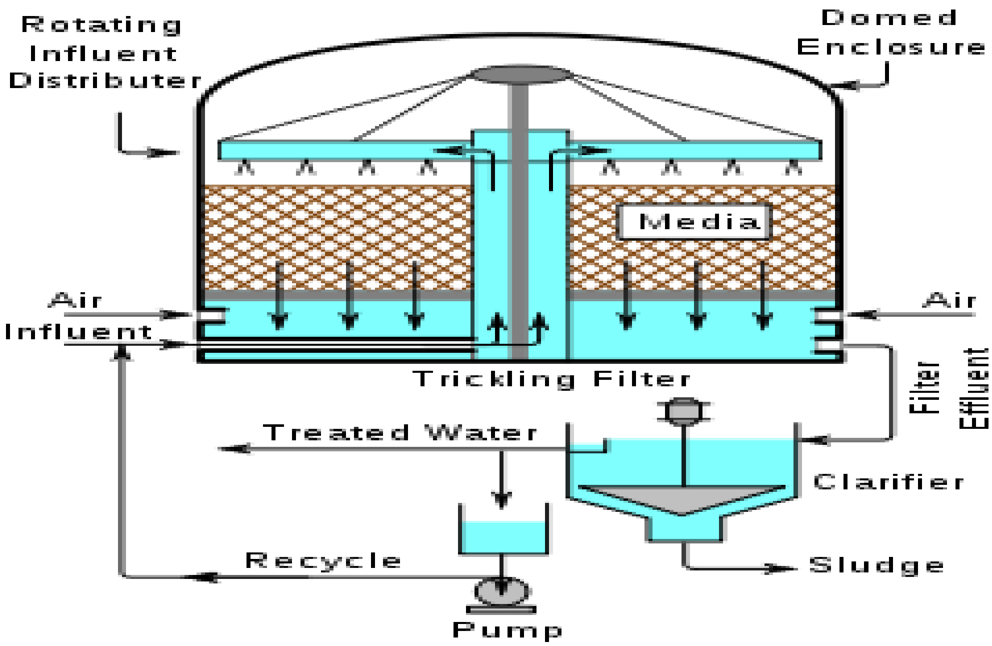
In complete mixing, the contents of aeration tank are well stirred and uniform throughout. Thus, at steady state, the effluent from the aeration tank has the same composition as the aeration tank contents.

The type of mixing regime is very important as it affects (1) oxygen transfer requirements in the aeration tank, (2) susceptibility of biomass to shock loads, (3) local environmental conditions in the aeration tank, and (4) the kinetics governing the treatment process.

2. TRICKLING FILTER

Trickling filter is a type of wastewater treatment system. It consists of a fixed bed of rocks, lava, coke, gravel, slag, polyurethane foam, sphagnum peat moss, ceramic, or plastic media over which sewage or other wastewater flows downward and causes a layer of microbial slime (biofilm) to grow, covering the bed of media. Aerobic conditions are maintained by splashing, diffusion, and either by forced air flowing through the bed or natural convection of air if the filter medium is porous.

The terms trickle filter, trickling biofilter, biofilter, biological filter and biological trickling filter are often used to refer to a trickling filter



Operation

The removal of pollutants from the wastewater stream involves both absorption and adsorption of organic compounds by the layer of microbial biofilm. The filter media is typically chosen to provide a very high surface area to volume. Typical materials are often porous and have considerable internal surface area in addition to the external surface of the medium. Passage of the wastewater over the media furnishes dissolved air, the oxygen which the slime layer requires for the biochemical oxidation of the organic compounds and releases carbon dioxide gas, water and other oxidized end products. As the biofilm layer thickens, it eventually sloughs off into the treated effluent and subsequently forms part of the secondary sludge. Typically, a trickling filter is followed by a clarifier or sedimentation tank for the separation and removal of the sloughing. Other filters utilizing higher-density media such as sand, foam and peat moss do not produce a sludge that must be removed, but require forced air blowers and backwashing or an enclosed anaerobic environment.

The treatment of sewage or other wastewater with trickling filters is among the oldest and most well characterized treatment technologies.

Industrial wastewater treatment trickle filters

Wastewaters from a variety of industrial processes have been treated in trickling filters. Such industrial wastewater trickling filters consist of two types:

- Large tanks or concrete enclosures filled with plastic packing or other media.

- Vertical towers filled with plastic packing or other media.

  The availability of inexpensive plastic tower packings has led to their use as trickling filter beds in tall towers, some as high as 20 meters.[4] As early as the 1960s, such towers were in use at: the Great Northern Oil's Pine Bend Refinery in Minnesota; the Cities Service Oil Company Trafalgar Refinery in Oakville, Ontario and at a kraft paper mill.

  The treated water effluent from industrial wastewater trickling filters is very often subsequently processed in a clarifier-settler to remove the sludge that sloughs off the microbial slime layer attached to the trickling filter media (see Image 1 above).

  Currently, some of the latest trickle filter technology involves aerated biofilters which are essentially trickle filters consisting of plastic media in vessels using blowers to inject air at the bottom of the vessels, with either downflow or upflow of the wastewater.

3. PACKED BED/COLUMN REACTOR

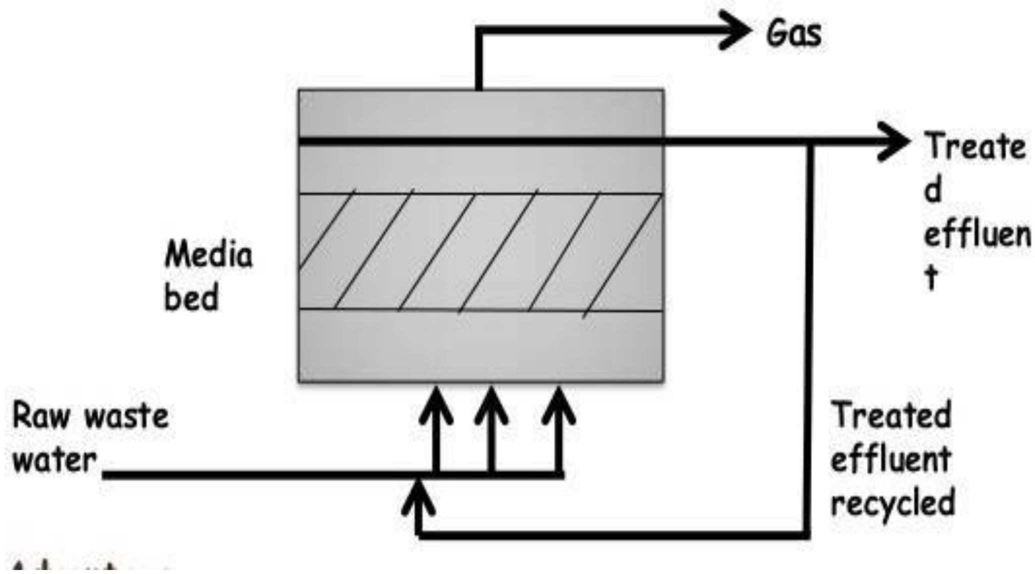
Packed bed ractors recently have been suggested for a number of wastewater treatment applications. Young and Mc-Carty in 1967 reported successful anaerobic treatment of concentrated soluble waste in an upflow submerged anaerobic filter. Tamblyn and Sword and Seidel and Crites reported that satisfactory anaerobic denitrification could be accomplished in similar upflow reactors. Johnson and Baumann described an aerobic sand-medium “pulsed adsorption bed” process for providing additional treatment of low quality effluents from secondary wastewater treatment plants.

The basic designs of the packed bed reactors (PBR’S) used for these applications are essentially the same. Each system consists of a unit containing a porous medium supported over an inlet chamber through which the influent wastewater is distributed (Figure 1).

In aerobic units, air (in some cases, oxygen-enriched air) is passed upward through the unit from an air distribution grid located immediately below or slightly above the bottom of the media bed. The selection of a medium depends on the particular application of the system. The PBR unit may be followed by granular-medium filtration or other treatment units to provide additional effluent polishing through suspended solids (ss) removal.

Johnson and Baumann and Wall reported and removal of carbonaceous biochemical oxygen demand (BODc) in laboratory and pilot scale aerobic PBR units using sand as a medium, but no nitrification forms was observed. Haug and Mccarty obtained satisfactory nitrification in laboratory scale upflow PBR’S using pure oxygen to maintain a high dissolved oxygen (DO) concentration throughout the reactor.

Research by the authors has demonstrated that considerable BODc removal and biological nitrification may be accomplished at low wastewater temperatures in PBR’S using air to maintain an aerobic environment if proper control is maintained over the influent conditions.



The column packed bed reactor used for the removal of BOD and nitrification. Microorganisms gets attached and form the bottom of the reactor.

ADVANTAGE

Easy construction

East operation and

Management Organisms do not

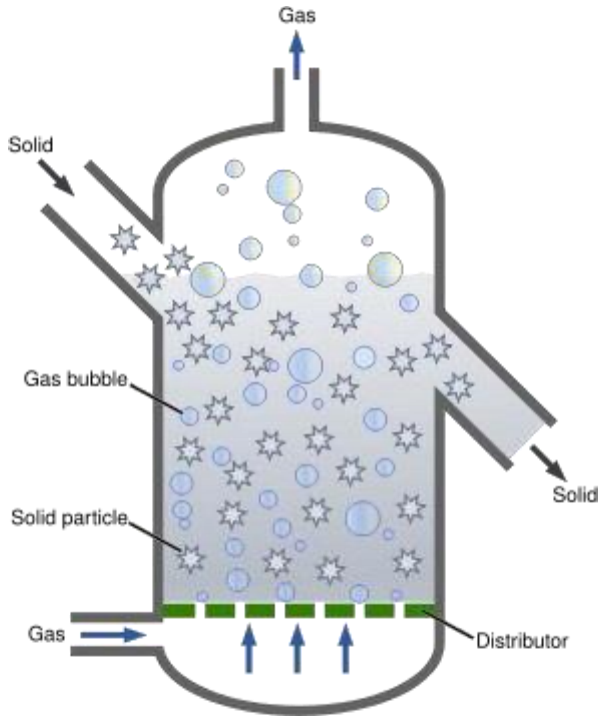
form slime on packing

APPLICATION

Column packed with anthracite, coal particle are used for denitrification of uranium recovery waste water.

4. FLUIDIZED BED REACTOR

Combination of attached growth and suspended growth. Reactor is cylindrical with perforated distribution plates. Biological slime film is developed and maintained on a solid support medium. Support media maintained in suspension by upward flow of influent. Fabricated media developed to support biomass growth within porous internal structure.



Types: 1. Captor: biomass is grown inside polyester foam pads. It can be removed periodically sequeezed. Simple sparger is used.

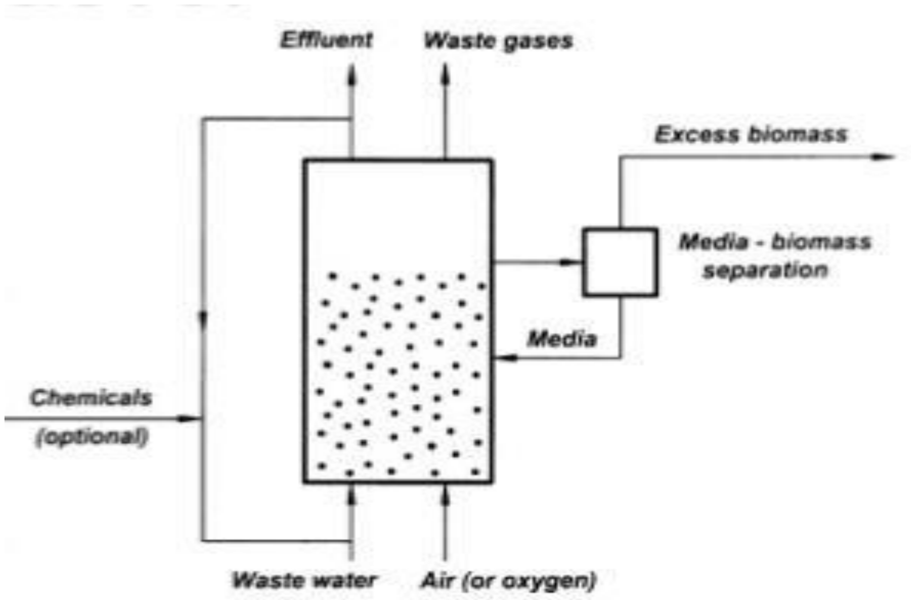
2. Oxitron: sand particles overflow/clean recycled. High purity oxygen is used.

Advantage: low biomass sludge production.

High biomass concentration, high strength waste water treatment, low sludge transport and disposal, high exposed liquid/biofilm surface area, low operation cost. Application: Aerobic FBR used for nitrate removal in petroleum industry, coke plant, explosive factory nuclear plant. Anoxic FBR used for de-nitrification.

5. INVERSE FLUIDIZED BED BIOFILM

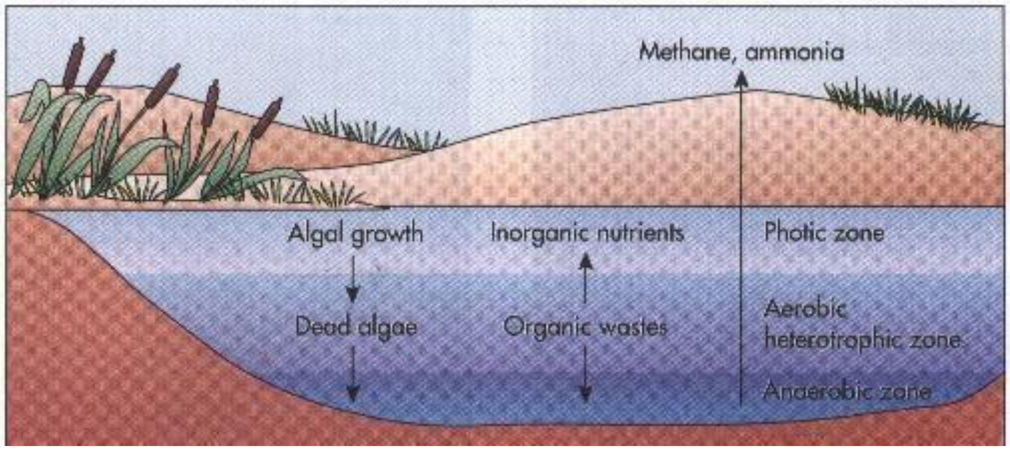
Reactor bed is consisting of biofilm attached to low density polypropylene particles. It expands downwards during fluidization. Allows the control of biomass loading and provides the high oxygen concentration in the reacting liquid media. Fluidization can be conducted by an upward co-current flow of gas and liquid through bed.



ADVANTAGES: Large specific support surface area. 2. Fast biofilm formation, 3. Effective and simple control of biofilm thickness.

6. OXIDATION PONDS

Oxidation Ponds are also known as stabilization ponds or lagoons. They are used for simple secondary treatment of sewage effluents. Within an oxidation pond heterotrophic bacteria degrade organic matter in the sewage which results in production of cellular material and minerals. The production of these supports the growth of algae in the oxidation pond. Growth of algal populations allows furthur decomposition of the organic matter by producing oxygen. The production of this oxygen replenishes the oxygen used by the heterotrophic bacteria. Typically oxidation ponds need to be less than 10 feet deep in order to support the algal growth. In addition, the use of oxidation ponds is largely restricted to warmer climate regions because they are strongly influenced by seasonal temperature changes. Oxidation ponds also tend to fill, due to the settling of the bacterial and algal cells formed during the decomposition of the sewage. Overall, oxidation ponds tend to be inefficient and require large holding capacities and long retention times. The degradation is relatively slow and the effluents containing the oxidized products need to be periodically removed from the ponds. An oxidation pond can be seen in the figure below.



7. Lagoons

An **aerated lagoon** or **aerated basin** is a holding and/or treatment pond provided with artificial aeration to promote the biological oxidation of wastewaters.

Types of aerated lagoons or basins

Suspension mixed lagoons, where there is less energy provided by the aeration equipment to keep the sludge in suspension.

Facultative lagoons, where there is insufficient energy provided by the aeration equipment to keep the sludge in suspension and solids settle to the lagoon floor. The biodegradable solids in the settled sludge then degrade as in an anaerobic lagoon.

Suspension mixed lagoons

Suspension mixed lagoons flow through activated sludge systems where the effluent has the same composition as the mixed liquor in the lagoon. Typically the sludge will have a residence time or sludge age of 1 to 5 days. This means that the chemical oxygen demand (COD) removed is relatively little and the effluent is therefore unacceptable for discharge into receiving waters. The objective of the lagoon is therefore to act as a biologically assisted flocculator which converts the soluble biodegradable organics in the influent to a biomass which is able to settle as sludge. Usually the effluent is then put in a second pond where the sludge can settle. The effluent can then be removed from the top with a low COD, while the sludge accumulates on the floor and undergoes anaerobic stabilization.

Methods of aerating lagoons or basins

There are many methods for aerating a lagoon or basin: Motor-driven floating surface aerators

Motor-driven submerged or floating
jet aerators Motor-driven fixed-in-
place surface aerators
Injection of compressed air through submerged diffusers

Floating surface aerators

A Typical Surface-Aerated Basin (using motor-driven floating aerators)

Ponds or basins using floating surface aerators achieve 80 to 90% removal of BOD with retention times of 1 to 10 days.[5] The ponds or basins may range in depth from 1.5 to 5.0 metres. **In a surface-aerated system**, the aerators provide two functions: they transfer air into the basins required by the biological oxidation reactions, and they provide the mixing required for dispersing the air and for contacting the reactants (that is, oxygen, wastewater and microbes). Typically, the floating surface aerators are rated to deliver the amount of air equivalent to 1.8 to 2.7 kg O_2/kWh. However, they do not provide as good mixing as is normally achieved in activated sludge systems and therefore aerated basins do not achieve the same performance level as activated sludge units.

Biological oxidation processes are sensitive to temperature and, between 0 °C and 40 °C, the rate of biological reactions increase with temperature. Most surface aerated vessels operate at between 4 °C and 32 °C.

Submerged diffused aeration

Submerged diffused air is essentially a form of a diffuser grid inside a lagoon. There are two main types of submerged diffused aeration systems for lagoon applications: floating lateral and submerged lateral. Both these systems utilize fine or medium bubble diffusers to provide aeration and mixing to the process water. The diffusers can be suspended slightly above the lagoon floor or may rest on the bottom. Flexible airline or weighted air hose supplies air to the diffuser unit from the air lateral (either floating or submerged).

COMPOSTING

The process of biological degradation of solid organic waste material to stable end products. This can be done in aerobic and anaerobic, aerobic method is more frequently used. In composting solid organic waste converted by aerobic microbiological process in to stable sanitary, nuisance free, humus like material that can be safely disposed into the environments. Composting is cost effective, eco friendly for stable ultimate disposal of sludge. The composting product can be used for soil improvement and used for production of mushroom, helpful for reuse and recycling of organic waste materials from domestic, agriculture and industry.

Organisms involved in composting

Bacteria 80-90% found in the compost possesses a broad range of enzymes to degrade a wide range of organic compounds. Other organisms are actinomycetes, fungi, protozoa, mites, insects, ants and earth worms.

Mechanism of composting

Proteins and lipids decomposed by bacteria, cellulose and other complex organic compounds degraded by actinomycetes and fungi.

Compositing done in 3 stages due to changes in temperature

1. Mesophilic stage: fungi and acid produceing bacteria are active in this stage and the temperature increases from ambient to about 40ºC.
2. Thermophilic stage: as the composting proceeds the temperature raises from 40ºC-70ºC. thermophilic bacteria, thermophilic fungi and actinomycetes are active in this stage.

   thermophilic stage is associated with high rate and maximum degrading organic materials.
3. Cooling stage: degradative activity slows down and the thermophilic organisms are replaced by mesophilic bacterial and fungi. Cooling stage is associated with the formationof water, pH stabilization and completion of humic acid formation.

Methods of composting:

Operation of composting involves the following steps.

1. Mixing of dewatered sludge with a bulking agent (saw dust, rice hulls, straw or recycle compost). Improve the porosity of the mixture for good aeration.
2. Creating aerobic conditions (aeration) by mechanical or other means. This is needed for the supply of oxygen to control temperature for the removal of water.
3. Removal of bulking agent, if possible.
4. Storage and disposal of the composite.

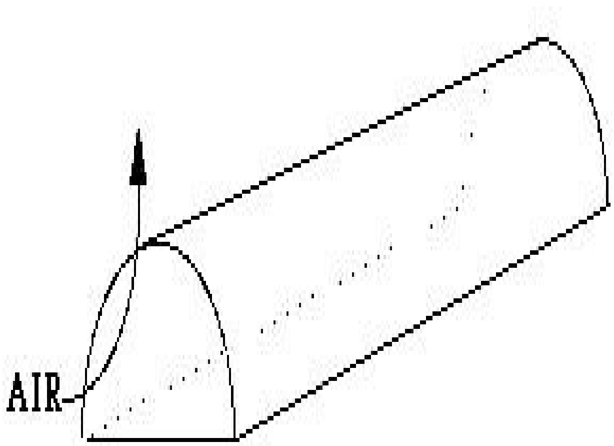
Composting systems

1. Non-reactor: 2 types i. Field composting, ii. Windrow composting
2. Reactor system:i. Horizontal flow reactor, ii. Vertical flow reactors, iii. Rotating drum

reactors

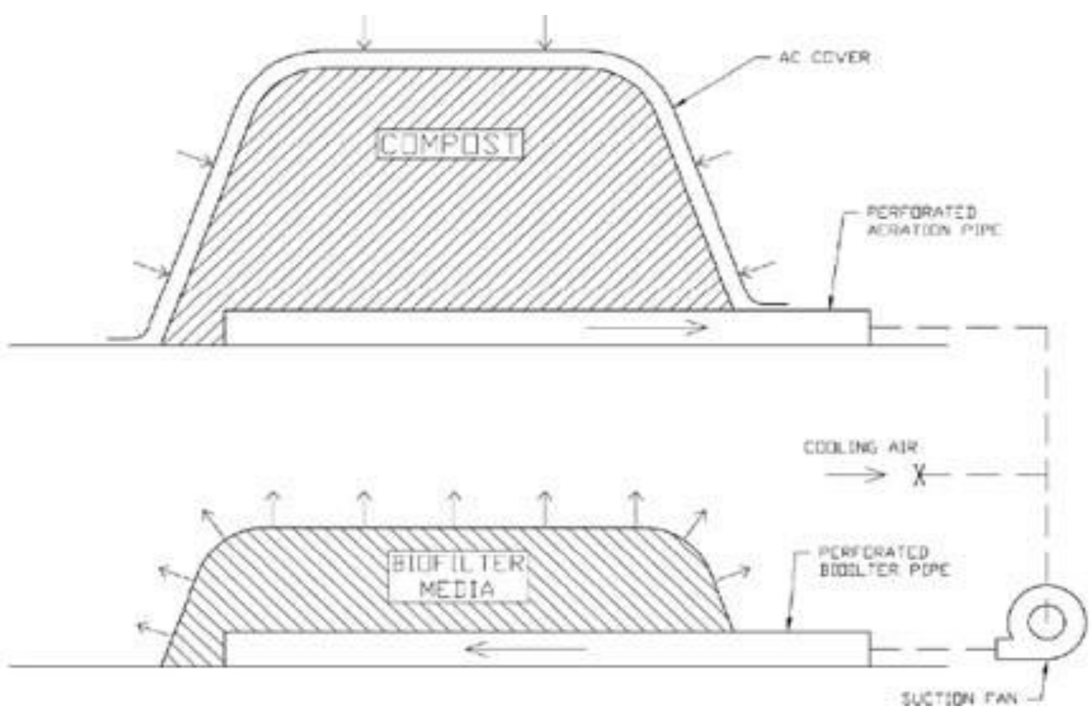
i. FIELD COMPOSTING

It is a natural organic decomposting process, best for hygienic waste. All microbial activity takes place in a thin layer on the soil surface or within a few centimeter of the soil surface. This system is useful for treating both sludge and green wastes, grass, straw, bushwood. To enhance the reaction the waste need to be chopped so the surface of waste exposed to the atmosphere in large, thermal heating does not occur i.e thermal disinfection or killing of weed seeds does not occur.



ii. WINDROW COMPOSTING

This composting is carried out due to the direct contact between the waste material and the atmosphere. The composting process influences the atmosphere by emitting odour, green house gases, spores, germs and dust. The atmosphere carrying oxygen influences the composting by supplying rain water, changes in the temperature, changes in the air humidity and proper aeration. The extent of contact between waste material and the atmosphere can be limited by covering the pile with mature compost material, straw or special textile that allow gas exchange, but reduce the infiltration of rain water. To get high quality compost windrow are disturbed from time –time by turning. This mixing of the material for homogenization dry or wet zones at the surface, wet zones at the bottom. For killing of pathogenic of microorganisms and weed seeds, renewing the structure and free air space and increase evaporation to dry the waste material or the mature compost. The turning frequency depends on the kind and structure of waste and quality requirement of finished compost.



REACTOR COMPOSTING

Composting in an enclosed space (eg. Container, box, bin, tunnel, shed with forced exchange of respiration gases is a type of reactor composting. According to the material flow reactor composting is divided in to

1. Horizontal flow reactor
2. Vertical flow reactor
3. Rotating drum reactor

The reactor have controlled forced aeration and enable the waste air and drainage water to be collected and treated. Addition of water or other additive is possible.

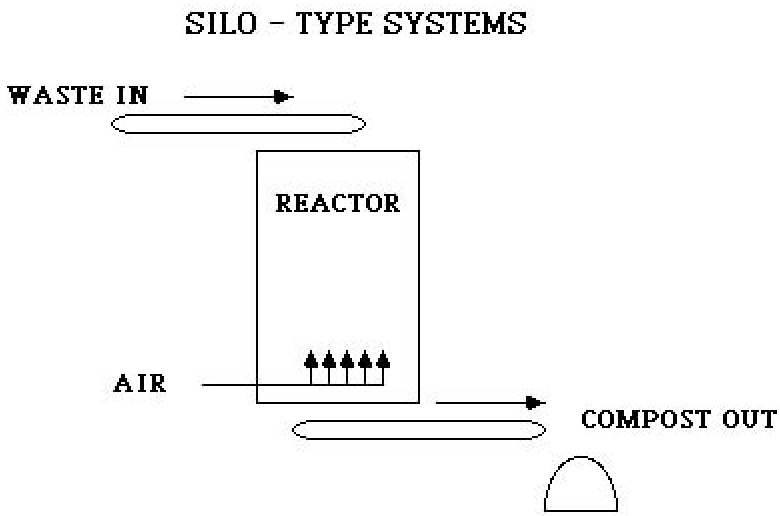
1. Horizontal flow reactor: Static reactor is a batch system where the waste material is loaded with a wheel loader or transport devices in to a horizontal reactor or is covered by a foil or textile material. The waste is placed over a grid and forced aeration was given. It can also pass air through pipes at the bottom of the material or from holes in the floor. The waste air and its odour components and waste water can be treated in a biofilter. In some systems waste air is recycled. Air flow rate is controlled base on oxygen, carbon dioxide and temperature of the material. The retention time in the reactor is between several days to several weeks.

Disadvantages: No turning/mixing, no water addition, forced aeration only from one direction. The end product may be heterogenous (partially too dry) and biologically not stabilized.

2. **Vertical composting reactors** : are generally over 4 meters (yards) high, and can be housed in silos or other large structures. Organic material is typically fed into the reactor at the top through a distribution mechanism, and flows by gravity to an unloading mechanism at the bottom. Process control is usually by pressure-induced aeration, where the airflow is opposite to the downward materials flow. The height of these reactors makes process control difficult due to the high rates of airflow required per unit of distribution surface area. Neither temperature nor oxygen can be maintained at optimal levels throughout the reactors, leading to zones of non-optimal activity. Some manufacturers have minimized these difficulties by enhanced air distribution and collection systems, including changing the airflow direction from vertical to horizontal between alternating sets of inflow and exhaust pipes. As with static pile composting, a stable porous structure is important in vertical reactors which usually lack internal mixing. Tall vertical reactors have been successfully used in the sludge

composting industry where uniform feedstocks and porous amendments can minimize these difficulties in process control, but are rarely used for heterogeneous materials like MSW.

Vertical Reactor



Vermicompost

Vermicomposting is basically a managed process of worms digesting organic matter to transform the material into a beneficial soil amendment. As per the USDA guidelines for compost practices (with effect from Oct 21, 2002), vermicomposts are defined as organic matter of plant and/or animal origin consisting mainly of finely-divided earthworm castings, produced nonthermophilically with biooxidation and stabilization of the organic material, due to interactions between aerobic microorganism and earthworms, as the materials pass through the earthworm gut. Good quality compost production in ambient temperature can be accomplished in shorter time by the process of vermicomposting that involves use of proper species of earthworms. The native cellulase activity of earthworms and microorganisms in earthworm gut promote faster decomposition of ingested organic material. The combined effect of enzymatic activity and grinding of organic materials to fineness by earthworms produces the vermicomposting and this is not observed in compost pits without earthworm.

The earthworms being voracious eaters consume the biodegradable matter and give out a part of the matter as excreta or vermi-castings. The vermi-casting containing nutrients is a rich manure for the plants. Vermicompost, apart from supplying nutrients and growth enhancing hormones to plants, improves the soil structure leading to increase in water and nutrient holding capacities of soil. Fruits, flowers and vegetables and other plant products grown using vermicompost are reported to have better keeping quality. A growing number of individuals and institutions are taking interest in the production of vermicompost utilising earthworm activity. As the operational cost of production of this compost works out to less than ` 2.0/Kg., it is quite profitable to sell the compost even at

` 4.00 to ` 4.50/Kg.

PROCESS

The process of composting crop residues / agri wastes using earthworms comprise spreading the agricultural wastes and cow dung in gradually built up shallow layers. The pits are kept shallow to avoid heat built-up that could kill earthworms. To enable earthworms to transform the material relatively faster a temperature of around 300C is maintained. The final product generated by this process is called vermicompost which essentially consist of the casts made by earthworms eating the raw organic materials. The process consists of constructing brick lined beds generally of 0.9 to 1.5 m width and 0.25 to 0.3 m height are constructed inside a shed open from all sides. For commercial production, the beds can be prepared with 15 m length, 1.5 m width and 0.6 m height spread equally below and above the ground. While the length of the beds can be made as per convenience, the width and height cannot be increased as an increased width affects the ease of operation and an increased height on conversion rate due to heat built up.

Cow dung and farm waste can be placed in layers to make a heap of about 0.6 to 0.9 m height. Earthworms are introduced in between the layers @350 worms per m3of bed volume that weighs nearly 1 Kg. The beds are maintained at about 240-50% moisture content and a temperature of 20–30oC by sprinkling water over the beds.

When the commercial scale production is aimed at, in addition to the cost of production, considerable amount has to be invested initially on capital items. The capital cost may work out to about `5000 to `6000 for every tonne of vermicompost production capacity. The high unit capital cost is due to the fact that large units require considerable expenditure on preparation of vermi beds, shed to provide shelter to these beds and machinery. However these expenditures are incurred only once.

Under the operational cost, transportation of raw materials as also the finished product are the key activities. When the source organic wastes and dung are away from the production facility and the finished product requires transportation to far off places before being marketed, the operational cost would increase. However, in most of the cases, the activity is viable and bankable. Following are the items required to be considered while setting up a unit for production of vermi-compost.

EARTH WORMS

Of about 350 species of earth worms in India with various food and burrowing habits Eisenia fetida, Eudrilus eugeniae and Perionyx excavatus are some of the species that are reared to convert organic wastes into manure. A combination of epigeic species that form no permanent burrows and live on the surface, anecicthat form semi-permanent and vertical burrows extending from the surface and endogeic that typically live throughout the deeper layers may be considered.

The worms feed on any biodegradable matter and vermicomposting units are ideally suited for locations / units with generation of considerable quantities of organic wastes. One earthworm reaching reproductive age of about six weeks lays one egg capsule (containing 7 embryos) every 7-10 days. Three to seven worms emerge out of each capsule. Thus, the multiplication of worms under optimum growth conditions is very fast. The worms live for about 2 years. Fully grown

worms could be separated and dried in an oven to make 'worm meal' which is a rich source of protein (70%) for use in animal feed.

Location:Rural areas with predominance of agriculture, suburbs of cities and peri urban villages are considered ideal locations for setting up of vermicomposting units on a larger scale from the view point of availability of raw material and marketing of the produce. As use of the compost is said to have ameliorative effect more particularly on fruit, vegetable, plantation and ornamental crops, vermicomposting units may be located in areas with concentration of fruit and vegetable growers and floriculture units. Further, the nearness to a commercial dairy unit or large concentration of cattle population will have an added advantage of cheap raw material i.e. cow dung.

BIOFERTILIZER

Biofertilizer is a type of fertilizer containing living microorganisms, that enrich the nutrient quality of the soil. The main sources of biofertilizers are bacteria, fungi, and cyanobacteria (blue-green algae). They form a symbiotic relationship with plants, in which the partners derive benefits from each other.

Biofertilizer are classified based on

1) For nitrogen -Rhizobium for legumes crops -Azotobacter/ Azospirilium for non legume crops -Acetobacter for sugarcane only. -Blue –Green Algae (BGA) and Azolla for low land paddy.

2) For phosphorus -Phosphate solubilizing Bacteria (PSB) for all crops to be applied with Rhizobium, Azotobacter, Azospirilium and Acetobacter

3) For enriched compost -Phosphate solubilizing Bacteria (PSB) and Azatobacter culture - Cellulolytic fungal culture

Biofertilizers in agriculture includes the following:

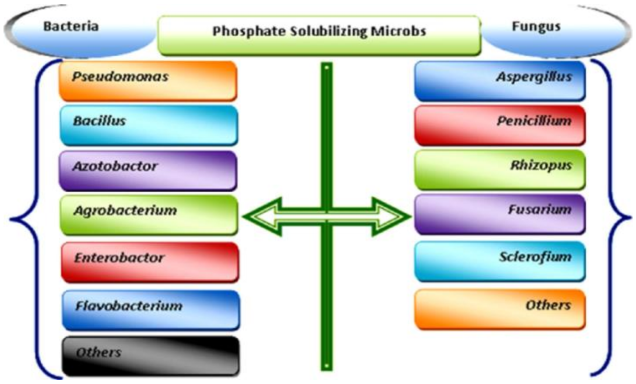
- Symbiotic nitrogen fixers, Rhizobium spp.;
- Non-symbiotic, free-living nitrogen fixers (Azotobacter, Azospirillum, etc.)

- Algal biofertilizers (blue-green algae or blue-green algae in association with Azolla)
- Phosphate-solubilising bacteria
- Mycorrhizae
- Organic fertilizers

PHOSPHATE SOLUBILIZER

Phosphate solubilizing bacteria (PSB) are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds. They have the capability of dissolving soil phosphorus which have been adsorbed and can mineralize organic P to become inorganic P, hence increasing the avalibility of P in the soil.

Phosphate solubilizing bacteria such as *Bacillus, Rhizobium, and Pseudomonas, flavobacterium,micrococcus,Streptomyces* and fungi belongs to *Aspergillus,Penicillium, Trichoderma* are the potent genera which are efficient to hydrolyze the inorganic phosphorus into soluble form and easily made available to the plant for growth promotion and root formation.



Plants absorb phosphate in the form $H_2PO_4^-$ and HPO_4^{-2} ions.Plants absorb only 10-15% -rest remains in insoluble form of phosphate. **Phosphobacteria** secrete organic acids which dissolve this unavailable phosphate into soluble form and make it available to the plants Phosphobacteria means microbial inoculants capable of phosphate solubilizing nature. Commonly used Phosphobacteria is Bacillus megaterium. Phosphobacteria is suitable for all crops. This has to be mixed with rhizobium.

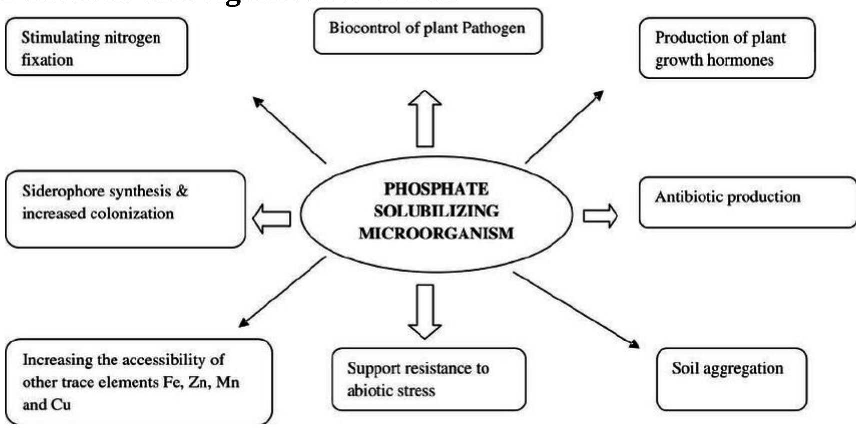
Media Preparation

Prepare appropriate media for specific to the bacterial inoculant in 250 ml, 500 ml, 3 litre and 5 litre conical flasks and sterilize. -The media in 250 ml flask is inoculated with efficient bacterial strain under aseptic condition Keep the flask under room temperature in rotary shaker (200 rpm) for 5- 7 days. -Observe the flask for growth of the culture and estimate the population, which serves as the starter culture. Using the starter culture (at log phase) inoculate the larger flasks (500 ml, 3 litre and 5 litre) containing the media, after obtaining growth in each flask. -The above media is prepared in large quantities in fermentor, sterilized well, cooled and kept it ready.

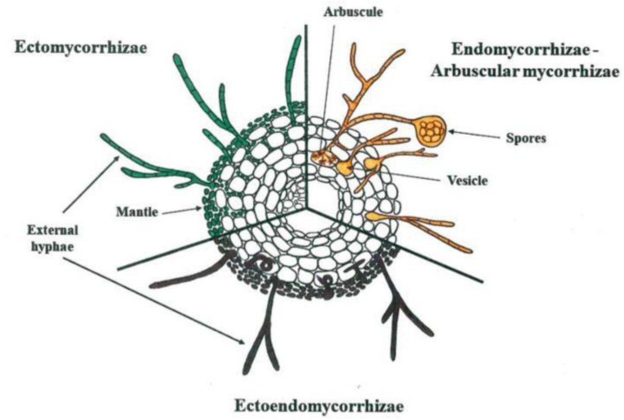
Inoculum preparation

The media in the fermentor is inoculated with the log phase culture grown in 5 litre flask. Usually 1 -2 % inoculum is sufficient, however inoculation is done up to 5% depending on the growth of the culture in the larger flasks. -The cells are grown in fermentor by providing aeration (passing sterile air through compressor and sterilizing agents like glass wool, cotton wool, acid etc.) and given continuous stirring. -The broth is checked for the population of inoculated organism and contamination if any at the growth period. -The cells are harvested with the population load of 109 cells ml-1 after incubation period. -There should not be any fungal or any other bacterial contamination at 10-6 dilution level -It is not advisable to store the broth after fermentation for periods longer than 24 hours. Even at 4^{o} C number of viable cells begins to decrease**.** PSB biofertlizer enhances the yield of wheat, rice and chickpea

Functions and significance of PSB



MYCORRHIZAL ASSOCIATION

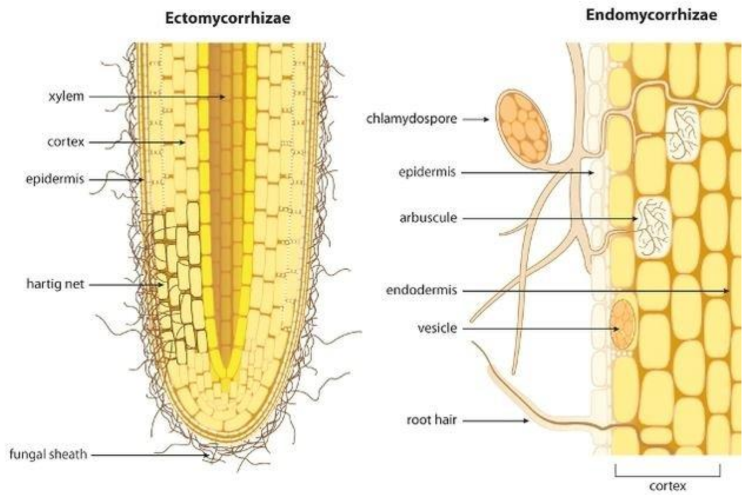


A **mycorrhiza** (the term 'mycorrhiza' comes from Greek – mycos meaning fungus and rhiza meaning roots) is a symbiotic **association** between a green plant and a fungus. The plant makes organic molecules such as sugars by photosynthesis and supplies them to the fungus, and the fungus supplies to the plant water and mineral nutrients, such as phosphorus, taken from the soil.

Based on mycorrhizae association pattern of fungi with plants are broadly classified into

1.Ectomycorrhizae

2.Endomycorrhizae



Features of Mycorrhiza

(i) Absence of any phytopathological symptoms in the partners during the active phase of mutualism,

(ii) Presence of complex interfaces between cells of the partners with a predominant type of perisymbiotic membrane, sur-rounding intracellular symbionts,

(iii) Presence of various types of phagocyte--like structures during establishment of symbionts and during harvesting phase to control the symbiotic population by the host.

Types of Mycorrhiza:

There are seven types of mycorrhizae. These are

1. Ectomycorrhizae,
2. Vesicular-arbuscular mycorrhizae,
3. Ectendomycorrhizae (Arbutoid),
4. Ericoid mycorrhizae,
5. Arbutoid mycorrhizae
6. Orchidoid mycorrhizae, and
7. Monotropoid mycorrhizae.

Ectomycorrhizae:

Ectomycorrhiza is commonly called “sheathing mycorrhiza”. They occur in 3% of all seed plants in forests of tem-perate regions, especially on pine, beech, spruce, birch etc

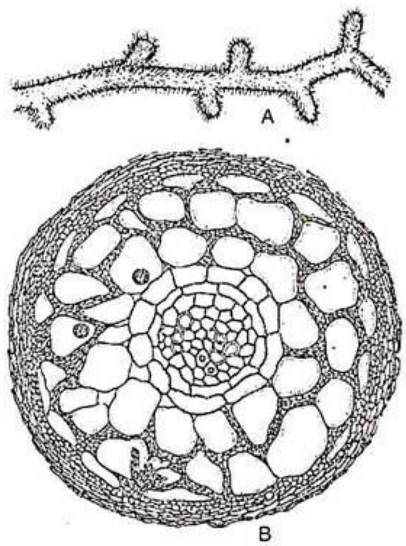


Fig. 4.103 : Ectomycorrhiza : A. *Pinus* root covered with mycorrhizal fungi, B. T.S. of *Pinus* root showing ectotrophic mycorrhizal fungi forming a mantle covering and growth of fungi between cortical cells

They cause exten-sive branching and growth of roots and modi-fication of branching pattern, such as racemose type in dicots (Fagus) and dichotomous in gym- nosperms (Pinus). In beech (Fagus) the ultimate lateral rootlets are differentiated into 'long' and 'short' roots.

The long roots show indefinite growth and their branches are the short roots that are thickened, forked and mycorrhizal. They appear in various colours like white, brown, yellow, black etc., depending on the colour of the fungus. The fungus enters the cortex forming '**Hartig net(is a network of inward growing hyphae, that extends into the root, penetrating between the epidermis and cortex of ectomycorrhizal plants.)**, but never goes inside the endodermis or stele. They form a mantle of varying thickness.

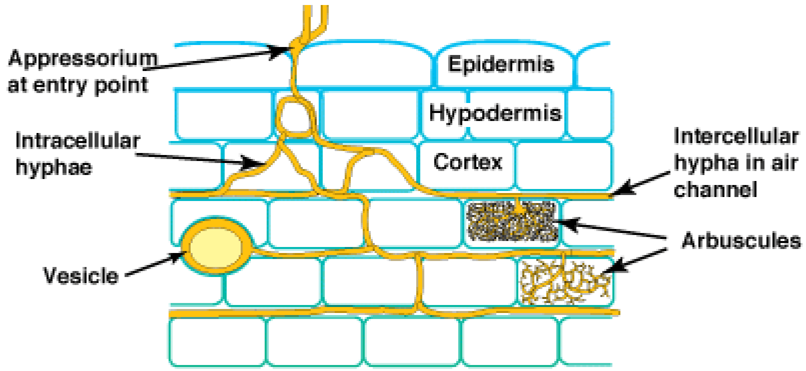
Vesicular-arbuscular mycorrhizae(VAM)

It is a type of endomycorrhizal asso-ciation, where both vesicles and arbuscles are developed together. VAM is by far the common-est of all mycorrhizae and has been reported in more than 90% of land plants.They are found in bryophytes, pteridophytes, gymnosperm (except Pinaceae) and most of angiosperms, commonly in Leguminosae (Fabaceae), Rosaceae, Gramineae (Poaceae) and Palmae (Arecaceae). VAM is not found in Ericaceae and Orchidaceae, where other type of association is available. VAM has even been reported in Lower Devonian plant, Rhynia.

VAM is produced by aseptate mycelial fungi belong to Endogonaceae under Mucorales of Zygomycotina and those members produced zygospores. The important genera involved in VAM are Glomus, Gyrospora, Acaulospora etc. Most of the members are not culturable Ectendomycorrhizae (Arbutoid). The VAM is so named because of the presence of two characteristic structures namely

(i) **The vesicles** are thin or thick walled vesicular structures produced intra-cellularly and stored materials like poly-phosphate and other minerals and arbuscles.

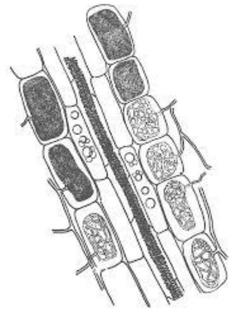
(ii) **The arbuscles** are repeated dichotomously branched haustoria which grow intracellularly The arbuscles live for four days and then get lysed releasing the stored food as oil droplets, mostly polyphosphate



There is no fungus mantle, but only a loose and very sparse network of septate hyphae spread into the soil. These hyphae bear different types of spores, chlamydospores, or aggregation of spores in sporocarp or zygospores. The super-ficial hyphae bear branches that penetrate the epidermis and then grow intercellularly only in cortex.

Intercellular hyphae form arbuscles inside the parenchyma of cortex by repeated dichotomous branching of the penetrating hyphae. The cell membrane of the penetrated cell is invaginated and covers the arbuscles. The hyphae also develop both inter- and intracellular thick-walled vesicles. The chlamydospores may germinate on nutrient agar, but the hyphae stop growing when food inside the spore is used up, thus they cannot be subcultured.

Ericoid mycorrhizae

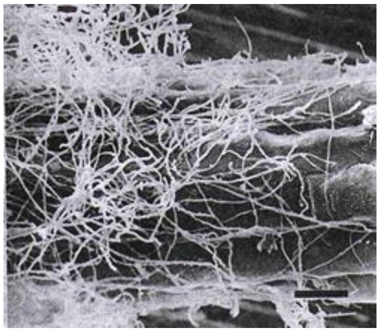


This is actually a type of endomycorrhiza. Ericoid mycorrhizae are found in the different members of Ericaceae like Erica, Calluna, Vaccinum, Rhododendron etc. The fungi are slow-growing, septate and mostly sterile. They are mostly culturable. Both *Pezizella ericae* (Ascomycotina) and *Clavaria vermiculata* (Basidiomycotina) have been iso-lated from Rhododendrons.

During this association the rootlets of the plants are covered by very sparse, loose, dark, septate hyphae that penetrate the cortex forming intercellular coils. (After 3-4 weeks the coils degenerate like arbuscles of vesicular- asbuscular mycorrhiza (VAM).

Most of the members of Ericaceae grow in acid soil with less amount of P and N nutrition. The fungus gets the photosynthate from the host and improves the mineral uptake and nutrition of the host, especially P and N. Many mycotrophs of Ericaceae show high resistance to metals like Zn and Cu. The mycorrhizal plants also show high tolerance to these metals, which is totally absent in non-infected plants.

Arbutoid mycorrhizae



It is a type of endomycorrhizal fungi that look similar to ectomycorrhizal fungi. They form a fungal sheath that encompasses the roots of the plant; however, the hyphae of the arbutoid mycorrhiza penetrate the cortical cells of plant roots, differentiating it from ectomycorrhizal fungi.

Arbutoid mycorrhizas are, like those of Ericoid and Monotropoid mycorrhizas, found in the plant order Ericales. Like ericoid mycorrhizas, the family Ericaceae is represented, with arbutoid mycorrhizas being formed in the genera Arctostaphylos and Arbutus. Arbutoid associations are also found in the Pyrolaceae family of the Order Ericales

Orchidoid mycorrhizae

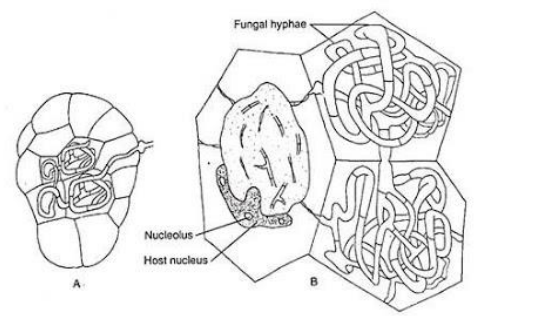


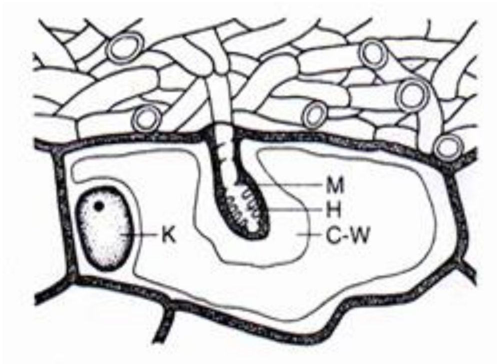
Fig. 4. 106 : Orchidoid mycorrhizae : A. Swollen embryo with initial infection, B. Cortical cells of *Dactylorchis* root showing coiled active and lysed hypae

Orchids produce millions of tiny seeds per capsule, weighing about 0.3-14µg. The embryo of seeds contains 10-100 cells and there is virtually no storage of food. The embryo is encircled in a thin-walled net-like testa that helps in their dispersal.Thus, majority of seeds are unable to germi-nate without exogenous supply of carbohydrates. Therefore, mycorrhizal association is obligatory for the seeds to germinate. The fungus provides C-nutrition to the seeds.

Initially the fungus enters the embryo and colonises, being restric-ted to the cortical cells and provides the nutrition (Fig. 4.106). For non-green orchids, this is obli-gatory throughout their lives. Apparently, it is a case of parasitism by orchids on the mycorrhizal fungi.

Fungi like *Rhizoctonia* (Basidiomycotina), are recognised by hyphal characteristics. Corticium, Ceratobasidium of Aphylloporales are associated in this type of mycorrhiza.

Monotropoid mycorrhizae



Monotropa hypopitys is a non-green saprophytic herb. It has short fleshy roots that are invested with a hyphal sheath and often forming Hartig net in the corti-cal zone. Due to absence of chlorophyll, they are unable to synthesise and supply carbohydrate to the fungus. *Boletus* is a mycorrhizal fungus associated with roots of both pine and Monotropa.

Significance of mycorrhizae association.

- Absorption of water,
- Dissolving essential minerals present in the organic debris and handing over the same to the plant. This allows the plant to grow in areas deficient in inorganic minerals,
- Absorbing inorganic salts present in the soil from over a large area. The forest trees like pines and birches are known to absorb 2-3 times more of potassium, nitrogen and phosphorus in the presence of mycorrhiza than in its absence,
- The fungus produces various growth promoting substances,
- It secretes antimicrobial substances which protect the young roots from attack of pathogens.

Role in Agriculture:

- The mycorrhizal association helps in the formation of dichotomous bran-ching and profuse root growth, thus enhances plant growth.
- Ectotrophic mycorrhiza helps in uptake of mineral ions and also acts as reservoir.
- They also help in absorption of nutri-ents.
- In nutrient deficient soil, the mycelial association helps in the absorption of N, Ca, P, Zn, Fe, Na and others.
- Mycorrhizal association is obligatory for the germination of orchid seeds.

- Mycorrhizal growth in orchids (Rhizoctonia repens with Orchis militaris tuber tissues) causes the synthesis of phytoalexins — orchinol and hirsinol. Both the compounds act as a barrier to pro-tect infection by other pathogens.
- Inoculation of VAM as biofertiliser provides a distinct possibility for the uptake of P in phosphorus-deficient soil.

BIOPESTICIDE

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. The micro-organisms used as bio-pesticides are viruses, bacteria, protozoa, fungi and mites. Some of the bio-pesticides are being used on a commercial scale.

Most important example is the soil bacterium, Bacillus thuringiensis (Bt). Spores of this bacterium possess the insecticidal Cry protein.Therefore, spores of this bacterium kill larvae of certain insects. The commercial preparations of B. thuringiensis contain a mixture of spores, Cry protein and an inert carrier.This bacterium was the first bio-pesticide to be used on a commercial scale in the world, and is the first bio-pesticide being produced on a commercial scale in India.

Bio-pesticides are of two types: bio-herbicides and bio-insecticides.

(i) Bio-herbicides:

Herbicides are chemicals that are used for inhibiting the growth of plants in unwanted places. Herbicides used for controlling weeds in the cultivated areas are called weedicides. A number of risks are involved in the use of chemical herbicides. This can be avoided if herbicide resistance can be introduced in the crop plants. It is possible through genetic engineering or recombinant DNA technology. Transgenic Tomato and Tobacco plants have been developed which show tolerance to specific herbicides.

Certain crop plants do not allow the weeds to grow nearby. They are called smoother crops, e.g., Barley, Rye, Sorghum, Millet, Sweet clover, Alfalfa, Soybean, Sunflower. Smoother crops eliminate weeds through chemicals. Crop rotation with these crops will naturally reduce the incidence of weeds.

Another way of weed control is the introduction of specific insects which feed on the weeds. Extensive growth of Opuntia in India and Australia was checked through the intro-duction of its natural herbivore, cochineal insect (Cactoblastis cactorum). Similarly, growth of Hypericum perforatum or Klamath weed was checked by U.S.A. through the introduction of Chrysolina beetles.

An organism which controls or destroys unwanted plant growth without harming the useful plant is called bioherbicide. The first bioherbicide happened to be mycoherbicide. It was put to use in 1981. The herbicide is Phytophthora palmivora. The fungus does not allow the Milkweed Vine to grow in Citrus orchards. Growth of Eichhornia crassipes (Water Hyacinth) is being controlled by Cercospora rodmanii in USA and Alternaria eichhorniae in India.

Puccinia chondrilla has controlled the growth of skeleton weed, Chondrilla juncea in Australia. Fungal spores are now available to be sprayed over weeds for their elimination. Two of them are

‘Devine’ and ‘Collego’. The spores are ideal for marketing because they can tolerate adverse conditions and can remain viable for long periods.

(ii) Bio-insecticides:

Bio-insecticides are those biological agents that are used to control harmful insects. They include the following.

(a) Predators:

Destructive insects or plant pests can be brought under control through introduction of their natural predators. The predators should be specific and unable to harm the useful insects. Introduction of ladybugs (Lady Bird Beetles) and Praying Mantis has been successful in combating scale insects or aphids which feed on plant sap.

(b) Parasites and Pathogens:

This is alternate biological control of plant pests through the search of their natural parasites and pathogens. They include viruses, bacteria, fungi and insect parasitoids. Parasitoids are organisms that live as parasites for some time (as early or larval stage) and free living at other times, e.g., Trichogramma. Nucleopolyhedrovirus (NPV) are species specific.

For example, Baculovirus heliothis (a virus) can control Cotton bollworm (Heliothis Zea). Similarly, Bacillus thuringenesis (a bacterium) is effective against the cabbage looper (Trichoplausiani) and Entomophthora ignobilis (a fungus) the green peach aphid of Potato (Myzus persicae). In U.S.S.R. the fungus Beauveria bassiana has been successfully employed in controlling Potato beetle and Codling moth.

(c) Natural Insecticides:

They are insecticides and related pesticides which are ob-tained from microbes and plants. A number of natural insecticides are available. The common ones include (i) Azadirachtin from Margosa or Neem (Azadirachta indica). It occurs in Margosa extract. Spray of the same keeps away the Japanese beetles and other leaf eating pests because of the antifeedant property of azadirachtin. (ii) Rotenones. They are powerful insecticides which are harmless to warm blooded animals. Chinese are believed to be first to discover their insecticidal properties. Rotenones are obtained from the roots of Derris elliptica and Lonchocarpus nicou. (iii) Squill. The red variety of Sea Onion (Red Squill, Ureginea maritima) produces a radicide which does not have any harmful effect on other animals, (iv) Nicotine. It is obtained from Nicotiana species. The purified chemical is highly poisonous. Nicotine sulphate is one of the most toxic insecticides, (v) Pyrethrum.

It is an insecticide which is obtained from the inflorescence of Chrysanthemum cinerarifolium (Dalmation Pyrethrum), C. coccineum and C. marshallii. The active compounds are pyrethrin and cinerin. Pyrethrin is also used in fly sprays, aerosols, mosquito coils, etc. (vi) Thurioside. It is a toxin produced by bacterium Bacillus thuringenesis. The toxin is highly effective against different groups of insects like moths, flies, mosquitoes and beetles. It does not cause any adverse environmental pollution or disturbance.

Thurioside occurs as crystals in the bacterium. It kills the susceptible insects through inhibiting ion transport in the midgut, formation of pores in gut epithelium, swelling and bursting of cells, (vii) Transgenic Plants. They are crop plants which are modified through genetic engineering to develop natural resistance to insects by inserting cry genes of Bacillus thuringenesis into them, e.g., Bt Cotton. Similarly, transgenic Tomato has been developed which is resistant to homworm larvae.

BIOFUEL PRODUCTION

A biofuel is a fuel that is produced through contemporary processes from biomass, rather than a fuel produced by the very slow geological processes involved in the formation of fossil fuels, such as oil. Since biomass technically can be used as a fuel directly (e.g. wood logs), some people use the terms biomass and biofuel interchangeably**.**

The word biofuel is usually reserved for liquid or gaseous fuels, used for transportation. The U.S. Energy Information Administration (EIA) follows this naming practice. Drop-in biofuels are functionally equivalent to petroleum fuels and fully compatible with the existing petroleum infrastructure. They require no engine modification of the vehicle.

Biofuels can be produced from plants (i.e. energy crops), or from agricultural, commercial, domestic, and/or industrial wastes (if the waste has a biological origin). Renewable biofuels generally involve contemporary carbon fixation, such as those that occur in plants or microalgae through the process of photosynthesis. If the biomass used in the production of biofuel can regrow quickly, the fuel is generally considered to be a form of renewable energy. The greenhouse gas mitigation potential of biofuel varies considerably, from emission levels comparable to fossil fuels in some scenarios to negative emissions in others.

The two most common types of biofuel are bioethanol and biodiesel.

Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn, sugarcane, or sweet sorghum. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form (E100), but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Bioethanol is widely used in the United States and in Brazil.

Biodiesel is produced from oils or fats using transesterification and is the most common biofuel in Europe. It can be used as a fuel for vehicles in its pure form (B100), but it is usually used as a diesel additive to reduce levels of particulates, carbon monoxide, and hydrocarbons from diesel-powered vehicles.

In 2019, worldwide biofuel production reached 161 billion liters (43 billion gallons US), up 6% from 2018, and biofuels provided 3% of the world's fuels for road transport. The International Energy Agency want biofuels to meet more than a quarter of world demand for transportation fuels by 2050, in order to reduce dependency on petroleum. However, the production and consumption of biofuels are not on track to meet the IEA's sustainable development scenario. From 2020 to 2030 global biofuel output has to increase by 10% each year to reach IEA's goal. Only 3% growth annually is expected the next 5 years.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – V– Environmental Biotechnology – SBTA1304

BIODEGRADATION

Biodegradation or biological degradation is the phenomenon of biological transformation of organic compounds by living organisms, particularly the microorganisms.

Biodegradation basically involves the conversion of complex organic molecules to simpler (and mostly non-toxic) ones. The term biotransformation is used for incomplete biodegradation of organic compounds involving one or a few reactions. Biotransformation is employed for the synthesis of commercially important products by microorganisms.

Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc. The microbes serve as scavengers in bioremediation. The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation. The other names used (by some authors) for bioremediation are bio-treatment, bio-reclamation and bio-restoration.

It is rather difficult to show any distinction between biodegradation and bioremediation. Further, in biotechnology, most of the reactions of biodegradation/bioremediation involve xenobiotic.

Xenobiotic:

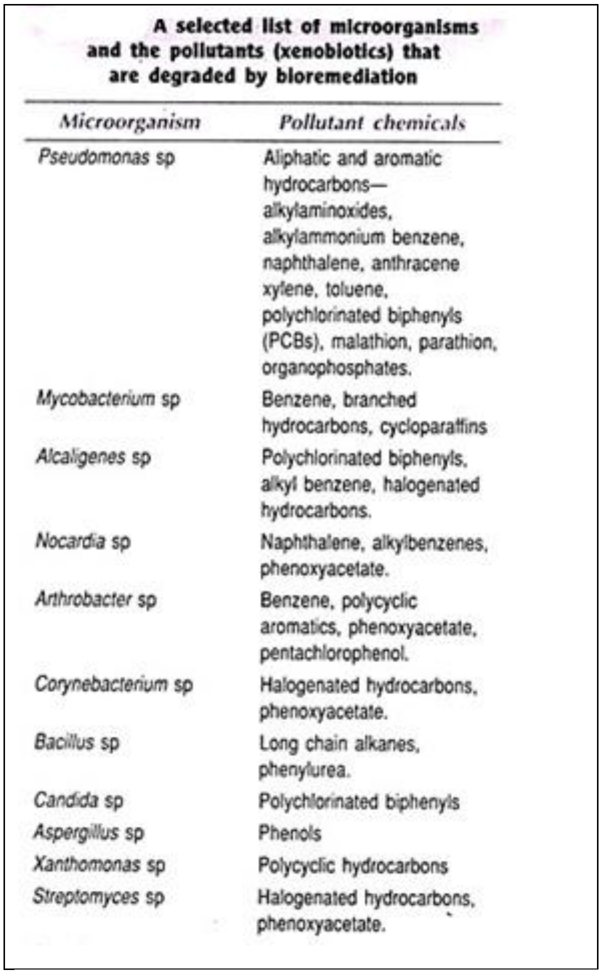
Xenobiotic (xenos-foregin) broadly refer to the unnatural, foreign and synthetic chemicals such as pesticides, herbicides, refrigerants, solvents and other organic compounds. Microbial degradation of xenobiotic assumes significance, since it provides an effective and economic means of disposing of toxic chemicals, particularly the environmental pollutants.

Pseudomonas — The Predominant Microorganism For Bioremediation:

Members of the genus Pseudomonas (a soil microorganism) are the most predominant microorganisms that degrade xenobiotic. Different strains of Pseudomonas, that are capable of detoxifying more than 100 organic compounds, have been identified. The examples of organic compounds are several hydrocarbons, phenols, organophosphates, polychlorinated biphenyls (PCBs) and polycylic aromatics and naphthalene.

About 40-50 microbial strains of micro-organisms, capable of degrading xenobiotics have been isolated. Besides Pseudomonas, other good examples are Mycobacterium, Alcaligenes, and Nocardia. A selected list of microorganisms and the xenobiotics degraded is given in Table

List of Microorganisms and the Pollutants



A selected list of microorganisms and the pollutants (xenobiotics) that are degraded by bioremediation

Microorganism	*Pollutant chemicals*
Pseudomonas sp	Aliphatic and aromatic hydrocarbons—alkylaminoxides, alkylammonium benzene, naphthalene, anthracene xylene, toluene, polychlorinated biphenyls (PCBs), malathion, parathion, organophosphates.
Mycobacterium sp	Benzene, branched hydrocarbons, cycloparaffins
Alcaligenes sp	Polychlorinated biphenyls, alkyl benzene, halogenated hydrocarbons.
Nocardia sp	Naphthalene, alkylbenzenes, phenoxyacetate.
Arthrobacter sp	Benzene, polycyclic aromatics, phenoxyacetate, pentachlorophenol.
Corynebacterium sp	Halogenated hydrocarbons, phenoxyacetate.
Bacillus sp	Long chain alkanes, phenylurea.
Candida sp	Polychlorinated biphenyls
Aspergillus sp	Phenols
Xanthomonas sp	Polycyclic hydrocarbons
Streptomyces sp	Halogenated hydrocarbons, phenoxyacetate.

Consortia of microorganisms for biodegradation:

A particular strain of microorganism may degrade one or more compounds. Sometimes, for the degradation of a single compound, the synergetic action of a few microorganisms (i.e. a consortium or cocktail of microbes) may be more efficient. For instance, the insecticide parathion is more efficiently degraded by the combined action of Pseudomonas aeruginosa and Psudomonas stulzeri.

Co-metabolism in biodegradation:

In general, the metabolism (breakdown) of xenobiotics is not associated with any advantage to the microorganism. That is the pollutant chemical cannot serve as a source of carbon or energy for the organism. The term co-metabolism is often used to indicate the non-beneficial (to the microorganism) biochemical pathways concerned with the biodegradation of xenobiotics. However, co- metabolism depends on the presence of a suitable substrate for the microorganism. Such compounds are referred to co-substrates.

Factors Affecting Biodegradation:

Several factors influence biodegradation. These include the chemical nature of the xenobiotic, the capability of the individual microorganism, nutrient and O2 supply, temperature, pH and redox potential. Among these, the chemical nature of the substrate that has to be degraded is very important.

Some of the relevant features are given hereunder:

i. In general, aliphatic compounds are more easily degraded than aromatic ones.

ii. Presence of cyclic ring structures and length chains or branches decrease the efficiency of biodegradation.

iii. Water soluble compounds are more easily degraded.

iv. Molecular orientation of aromatic compounds influences biodegradation i.e. ortho > para > meta.

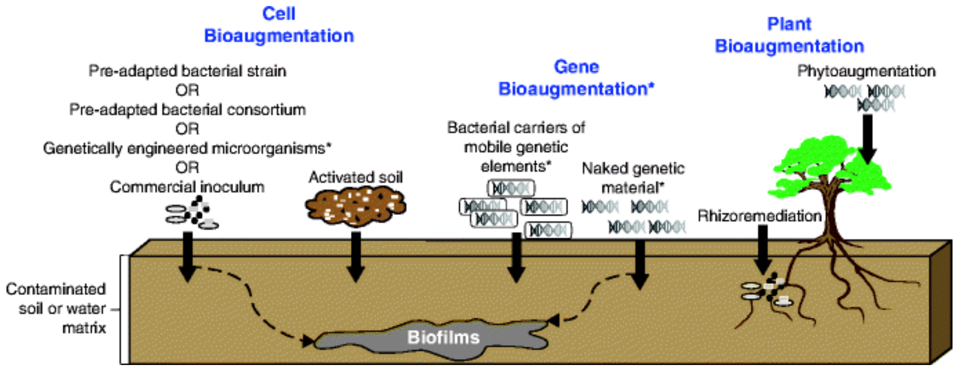
v. The presence of halogens (in aromatic compounds) inhibits biodegradation.

Besides the factors listed above, there are two recent developments to enhance the biodegradation by microorganisms.

Bio-stimulation:

This is a process by which the microbial activity can be enhanced by increased supply of nutrients or by addition of certain stimulating agents (electron acceptors, surfactants).

Bio-augmentation:



It is possible to increase biodegradation through manipulation of genes. More details on this genetic manipulation i.e. genetically engineered microorganisms (GEMs), are described later. Bio-augmentation can also be achieved by employing a consortium of micro-organisms.

Enzyme Systems for Biodegradation:

Several enzyme systems (with independent enzymes that work together) are in existence in the microorganisms for the degradation of xenobiotics. The genes coding for the enzymes of bio-degradative pathways may be present in the chromosomal DNA or more frequently on the plasmids. In certain microorganisms, the genes of both chromosome and plasmid contribute for the enzymes of biodegradation. The microorganism Pseudomonas occupies a special place in biodegradation.

List of Xenobiotics and Plasmids

Xenobiotic	Name of plasmid in Pseudomonas
Naphthalene	NAH
Xylene	XYL
Xylene and toluene	TOL, pWWO, XYL-K
Salicylate	SAL
Camphor	CAM
3-Chlorobenzene	pAC25

Recalcitrant Xenobiotics:

There are certain compounds that do not easily undergo biodegradation and therefore persist in the environment for a long period (sometimes in years). They are labeled as recalcitrant.

There may be several reasons for the resistance of xenobiotics to microbial degradation:

i. They may chemically and biologically inert (highly stable).

ii. Lack of enzyme system in the microorganisms for biodegradation.

iii. They cannot enter the microorganisms being large molecules or lack of transport systems.

iv. The compounds may be highly toxic or result in the formation highly toxic products that kill microorganisms.

There are a large number of racalcitrant xenobiotic compounds e.g. chloroform, freons, insecticides (DDT, lindane), herbicides (dalapon) and synthetic polymers (plastics e.g. polystyrene, polyethylene, polyvinyl chlorine).

It takes about 4-5 years for the degradation of DDT (75-100%) in the soil. A group of microorganisms (Aspergillus flavus, Mucor aternans, Fusarium oxysporum and Trichoderma viride) are associated with the slow biodegradation of DDT.

BIO-MAGNIFICATION:

The phenomenon of progressive increase in the concentration of a xenobiotic compound, as the substance is passed through the food chain is referred to as bio-magnification or bioaccumulation. For instance, the insecticide DDT is absorbed repeatedly by plants and microorganism.

When they are eaten by fish and birds, this pesticide being recalcitrant, accumulates, and enters the food chain. Thus, DDT may find its entry into various animals, including man. DDT affects the nervous systems, and it has been banned in some countries.

TYPES OF BIOREMEDIATION:

The most important aspect of environmental biotechnology is the effective management of hazardous and toxic pollutants (xenobiotics) by bioremediation. The environmental clean-up process through bioremediation can be achieved in two ways—in situ and ex situ bioremediation.

IN SITU BIOREMEDIATION:

In situ bioremediation involves a direct approach for the microbial degradation of xenobiotics at the sites of pollution (soil, ground water). Addition of adequate quantities of nutrients at the sites promotes microbial growth. When these microorganisms are exposed to xenobiotics (pollutants), they develop metabolic ability to degrade them.

The growth of the microorganisms and their ability to bring out biodegradation are dependent on the supply of essential nutrients (nitrogen, phosphorus etc.). In situ bioremediation has been successfully applied for clean-up of oil spillages, beaches etc. There are two types of in situ bioremediation-intrinsic and engineered.

Intrinsic bioremediation:

The inherent metabolic ability of the microorganisms to degrade certain pollutants is the intrinsic bioremediation. In fact, the microorganisms can be tested in the laboratory for their natural capability of biodegradation and appropriately utilized.

Engineered in situ bioremediation:

The inherent ability of the microorganisms for bioremediation is generally slow and limited. However, by using suitable physicochemical means (good nutrient and O_2 supply, addition of electron acceptors, optimal temperature), the bioremediation process can be engineered for more efficient degradation of pollutants.

Advantages of in situ bioremediation:

1. Cost-effective, with minimal exposure to public or site personnel.
2. Sites of bioremediation remain minimally disrupted.

Disadvantages of in situ bioremediation:

1. Very time consuming process.
2. Sites are directly exposed to environmental factors (temperature, O_2 supply etc.).
3. Microbial degrading ability varies seasonally.

EX SITU BIOREMEDIATION:

The waste or toxic materials can be collected from the polluted sites and the bioremediation with the requisite microorganisms (frequently a consortium of organisms) can be carried out at designed places. This process is certainly an improvement over in situ bioremediation, and has been successfully used at some places.

Advantages of ex situ bioremediation:

1. Better controlled and more efficient process.
2. Process can be improved by enrichment with desired microorganisms.
3. Time required in short.

Disadvantages of ex situ bioremediation:

1. Very costly process.
2. Sites of pollution are highly disturbed.
3. There may be disposal problem after the process is complete.

Metabolic Effects of Microorganisms on Xenobiotics:

Although it is the intention of the biotechnologist to degrade the xenobiotics by microorganisms to the advantage of environment and ecosystem, it is not always possible. This is evident from the different types of metabolic effects as shown below.

Detoxification:

This process involves the microbial conversion of toxic compound to a non-toxic one. Biodegradation involving detoxification is highly advantageous to the environment and population.

Activation:

Certain xenobiotics which are not toxic or less toxic may be converted to toxic or more toxic products. This is dangerous.

Degradation:

The complex compounds are degraded to simpler products which are generally harmless.

Conjugation:

The process of conjugation may involve the conversion of xenobiotics to more complex compounds. This is however, not very common.

Types of Reactions in Bioremediation:

Microbial degradation of organic compounds primarily involves aerobic, anaerobic and sequential degradation.

Aerobic bioremediation:

Aerobic biodegradation involves the utilization of O2 for the oxidation of organic compounds. These compounds may serve as substrates for the supply of carbon and energy to the microorganisms. Two types of enzymes namely mono-oxygenases and- di-oxygenases are involved in aerobic biodegradation. Mono-oxygenases can act on both aliphatic and aromatic compounds while di-oxygenases oxidize aliphatic compounds.

Anaerobic bioremediation:

Anaerobic biodegradation does not require O2 supply. The growth of anaerobic microorganisms (mostly found in solids and sediments), and consequently the degradation processes are slow. However, anaerobic biodegradation is cost- effective, since the need for continuous O2 supply is not there. Some of the important anaerobic reactions and examples of organic compounds degraded are listed below.

Hydrogenation and dehydrogenation — benzoate, phenol, catechol.

Dehaiogenation — Polychlorinated biphenyls (PCBs), chlorinated ethylene's. The term de-chlorination is frequently used for dehaiogenation of chlorinated compounds.

Carboxylation and decarboxylation — toluene, cresol and benzoate.

Sequential Bioremediation:

In the degradation of several xenobiotics, both aerobic and anaerobic processes are involved. This is often an effective way of reducing the toxicity of a pollutant. For instance, tetra chloromethane and tetrachloroethane undergo sequential degradation.

Biodegradation of Hydrocarbons:

Hydrocarbon are mainly the pollutants from oil refineries and oil spills. These pollutants can be degraded by a consortium or cocktail of microorganisms e.g. Pseudomonas, Corynebacterium, Arthrobacter, Mycobacterium and Nocardia.

Biodegradation of Aliphatic Hydrocarbons:

The uptake of aliphatic hydrocarbons is a slow process due to their low solubility in aqueous medium. Both aerobic and anaerobic processes are operative for the degradation of aliphatic hydrocarbons. For instance, unsaturated hydrocarbons are degraded in both anaerobic and aerobic environments, while saturated ones are degraded by aerobic process. Some aliphatic hydrocarbons which are reclacitrant to aerobic process are effectively degraded in anaerobic environment e.g. chlorinated aliphatic compounds (carbon tetrachloride, methyl chloride, vinyl chloride).

Biodegradation of Aromatic Hydrocarbons:

Microbial degradation of aromatic hydrocarbons occurs through aerobic and anaerobic processes. The most important microorganism that participates in these processes is Pseudomonas.

The biodegradation of aromatic compounds basically involves the following sequence of reactions:

1. Removal of the side chains.

2. Opening of the benzene ring.

Most of the non-halogenated aromatic compounds undergo a series of reactions to produce catechol or protocatechuate. The bioremediation of toluene, L-mandelate, benzoate, benzene, phenol, anthracene, naphthalene, phenanthrene and salicylate to produce catechol is shown in Fig. 59.1. Likewise, Fig. 59.2, depicts the bioremediation of quinate, p-hydroxymandelate, p-hydroxybenzoyl formate, p-toluate, benzoate and vanillate to produce protocatechuate.

Catechol and protocatechuate can undergo oxidative cleavage pathways. In ortho-cleavage pathway, catechol and protocatechuate form acetyl CoA (Fig. 59.3), while in meta-cleavage pathway (Fig. 59.4), they are converted to pyruvate and acetaldehyde. The degraded products of catechol and protocatechuate are readily metabolised by almost all the organisms.

Biodegradation of Pesticides and Herbicides:

Pesticides and herbicides are regularly used to contain various plant diseases and improve the crop yield. In fact, they are a part of the modern agriculture, and have significantly contributed to green revolution. The common herbicides and pesticides are propanil (anilide), propham (carbamate), atrazine (triazine), picloram (pyridine), dichlorodiphenyl trichloroethane (DDT) monochloroacetate (MCA), monochloropropionate (MCPA) and glyphosate (organophosphate). Most of the pesticides and herbicides are toxic and are recalcitrant (resistant to biodegradation). Some of them are surfactants (active on the surface) and retained on the surface of leaves.

Biodegradation of Halogenated Aromatic Compounds:

Most commonly used herbicides and pesticides are aromatic halogenated (predominantly chlorinated) compounds. The bio-degradative path-ways of halogenated compounds are comparable with that described for the degradation of non-halogenated aromatic compounds (Figs. 59.1, 59.2, 59.3 and 59.4). The rate of degradation of halogenated compounds is inversely related to the number of halogen atoms that are originally present on the target molecule i.e. compounds with higher number of halogens are less readily degraded.

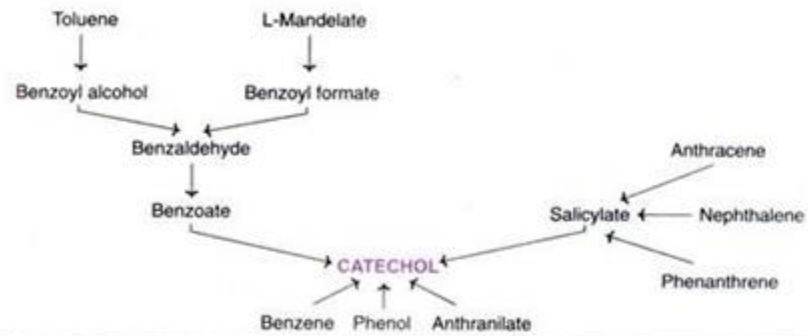


Fig. 59.1 : Bioremediation of certain aromatic compounds by bacteria to produce catechol.

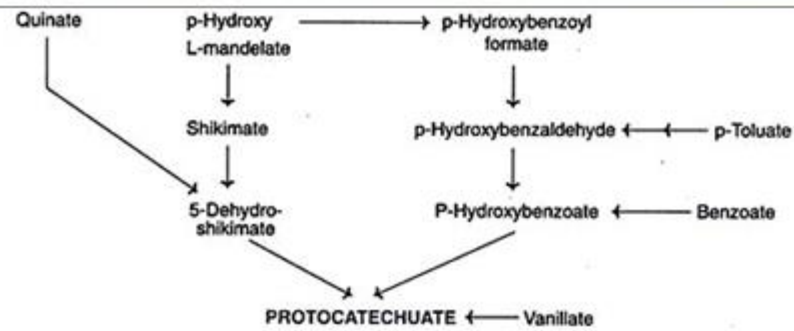
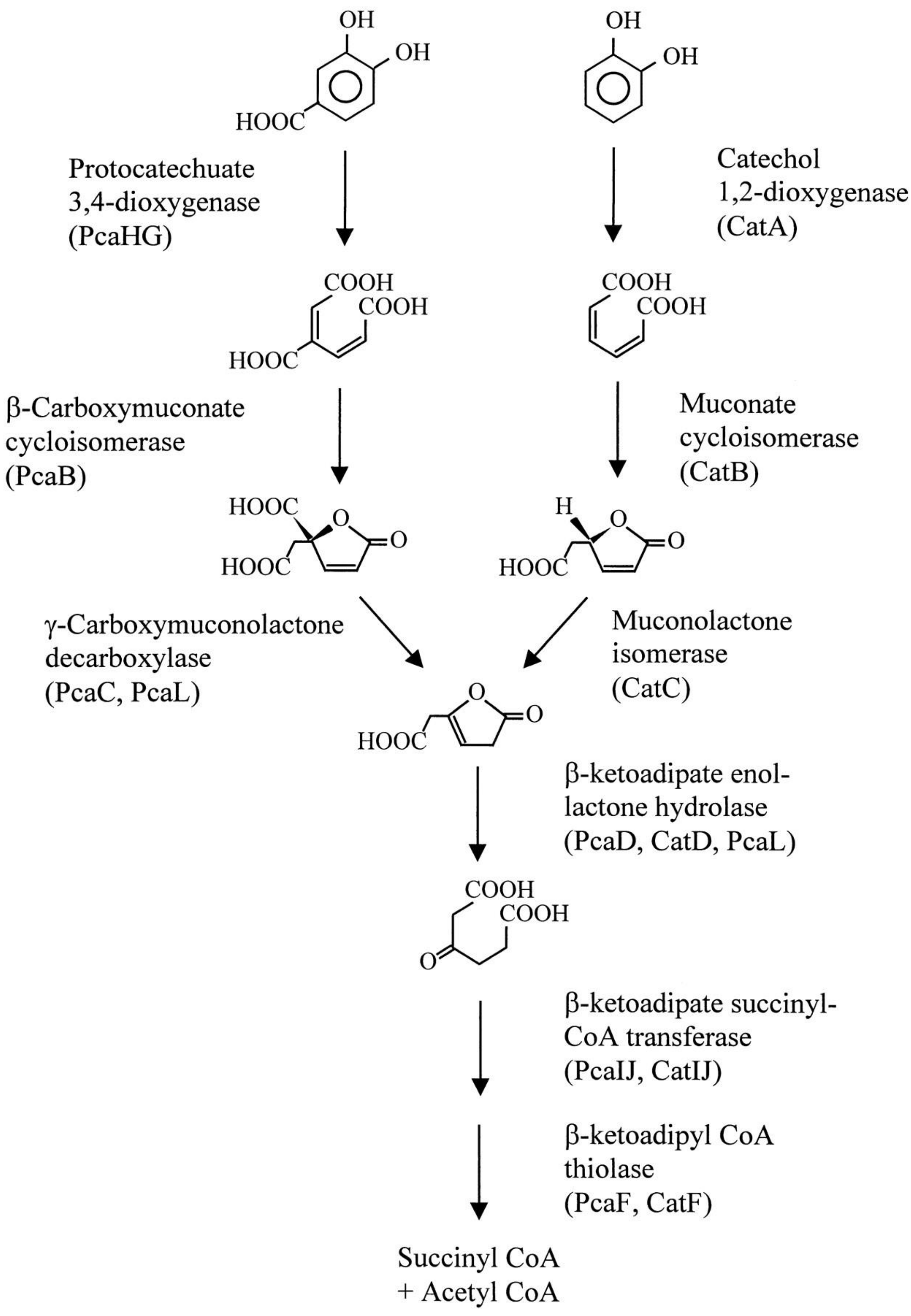


Fig. 59.2 : Bioremediation of certain organic compounds by bacteria to produce protocatechuate.

Bioremediation of Certain Aromatic Compounds Bioremediation of Certain Organic Compounds

Conversion of Catechol and Protocatechuateto Acetyl CoA and Succinate



Conversion of Catechol and Protocatechuate

Dehalogenation (i.e. removal of a halogen substituent from an organic compound) of halogenated compounds is an essential step for their detoxification. Dehalogenation is frequently catalysed by the enzyme di-oxygenase. In this reaction, there is a replacement of halogen on benzene with a hydroxyl group.

Most of the halogenated compounds are also converted to catechol and protocatechuate which can be metabolise. Besides Pseudomonas, other microorganisms such as Azotobacter, Bacilluefs and E. coli are also involved in the microbial degradation of halogenated aromatic compounds.

Biodegradation of Polychlorinated Biphenyls (PCBs):
The aromatic chlorinated compounds possessing biphenyl ring (substituted with chlorine) are the PCBs e.g. pentachlorobiphenyl. PCBs are commercially synthesized, as they are useful for various purposes — as pesticides, in electrical conductivity (in transformers), in paints and adhesives. They are inert, very stable and resistant to corrosion.

However, PCBs have been implicated in cancer, damage to various organs and impaired reproductive function. Their commercial use has been restricted in recent years, and are now used mostly in electrical transformers.

PCBs accumulate in soil sediments due to hydrophobic nature and high bioaccumulation potential. Although they are resistant to biodegradation, some methods have been recently developed for anaerobic and aerobic oxidation by employing a consortium of microorganisms. Pseudomonas, Alkali genes, Corynebacterium and Acinetobacter. For more efficient degradation of PCBs, the microorganisms are grown on biphenyls, so that the enzymes of biodegradation of PCBs are induced.

Biodegradation of Some Other Important Compounds:

Organo-nitro Compounds:

Some of the toxic organo-nitro compounds can be degraded by microorganisms for their detoxification.2, 4, 6-Trinitrotoluene (TNT):

Certain bacterial and fungal species belonging to Pseudomonas and Clostrium can detoxify TNT.

Synthetic detergents:

They contain some surfactants (surface active agents) which are not readily biodegradable. Certain bacterial plasmid can degrade surfactants.

Genetic Engineering for More Efficient Bioremediation:

Although several microorganisms that can degrade a large number of xenobiotics have been identified, there are many limitations in bio-remediation:

i. Microbial degradation of organic compounds is a very slow process.

ii. No single microorganism can degrade all the xenobiotics present in the environmental pollution.

iii. The growth of the microorganisms may be inhibited by the xenobiotics.

iv. Certain xenobiotics get adsorbed on to the particulate matter of soil and become unavailable for microbial degradation.

It is never possible to address all the above limitations and carry out an ideal process of bioremediation. Some attempts have been made in recent years to create genetically engineered microorganisms (CEMs) to enhance bio-remediation, besides degrading xenobiotics which are highly resistant (recalcitrant) for breakdown. Some of these aspects are briefly described.

Genetic Manipulation by Transfer of Plasmids:

The majority of the genes responsible for the synthesis of bio-degradative enzymes are located on the plasmids. It is therefore logical to think of genetic manipulations of plasmids. New strains of bacteria can be created by transfer of plasmids (by conjugation) carrying genes for different degradative pathways.

If the two plasmids contain homologous regions of DNA, recombination occurs between them, resulting in the formation of a larger fused plasmid (with the combined functions of both plasmids). In case of plasmids which do not possess homologous regions of DNA, they can coexist in the bacterium (to which plasmid transfer was done).

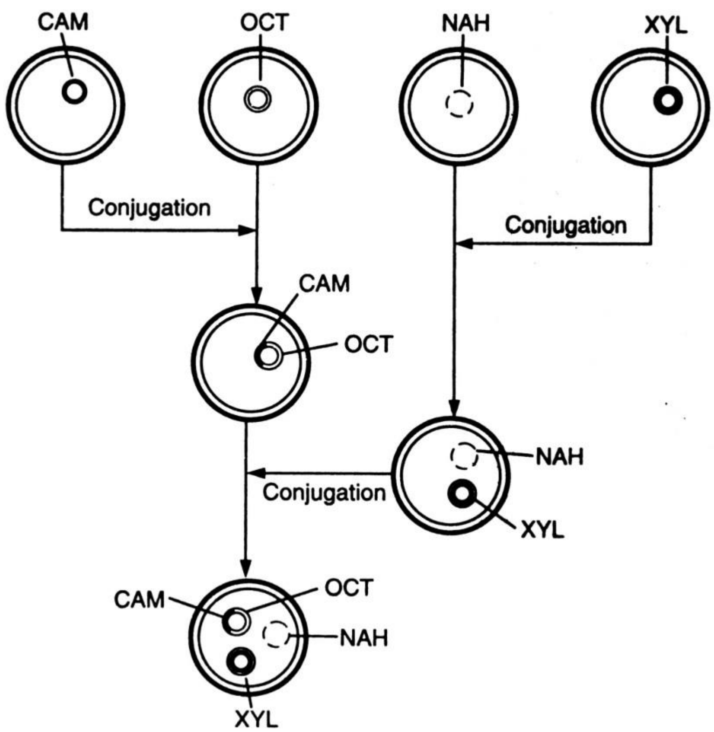
The first successful development of a new strain of bacterium (Pseudomonas) by manipulations of plasmid transfer was done by Chakrabarty and his co-workers in 1970s. They used different plasmids and constructed a new bacterium called as superbug that can degrade a number of hydrocarbons of petroleum simultaneously.

United States granted patent to this superbug in 1981 (as per the directive of American Supreme Court). Thus, superbug became the first genetically engineered microorganism to be patented. Superbug has played a significant role in the development of biotechnology industry, although it has not been used for large scale degradation of oil spills.

Creation of Superbug by Transfer of Plasmids:

Superbug is a bacterial strain of Pseudomonas that can degrade camphor, octane, xylene and naphthalene.

Creation of the Superbug



The bacterium containing CAM (camphor- degrading) plasmid was conjugated with another bacterium with OCT (octane-degrading) plasmid. These plasmids are not compatible and therefore, cannot coexist in the same bacterium. However, due to the presence of homologous regions of DNA, recombination occurs between these two plasmids resulting in a single CAM-OCT plasmid. This new bacterium possesses the degradative genes for both camphor and octane.

Another bacterium with XYL (xylene-degrading) plasmid is conjugated with NAH (naphthalene-degrading) plasmid containing bacterium. XYL and NAH plasmids are compatible and therefore can coexist in the same bacterium. This newly, produced bacterium contains genes for the degradation of xylene and naphthalene.

The next and final step is the conjugation of bacterium containing CAM-OCT plasmid with the other bacterium containing XYL and NAH plasmids. The newly created strain is the superbug that carries CAM-OCT plasmid (to degrade camphor and octane), XYL (xylene-degrading) plasmid and NAH (naphthalene-degrading) plasmid.

Development of Salicylate—Toluene Degrading Bacteria by Plasmid Transfer:

Some attempts have been made for the creation of a new strain of the bacterium Pseudomonas putida to simultaneously degrade toluene and salicylate. Toluene-degrading (TOL) plasmid was transferred by conjugation to another bacterium that is capable of degrading salicylate (due to the presence of SAL plasmid).

The newly developed strain of Pseudomonas can simultaneously degrade both toluene and salicylate. And this occurs even at a low temperature (0-5°C). However, the new bacterium is not in regular use, as more research is being conducted on its merits and demerits.

Genetic Manipulation by Gene Alteration:

Work is in progress to manipulate the genes for more efficient biodegradation. The plasmid pWWO of Pseudomonas codes for 12 different enzymes responsible for the meta-cleavage pathway (for the conversion of catechol and protocatechuate to pyruvate and acetaldehyde, for degradation of certain aromatic compounds. Some success has been reported to alter the genes of plasmid pWWO for more efficient degradation of toluene and xylene.

Genetically Engineered Microorganisms (GEMs) in Bioremediation:

Superbug is the first genetically engineered microorganism. Several workers world over have been working for the creation of GEMs, specifically designed for the detoxification of xenobiotics. Almost all these CFMs have been created by transferring plasmids.

Bio-surfactant Producing GEM:

A genetically engineered Pseudomonas aeruginosa has been created (by Chakarabarty and his group). This new strain can produce a glycolipid emulsifier (a bio-surfactant) which can reduce the surface tension of an oil water interface. The reduced interfacial tension promotes biodegradation of oils.