Introduction:

Among the various subfields within biotechnology, the one dealing with environmental problems which is called environmental biotechnology. Environmental biotechnologies are competing with great success against traditional techniques and are providing solution to acute environmental problems, managing, preserving and restoring the environmental quality. Biotechnological methodologies can be suitably, transformation of pollutants into harmless substances, generate biodegradable materials from renewable resources and develop ecofriendly manufacturing and disposal processes. All these aspects come under the purview of environmental biotechnology.

Environmental biotechnology in a broad term that: refers to techniques that use biological system, living organisms, or derivatives for remediation of contaminated environment (land, water, and air). Produce beneficial products of human need, benefits in health care, and in the treatment of municipal, agriculture and industrial wastes.

Environmental biotechnology very relevant field dealing with:

- Developing techniques which would consume fewer resources include recycling and reuse of component and reduce production of wastes.
- Treatment of organic and inorganic wastes. This aims to minimization of pollution load in the environment, restoration environmental quality, using different biological processes.
- Application updated information of bioremediation technologies for removal of recalcitrant compounds resulted from municipal, agriculture and industrial effluents.
- Application of different preventive approach to industries leading to cleaner manufacturing technology.
- Immobilization of living organisms, bacteria, fungi, and algae and their use for organic and inorganic material removal from waste.
- Technology include the cleanup of water and land areas polluted with petroleum product through (genetically upgraded bacterial species), rehabilitation of polluted aquatic reservoirs and land areas fouled by pollutants, including toxic metals from industrial effluents.
- Environmental biotechnology has provided better understanding the concept of biomethylation of toxic metals, their effect and biomagnifications.

Biomethylation: formation of organo-metalic complexes in the environment due to microbial action and metabolism, such conversion of:
Hg (inorganic) → CH3-Hg (organic Hg in the body of the organisms) could be more toxic than the inorganic form of mercury.

Biomagnifications: In this process the concentration of some toxicants gets magnified several folds in organisms through the food chain than their concentration in the environment e.g. DDT concentration in the dolphins is several (1000) times more than in the sea water.
Pollutants → Phytoplankton and plant → Fishes → higher animals.
(DDT,Cd,Hg)

Important terms in Environmental Biotechnology

Adhesions: Microbial surface antigens, often in the form of filamentous pili or proteins that bind one cell to another.

Alkane: Referring to saturated hydrocarbons with carbon atoms in a chain without double bonds
Alkene: Referring to unsaturated hydrocarbons with carbon atoms in a chain containing double bonds between the carbon atoms.

Anoxygenic: Referring to activity that contributes to the anaerobic environment.

Assimilation: The incorporation of compounds into cellular materials.

Augmentation: With respect to bioremediation, the addition of desired bacteria to a bioreactor or to a contaminated site.

Bacteriophages: Viruses that attack bacteria.

Biodiesel: An extract of algal cells containing oils; suitable for use in engines.

Biofarming: The addition of contaminated soil to agricultural soil with the purpose of soil microorganisms mineralizing the organic contaminant.

Biofilm: Film containing microbial cells of diverse genera that are localized on a surface by extracellular matrix material.

Biofuel: A biological product (ethanol, methane, H_2, etc.) that can be used as an engines fuel.

Biogeochemical cycle: The path that a nutrient or element takes as it moves through the biosphere, hydrosphere, lithosphere, and atmosphere.

Biomineralization: The process by which microorganisms form mineral phases.

Biomining: The use of microorganisms to aid in the extraction and recovery of metals from ores.

Bioremediation: The application of microorganisms (or biological material) to detoxify organic substances or inorganic compounds.

Biosorption: Metablism-independent binding of metal ions or radionuclide species to cellular components.

Cellulose: A biopolymer that consists of several dozen chains of microfibrils where each chain of glucose is held by **j3**-1,4-glucosidic bonds.

Chemoautotrophy: The process in which carbon dioxide is used as the source of carbon.

Chemolithotrophs: Microorganisms that couple electron flow to oxidation or reduction of inorganic materials.

Chemolithotrophy: The process in which inorganic compounds are oxidized to generate energy for organisms.

Chemoorganotrophs: Organisms that utilize organic compounds as their energy sources.

Compost: A process using aerobic microbial decomposition of plant material for the production of a soil conditioner.

Conjugation: The genetic exchange resulting from cell-cell contact; occurs in both prokaryotic and eukaryotic microorganisms.

Dehydrogenase: An enzyme that oxidizes molecules by transferring electrons to an electron carrier of NAD or cytochromes.

Denitrification: The conversion of nitrate to atmospheric nitrogen.

Dinitrogen: Atmospheric nitrogen, N_2.

Dissimilation: Activity leading to the conversion of an electron acceptor to a metabolic end product; not associated with incorporation of chemicals into cell biomass.

Dissimilatory reduction: In microbiology, the transfer of a large number of electrons to an electron acceptor with the consequence of producing a high quantity of product from respiration.

Dissimilatory sulfate reduction: The use of sulfate as the final electron acceptor by chemolithotrophic organisms with the production of H_2S.

Disturbance: An event that causes the death, displacement, or harm of or to individuals within a given population, community, or ecosystem; leads to opportunities for new individuals to replace them.

Extracellular polymeric matrix (EPM): Polysaccharide material surrounding bacterial cells along with other polymeric material.

Extremophiles: Organisms that live in and have adapted to extreme conditions of pH, temperature, or salinity.

Fermentation: An anaerobic metabolic process of bacteria and yeast resulting in the production of desired end products including ethanol and lactic acid.
Indigenous bacteria: Bacteria normally present in the environment.
Methanotroph; A microorganism that grows with methane as the electron donor.
nitrogen fixation: Also called **diazotrophy**; the process of reducing atmospheric nitrogen to ammonia, carried out by various bacteria and archaea in order to supply nitrogen for building proteins and nucleic acids.
Nitrogenase: The enzyme that converts atmospheric nitrogen to ammonia.
Rhizosphere: The area of soil surrounding plant roots.
Siderophores: Small organic compounds produced by bacteria or fungi; these compounds facilitate cellular uptake of $Fe^{3}+$.
Sludge: Solid material containing a high concentration of microorganisms, inorganic precipitates, and undigested organic solids.
Sorption: A term used to include adsorption and absorption; a process where a chemical moves from a soluble phase to an insoluble phase.
Xenobiotic: A chemical produced in the laboratory and not produced by any living system.

Biological Treatment Process:

A process consists of the application of a controlled natural process in which microorganisms remove soluble and colloidal organic matters from the waste water and are in turn removed them selves.

Most domestic and industrial effluents are heterogeneous, and contain both dissolved and suspended matters, both organic and inorganic. Treatment of such effluent or wastewater involves the removal of contaminants to prevent any adverse effects on the receiving water or allow its reuse.

Three treatment processes are usually fallowed:

1. Primary treatment: This is essentially a physio-chemical process where sedimentation, chemical coagulation and precipitation are fallowed to remove the coarse and fine suspended solids.
2. Secondary treatment: The process involves the removal of colloidal and dissolved organic substances and some toxic chemicals. It is essentially a biological treatment process.
3. Tertiary treatment: This is also called advanced treatment, where further purification is needed depending on the reuse of that water. At this stage, excess nutrients like nitrogen, phosphorous and toxic elements like metals are removed.

Biological treatment or secondary treatment mainly based on: The catabolic activities of microbes including bacteria, algae, fungi, protozoa, rotifers and nematodes.

Principle requirements of secondary treatment include: adequate supply of microbes, ensuring the contact of bacteria with influent, and oxygen availability.

Secondary treatment or biotreatment usually applied in: Controlled environment or bioreactor (offers possibility of shortening the treatment time). Although there could be natural system of treatment in lagoons and stabilization ponds, using both the microbes and higher plants.

Techniques used in biological treatment:

Two techniques used in this application with the same principles:

1. Attached film growth → like trickling filter.
2. Suspended growth process → like activated sludge.

Suspended growth process (Activated sludge):

Principles of suspended growth:

- Raw wastewater flowing into the biological reactor (aerated tank) containing organic matter (BOD) as a food supply.
- Bacteria metabolize the waste solids, producing new growth while taking in dissolved oxygen and releasing CO_2.
- Protozoa graze on bacteria for energy to reproduce. Some of the new microbial growth dies releasing cell contents, to solution for resynthesis.
- After the addition of a large population of microorganisms, aerating raw wastewater for a few hours removes organic matter from solution by synthesis into microbial cells.
- Mixed liquor (ML), is continuously transferred to a clarifier for gravity separation of the biological floc and discharge of the calrifiered effluent.
- Settled floc (activated sludge) is returned continuously to the aeration basin for mixing with entering raw waste as a seed or inoculums (Figure 1).

Mixed liquor (ML): is the liquid suspension of microorganisms in an aeration basin.

Mixed liquor suspended solids (MLSS): is the biological growth in the aeration basin,

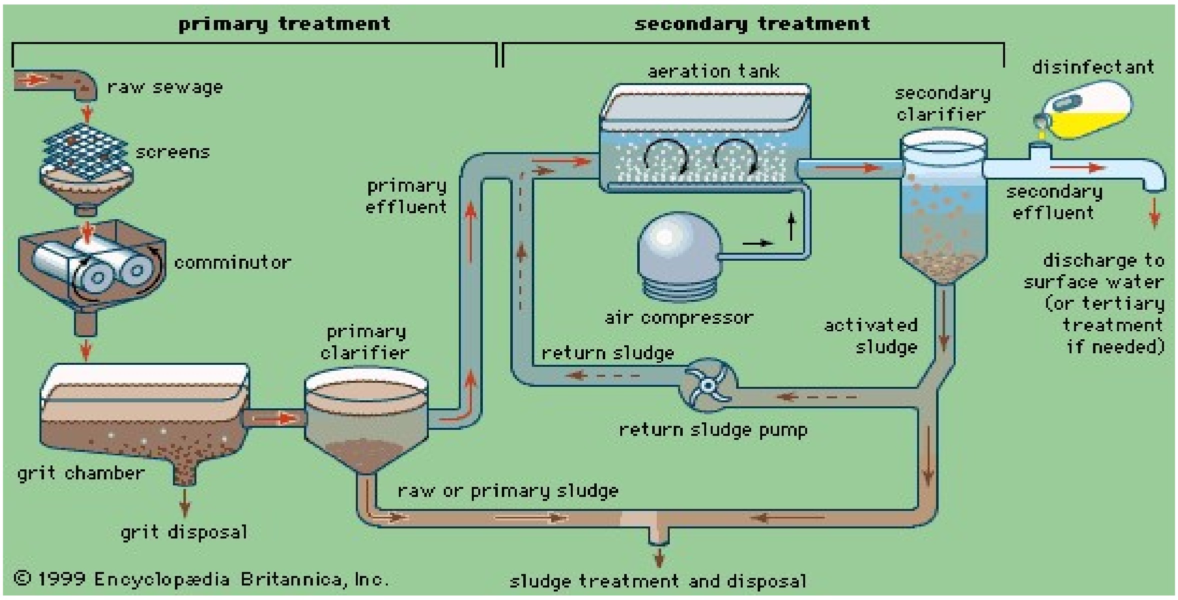


Figure (1): Activated sludge process.

Transfer of dissolved oxygen (DO) in aeration basin:

The DO transfer to the waste in a two step process:

1. The air bubbles are created by compressed air forced through submerged diffuser.
2. Mechanical aeration where turbulent mixing entrains air in the liquid.

Process variables used in control of the biological processes:

The used process variables that may be applied in control of the biological treatment are:

1. Hydraulic retention time (HRT) or aeration period:

This is the average time spent by the influent sewage in the aeration basin tank. It is calculated as the tank volume (m^3) divided by the flow rate:

$HRT(t) = \frac{V}{Q}$ Where: t= Aeration period (h), V= Tank volume (m^3)

Q= average daily flow rate (m^3/d).

The higher the inflow rate (Q) the sooner the sewage influent will reach the outlet and therefore the lower will be the residence time or hydraulic retention time.

2. BOD loading: this can be calculated by kg BOD applied to the tank per day divided by the liquid volume in the aeration tank.

$BODload = \frac{Q.BOD}{V}$ Where: Q, raw waste water flow (m^3 /day)

BOD load= kg BOD/ m^3. day.

BOD= daily flow of BOD (mg/L)

3. Food to microorganism's ratio (F/M):

It is calculated as the daily flow of BOD divided by the total MLSS in the aeration tank.

$F/M = \frac{Q.BOD}{MLSS.V}$ Where:

F/M= food to microorganisms ratio (kg BOD/day. kg MLSS).

BOD= raw wastewater BOD (mg/L).

V= volume of aeration basin or tank (m^3).

MLSS= Mixed liquor suspended solids in the tank (mg/L).

The higher the biomass growth in the tank, the higher rate of BOD removal. As the ratio of food (BOD) to microorganism's increases, so the rate of BOD removal, growth rate, and respiration rate increase.

$$BOD \quad efficiency = \frac{quantity \quad of \quad BOD \quad removed\,(effluent)}{quantity \quad of \quad BOD \quad entering\,(iffluent)} \times 100\%$$

Advantages of biological treatment plant:

1. Fast treatment or removal of sewage.
2. High rate of BOD removal, up to 95% of BOD_5 and 98% of bacteria particularly coli form and 95% of suspended solids.
3. Good settlement of sludge in clarifier.
4. Low rate of sludge production.
5. Minimal aeration costs.
6. High quality effluent – low in BOD suspended solid.

2. Attached film growth (Trickling Filter):

This filter is a bed of crushed stone, or gravel to which the settled sewage is applied in by sprinkling on the surface of the filter medium. The organic matter is absorbed and oxidized when passes through the filter medium containing microbial slim layer (figure 2)

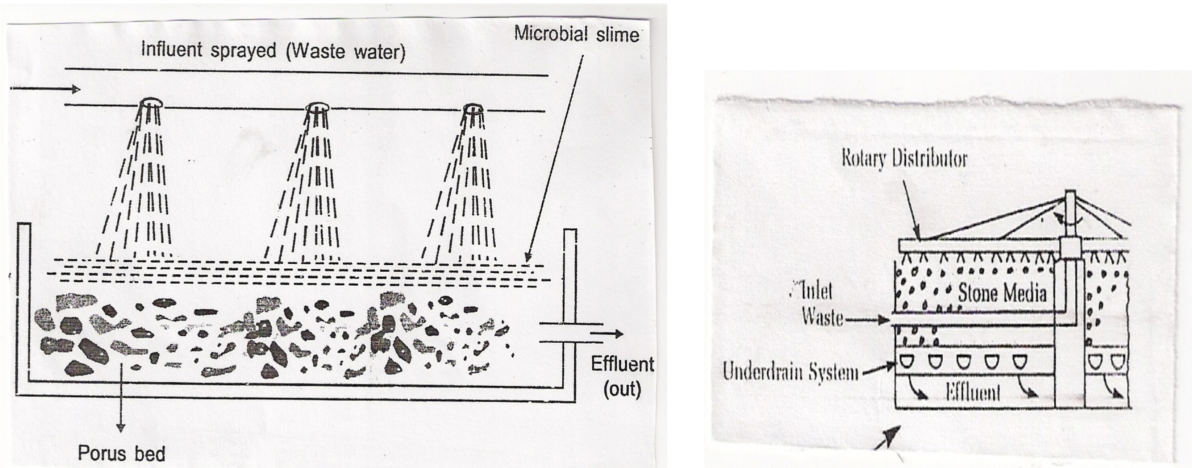


Figure (2): Schematic view of trickling filter.

Constituents of trickling filter:

1. Filter tank (rectangular or circular constructed of concrete).
2. Filter media are generally crushed stones or rocks, and plastic. The rock bed (1-3 m depth)
3. the influent pipes (the influent is sprinkled or sprayed over a biological film or slime or rocks)
4. distributors (rotary distributors or fixed – spray nozzles)

Principles of operation:

a) The influent wastewater sprinkled over a biological film or slim

b) As the liquid trickles through the biofilm, and while O_2 and organic matter (suspended and dissolved solids) diffuse in biofilm. Microbial metabolisms convert the organic matter into CO_2 and NO_2.
c) When the slim layer gets thickened, it is automatically sloughed or removed and settles in settling tank.

Microbial biofilm contains: bacteria (*Flavobacterium, Pseudomonas, Alcaligens*, and others) algae, fungi, protozoa, yeasts.
hem
The waste treated in Trickling filters:

1. Milk processing 2. Domestic sewage 3. Pharmaceuticals waste 4. Paper mill wastes.

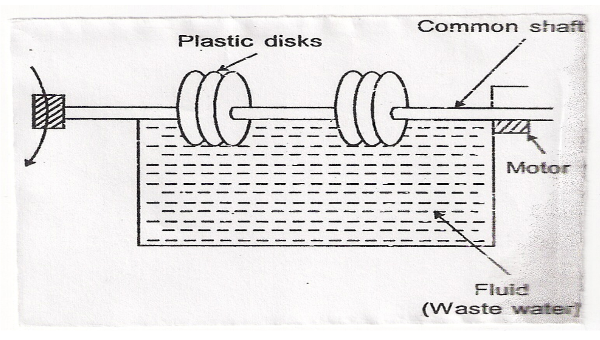
Difference between activated sludge and trickling filter:

Activated sludge	Trickling filter
1. Microbial growth is suspended flocs	Biofilm is fixed on a medium
2. solids from settlers are partially recycled	Solids are wasted
3. Effective in removing pathogens	Less effective
4. High operating cost	Low operating cost

Another attached film growth techniques are:

3. Rotating Biological Contactor (RBC)

In this bioreactor, microbial slim film is built upon a partly submerged support medium, which consists of a series of light weight disks, mounted on a horizontal shaft in a tank through which wastewater is allowed to flow (figure 3).



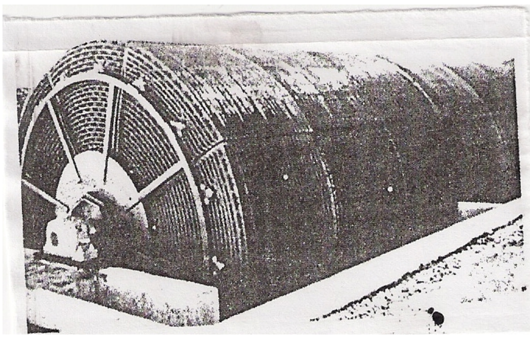


Figure (3): Photo and schematic diagram of rotating biological contactor (disk).
Constituent of the bioreactor:

1. wastewater tank
2. Biological disks mainly plastic disk 2-3 m diameter.
3. Horizontal shaft supplied with motor.

The operation of the bioreactor:

1. The waste tank filled with certain wastes, and the plastic disk which have 40% of the surface area submerged in the waste.
2. The medium (disk) is rotated at 1-7 revolution per minute and the slim biomass 1-4 mm layer is developed.
3. As the medium rotates, the biofilm is exposed to the nutrient in the waste water and air. Excess biomass is built on the medium in a manner similar to a trickling filter.
4. The biomass is removed in a clarifier.

The waste treated in rotating biological disk:

a. Municipal wastewater and industrial (like pulp, textile, meat, and vegetables)
b. Phenol from industrial effluents by suitable microbes.

Advantages: a. operation cost less than activated sludge b. more stable and efficient.
Disadvantages: higher BOD and suspended solids in the effluent

4. Fluidized beds:

These are column reactors in which waste water is pumped upwards through a particulate bed of material upon which biofilm have developed (figure 4).

Advantages: higher surface area of biofilm per unit of reactor.

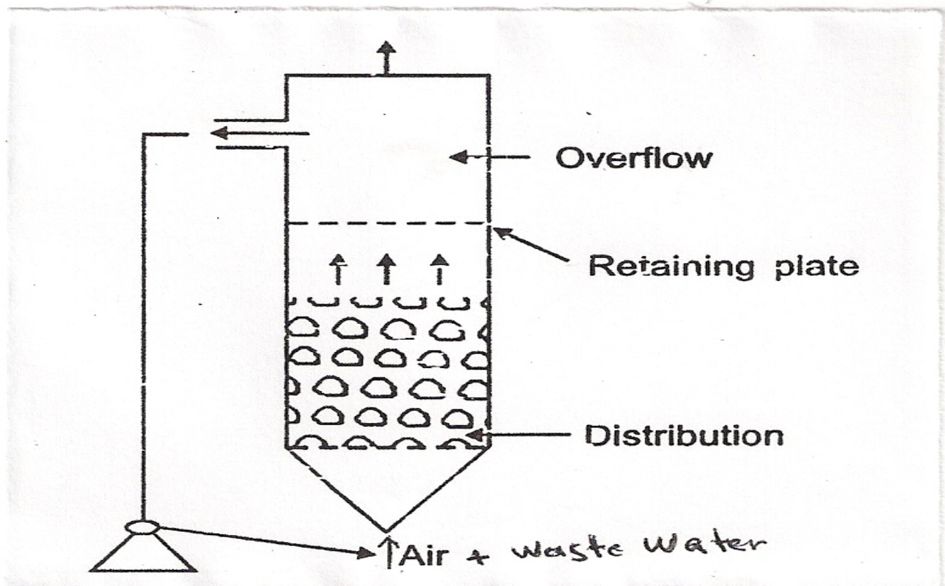


Figure (4): Fluidized bed reactor.

A. Calculate food to microorganisms ratio F/M in unit (kg BOD/kg MLSS. d) of activated sludge process if: average daily flow rate = 50 m^3/d, BOD load = 2.5 kg BOD/m^3.d, MLSS in the tank= 50 kg/m^3 and hydraulic retention time (HRT) = 6 hours.

B. Calculate HRT in h of activated sludge process if: Flow=100 m^3/d, BOD load= 0.5 kg BOD/m^3.d, Mlss= 30 kg/m^3 and BOD = 60 kg/m^3.

Biological treatment by activated sludge:

Source of wastewater:

Wastewater comes from two major sources: as human sewage and as process waste from manufacturing industries (wastewater from industry is about 7 times that of domestic sewage). If the wastewater untreated, and discharged directly to the environment, the receiving waters would become polluted and water-borne diseases would be widely distributed.

Biological treatment simply: involves applying naturally occurring bacteria at very much concentration in tanks.

The concept of treatment is very simple: The bacteria remove small organic carbon molecules by eating them. As a result, the bacteria grow, and the wastewater is cleansed.

The control of the biological treatment process is very complex, because of the large number of variable affect it, these include:

- Changes in the composition of the bacterial flora in the tank.
- The influent of the sewage to the process show variation inflow rate.
- Chemical composition and pH and temperature.
- Industrial wastewater contains the resistant chemicals that the bacteria degrade only very slowly.
- Toxic chemicals that inhibit the functioning of activated sludge bacteria.
- High concentration of toxic chemicals can produce a toxic shock that kills the bacteria.

When this happens the system may pass untreated effluent direct to the environment.

The nature and composition of waste water:

Domestic sewage mainly contains:

1. Organic carbon, either in solution or as particulate matter
2. About 60% is in particulate form may settle or suspended in the tank.
3. Particles of 1 nm to 100 µm remain in colloidal suspension and during treatment become adsorbed on to the flocs of the activated sludge.

Organic matter is easily biodegradable consisting of:

1. Protein, amino acid, peptides, carbohydrates, fats and fatty acid.
2. The average carbon to nitrogen to phosphorus ratio (C: N: P ratio) stated as approximately 100:20:6 which is ideal to growth of activated sludge bacteria.

While, industrial waste water are more variable in composition (such as pulp and paper) industries for example, are deficient in nitrogen and phosphorous. These nutrients need to be added to achieve correct ratio for microbial growth.

Soft and hard organic matter (BOD) digestion:

The time for the removal of organic carbon varies with the ability of activated sludge bacteria to ingest it:

- Soft BOD: this group of compounds is often referred to as the readily biodegradable or soft BOD, which is small molecular weight compounds will start to be removed from the sewage immediately after it has entered the activated sludge tank. Their removal may be completed in 1-2 hours.
- Hard BOD: higher molecular weight compound will take several hours to be degraded and removed. Compounds are more recalcitrant, and may still be present several days. This less readily biodegradable BOD is often referred to as hard BOD (figure 3-1)

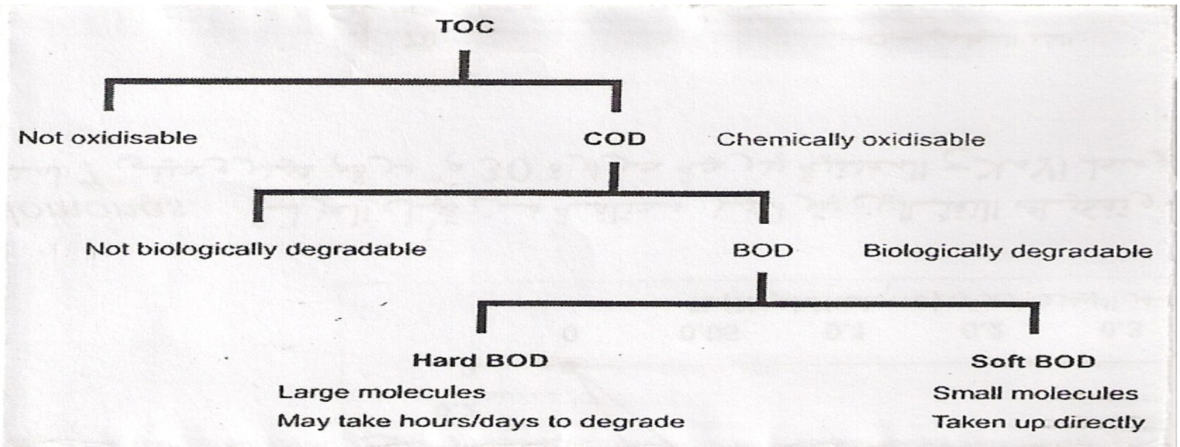


Figure (3-1): the relationship between the organic carbon fractions and their degradability.

Microbial ecology:

Activated sludge bacteria:

The activated sludge of the aeration basin of a waste water treatment works is a complex of competing organisms. The dominant organisms are the bacteria, of which there may be 300 species present. Bacteria are amongst the smallest and most abundant living organisms. Single cell, varying in size from about 0.5-2 µm the interior of the cell contains the cytoplasm and the thousands of different chemicals, whose reactions are regulated by enzymes. Most bacteria are spherical, but some may be rod shaped or spiral form and filamentous bacteria.

- Digestion process by bacteria:

Small molecular weight compound diffuse into the bacteria (ingestion) through the cell wall. At the same time, some large complex molecules that have been synthesized within the bacteria pass outwards. This process is refereed to as secretion. The secretions include slimes and gels that may bond the bacteria together and also enzymes. The enzymes break down large organic molecules into smaller monomers that are small enough to be ingested.
Then bacteria use the ingested molecules for the synthesis of new molecules, in the process of growth, reached normal size, and divided into two, and the process is repeated.

- Types of bacteria in activated sludge:

There are two types of bacteria according to the nutrition mode:

1. The heterotrophic or carbonaceous bacteria, which are the predominant groups of organisms. Feeding mainly on organic carbon molecules rather than inorganic ones.
2. The autotrophic bacteria, which take inorganic chemicals, and use these in the synthesis of organic carbon. For example nitrifying bacteria that remove ammonia from the waste water. There are few species of autotrophs, they have low growth rates, and they can not compete by the faster – growing heterotrophs.

Bacterial flocs:

The flocs are formed from aggregates of non-living organic polymers that are probably secreted by bacteria. They have open porous structure; vary in size from less than 10 µm up to 1 mm (1000 µm).

The bacteria are adsorbed on to the internal and external surfaces of the floc. Immediately after the waste water enters the aeration tank, the fine particulates, colloidal particles and large molecules, adsorb to the floc material. The enzymes that are secreted by the bacteria into the water will facilitate their digestion. For the bacteria living on or inside of the floc, a mixed liquor oxygen concentration of 1.2-2 mg O_2/l may be required, below this range the center of the flocs may become oxygen depleted and colonized by facultative anaerobic bacteria. The outer surface of the activated sludge flocs are colonized by microorganisms of a higher trophic level such as, protozoa and rotifers. These feed on bacteria and particulate material in the waste water.

Metabolism of bacteria:

Treatment of the sewage in the aeration tank involves removal of organic carbon from the mixed liquor by ingestion by the bacteria. Once inside the bacteria, the carbon compounds are metabolized. Metabolism comprises the thousands of simultaneous chemical reactions that are going on at one time inside the bacteria. In each of these reactions, a substrate in the presence of an enzyme (which acts as a catalyst), is converted into product.

Substrate $\xrightarrow{enzyme}$ product.

The product then becomes the substrate for the next step in the chain, and is almost immediately converted in the presence of another specific enzyme, into a different product.

The major divisions of metabolism are:

- Catabolism or energy metabolism: this comprises a serious of reactions in which carbon compounds are broken down to yield cellular energy. This is biological oxidation and involves oxygen uptake by the bacteria. This process referred to as respiration.
- Anabolism: this is a series of biosynthetic reaction in which small molecules are joined together to form large molecular weight macromolecules. This requires an input of energy from catabolism, and is the basis of the process of growth.

Microbial processes:

We can identify the three major processes that are relevant to the biological treatment:

1. ingestion
2. respiration
3. growth and division

These processes in an ingle bacterial cell can be shown in figure (3-2).

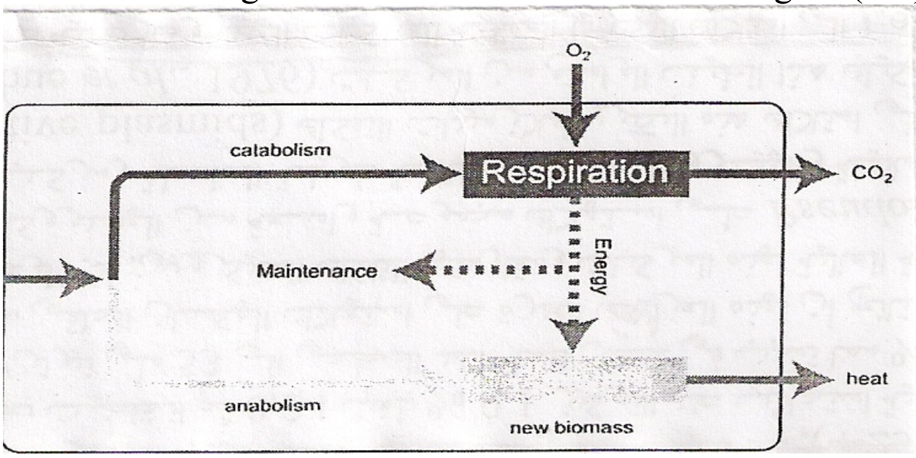


Figure (3-2): Respiration of a single bacterium showing the relationship between the three processes.

The three processes, ingestion, respiration and growth are very highly coupled. No one process can go faster than the other. It will be noted that the 3 processes correspond to the major processes that we shall see during the operation of the treatment system. They can be summarized as:

Bacterial process	Treatment plant process
Ingestion	Biodegradation
Respiration	Aeration requirement
Growth and division	Biomass production

Ingestion:

This involves the passage of organic carbon compounds, other molecules and ions from the mixed liquor into the bacterium, they have pass through the cell wall and inner membrane. Ions such as sodium diffuse in because the concentration in the mixed liquor is higher than inside the bacterium.

Small organic molecules: similarly pass in a long concentration by various mechanisms located in the inner membrane.
Large molecules: are excluded, in order to use these for their nutrition and growth. The bacteria secrete enzymes into the water to digest them into small monomers, which can then pass into the cell.

The bacterium required the presence of the particular chemical compound in the water to switch on the genes for the synthesis of the enzyme required for its digestion. This process also calls adaptation or acclimation.

Growth of bacteria:

Growth rate is measured as the increase in number of cells with time. Some bacteria may double their biomass in as little as 20 minutes, provided they have the right conditions of temperature, pH and an abundance of organic carbon, other nutrients, trace elements etc.

The growth rate observed is a result of both: genetic and environmental factors. The shape of the growth curves and the maximum rate of growth under optimal conditions are genetically determined. While the effects of environmental factors are described below:

1. **Substrate concentration:**

The main substrate for growth is the BOD or degradable organic carbon in the mixed liquor. With increase in the concentration of substrate, the growth rate increase exponentially and then levels off. So with further increase in concentration of substrate in the medium, there is no further increase in growth. So the bacteria are at their maximum growth rate (figure3-3)

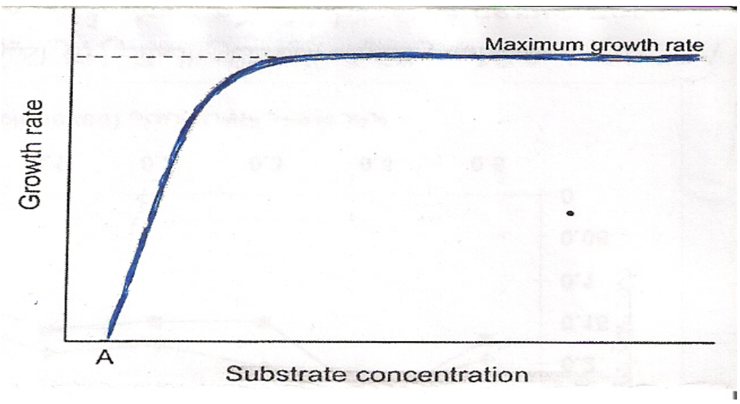


Figure (3-3): The growth rate increases exponentially with increase in substrate concentration, to a maximum.

The slope of the growth versus substrate concentration curve can be important. A steeper slope indicates a greater affinity or ability to use substrate. In the example in Figure (3-4), species Y has a greater affinity for substrate than species X at low concentrations. At this concentration, it will grow faster and out-compete X. at higher concentration; X has a higher maximum growth rate and will then out-compete Y.

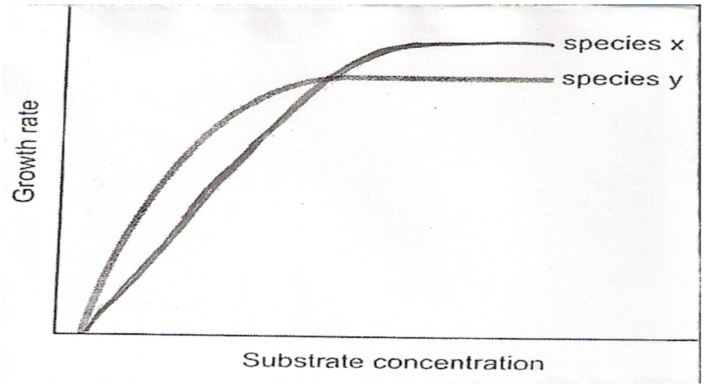


Figure (3-4): species X has a higher maximum growth rate than species y. However; Y has a higher affinity for the substrate than X at low concentration.

2. **Availability of the nutrients:**
   The major substrate requirement is for carbon, growth also dependent on the intake of:
   a. Macronutrients: like nitrogen and phosphorous. The optimum ratio of C: N: P in the mixed liquor is generally though to be 100:5:1. The ratio of these nutrients in domestic sewage reported as 100:20:6. This indicates that nitrogen and phosphorous will not be limiting for growth of bacteria.
   b. Micronutrients (trace) components: which include S, Ca, Mg, K and Fe. These elements are available in abundance in domestic sewage. By contrast, the waste water from pulp and paper, and food processing industries can be deficient in N and P. Nutrients therefore need to be added to the mixed liquor to obtain maximum bacterial growth and treatment. Lack or an insufficiency of a nutrient may result in incomplete treatment, because the bacteria are unable to grow optimally.

3. **Oxygen levels:**

If oxygen levels in the mixed liquor are too low, respiration will be inhibited and hence energy will not be available for growth. The higher the oxygen concentration in the water, the larger amount of oxygen inter to the inside of the bacterial cell. Oxygen is no limiting above concentration about 1.5 – 2 mg O_2/l for bacterial in flocs. Below these critical concentrations, the respiration rate falls rapidly due to the unavailability of oxygen.

4. **Temperature:**

As the temperature increases, the rate of growth, and hence requirement for oxygen for respiration increases. The respiration and growth rate approximately doubles for every 10 ˚C increase in temperature within optimum range. However, the solubility of oxygen in water decreases with increase in temperature. Optimum aeration becomes more difficult as the temperature in the tank rises. It is for this reason that most thermophilic plants, operating at 40 - 60 ˚C, have to use pure oxygen for aeration.

5. **Toxicity:** toxic chemical in the waste water can inter the bacteria and inhibit one or more enzymes of the pathways involved in either anabolism or catabolism. When pathway actually inhibited, all three processes of ingestion, growth and respiration will be similarly inhibited. Immediately after feeding, the respiration rate rises rapidly to its maximum value. When toxic waste water is introduced, the respiration rates falls to a new lower level. The difference between this new rate and the maximum rate is measure of the inhibition. The percentage of inhibition increases with increase in concentration of the toxic chemical in the mixed liquor.

Respiration:

This is a chain of metabolic reactions by which a substrate molecule is oxidized, and the energy made available to do work inside the cell. The energy contained in a substrate such as glucose is rapidly librated as a heat when it is oxidized by burning it in air. When glucose is metabolized in respiration, the same amount of energy is librated, but after some of it has been use to carry out cellular work. The energy is initially captured by the molecule adenosine diphosphate (ADP). This adds on another phosphate group to form adenosine triphosphate (ATP).

$C_6H_{12}O_6 + 6O_2 + 38\ ADP + 38\ P = 6Co_2 + 6\ H_2O + 38\ ATP$.

The main use of energy in bacteria is for biosynthesis for growth. Growth involves joining together of small molecular weight compounds to form macromolecules such as membranes, cell wall etc.

Respiration process is providing the energy for biosynthesis and for cell maintenance.

Environmental Treatment:

Metabolism of Nitrogen compounds:

Nitrogen containing substances in wastewater are organic or inorganic. Together with phosphate, they represent the main source for eutrophication of surface water. For this reason they must be eliminated together with the organic carbon during wastewater treatment. Where as phosphates form insoluble precipitates with many heavy metals ions can be separated by sedimentation.

Source of nitrogen compounds:

The major portion of nitrogen compounds in municipal wastewater is:

- Reduced nitrogen compounds, such as ammonia, urea, amines, amino acid, and proteins.
- Oxidizing nitrogen compounds, such as: nitrate and nitrite which represent the main nitrogen source in wastewater of certain food or metal industry.

Depending on the kind of nitrogen compounds present in wastewater, nitrogen removal requires up to three processes in sequence: ammonification, nitrification, and denitrification.

a. Ammonification:

The main organic nitrogen compound in wastewater is heterocyclic compound (e.g. nucleic acid and protein). Proteolyses and degradation of amino acids leads to librations of ammonia by the various mechanisms of ammonification including: hydrolytic, oxidative, reductive, and desaturative deamination (equation 1-4 respectively).

$R\text{-}\ NH_2 + H_2O \rightarrow R - OH + NH_3$ ------- (4-1)
$R\text{-}\ CHNH_2COOH + H_2O \rightarrow R - CO - COOH + 2(H) + NH_3$ ------- (4-2)
$R\text{-}\ CHNH_2 - COOH + 2H \rightarrow R - CH_2 - COOH + NH_3$ ------- (4-3)
$R\text{-}\ CH_2\ CHNH_2 - COOH \rightarrow R - CH = CH\text{-}\ COOH + NH3$ ------- (4-4)

It can be estimated that bacteria consist roughly 50% protein and the nitrogen content of protein is about 16%. Thus for synthesis of 1 g of bacterial biomass, about 0.08 g of ammonia-N is required.

To eliminate ammonia that is not used for cell growth during wastewater treatment, it must be first nitrified and then denitrified to molecular nitrogen or anaerobically oxidized with nitrite.

b. Nitrification of ammonia:

This process can accomplish by two types of nitrifiers organisms: Autotrophic and heterotrophic nitrifiers:

1. Autotrophic nitrification

Autotrophic nitrifiers are aerobic microorganisms oxidizing ammonia to nitrite and then nitrite oxidized to nitrate.

$NH^{+}_{4} + 1.5\ O_2 \rightarrow NO_2 + 2H^{+} + H_2O \rightarrow NO_2 + 1.5\ O_2 \rightarrow NO_3$ -------- (4-5)

Organisms catalyzing nitrification: belong to the genera *Nitrosomonas, Nitrococcus, Nitrosospira* and *Nitrosovibrio*.

In Autotrophic nitrification process:

- Ammonia oxidation to nitrite or nitrite oxidation to nitrate is energy yielding processes for autotrophic growth of nitrifying bacteria.
- Autotrophic nitrifiers cannot successfully compete with heterotrophic bacteria for oxygen.
- In a highly loaded (concentration) activated sludge the autotrophic nitrifiers are overgrown by the heterotrophic bacteria, and consume the oxygen.
- Ammonia oxidation starts only if the BOD_5 concentration in the wastewater is < 110 mg/l.
- During nitrification of ammonia the alkalinity increases (pH increase).

2. Heterotrophic Nitrification:

Heterotrophic nitrification organisms include: some bacteria of the genera *Arthrobacter, Flavobacterium* and *Thiosphaera* which are able to catalyze nitrogen – containing organic substances.

$R\text{-}NH_2 \rightarrow R - NHOH \rightarrow R - NO \rightarrow NO^{-}_{3}$ ------- (4-6).

Heterotrophic nitrifiers:

- Oxidize and reduce nitrogen compound from wastewater, such as hydroxyl amine and aliphatic and aromatic nitrogen-containing compound.
- In contrast to autotrophic nitrification, no energy is gained (obtained) by nitrate formation. For this reason an organic substrate must be respired to satisfy the energy metabolism.

c. Denitrification: Nitrate removal

Denitrification starts with the reduction of nitrate to nitrite by membrane-bound nitrate reductase (a). Then a membrane-bound nitrite reductase (b) catalyzes NO formation. Finally, NO reductase (c) and N_2O reductase (d) form gaseous nitrogen (N_2) which then escapes to the atmosphere.

$$NO_3^- \xrightarrow[a]{2e-} NO_2^- \xrightarrow[b]{e-} NO \xrightarrow[c]{e-} 0.5N_2 + 2H_2O + OH^- \quad \text{.......(4-7)}$$

The denitrifiers can thus complete the oxidation of organic substrate using the enzymes of the respiration in the boundary zones of the activated sludge flocs even if dissolved oxygen is not present in the aeration tank.

<u>Many types of bacteria are capable of performing denitrification include</u>: *Denitrobacillus, Micrococcus denitryficans, Spirillum, Bacillus, Pseudomonas* and *Escherichia.*

Metabolism of phosphorous compounds:

As phosphorous is significant in algae productivity, its removal from aquatic bodies is essential to protect them from eutrophication.

<u>Chemical treatment:</u> usual practice is to precipitate them chemically with salt of Ca, Fe, Al and Mg. with calcium salts, phosphorous is precipitated as hydroxyl apatite: $Ca_5\,OH\,(PO_4)_3 + 6H_2O$.

$$Ca^{+2} + PO_4^{-3} \rightarrow Ca_3(PO_4)_2 \xrightarrow{Apatite} Ca_5OH\,(PO_4)_3 \downarrow (\text{Pr}\,ecepitate)$$

<u>An alternative method is the biological phosphorous removal:</u> where phosphate metabolizing bacteria help in the process. The energy required for this is made available by the release of phosphorous bounds as polyphosphate in volute granules in the bacterial protoplasm.

The principle of biological treatment plant of phosphorous lies in the: exposure of the organisms to alternating anaerobic and aerobic condition.

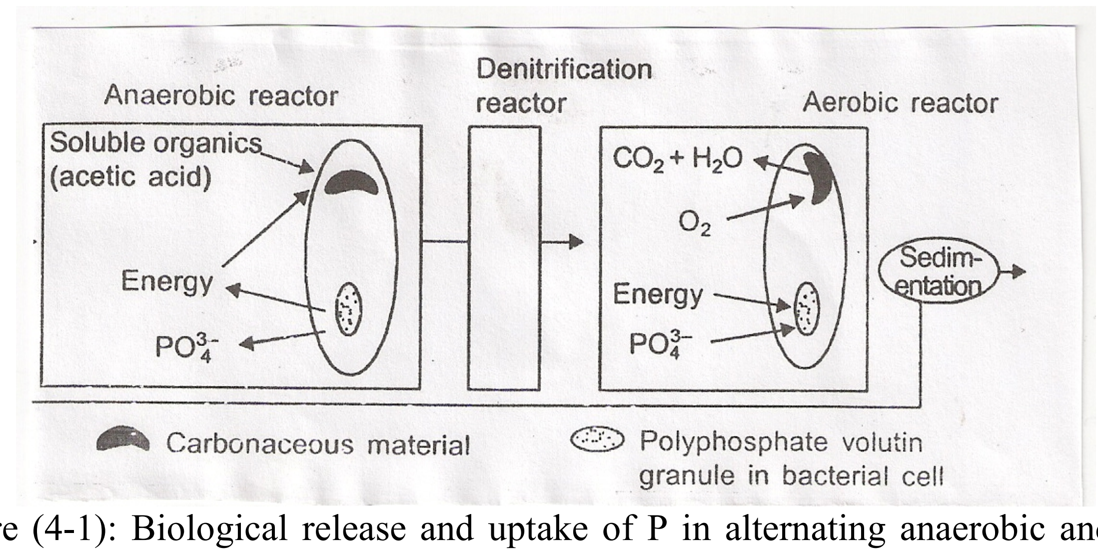


Figure (4-1): Biological release and uptake of P in alternating anaerobic and aerobic condition.

Under anaerobic conditions: transport and storage of simple organics such as acetate require energy, which is obtained from polyphosphate reserves of the bacteria with release of phosphorous.
While under aerobic condition: the organic matter is oxidized to produce energy and reaccumulation of phosphates into polyphosphates. The net effect is the excess of phosphorous in the bacterial cell.

Metabolism of sulphur compounds:

Municipal sewage and many industrial effluents contain sulphides, including hydrogen sulphide. About 1% of the organic fraction consists of sulphur.
Source of sulphur: The sulphur – containing amino acids cystine, cystein, methionin and lathionine are formed during the breakdown of proteins.

The reduction of sulphur in the treatment plant can be achieved:

1. Under strongly anaerobic conditions, even sulphate may be reduced to hydrogen sulphide.

$$SO_4^{-2} + 8H^+ \rightarrow H_2S + 2H_2O + 2OH^- \text{ …… (4-8)}$$

2. The resulting hydrogen sulphide may be oxidized under aerobic condition biologically by a few adapted bacteria for example: *Thiothrix* by supplying oxygen to form sulphide.

$$2H_2S + O_2 \rightarrow S_2 + 2H_2O + 251KJ \text{ …… (4-9)}$$

3. Where the oxidation stops, then sulphur granules are deposited in the bacterial cells.
4. when discharges from final clarifier of treatment systems containing H_2S, this indicative of operating failures, due to oxygen deficiency in the tank, septic zones in the tank.

Reduction of CO_2 emission through biological treatment:

This is achieved with the help of calcifying organisms like corals and mostly member of green algae and red algae. They live in symbiotic relationship and the calcification process is rapid:
$H_2O + CO_2$ (aq) → H_2CO_3 (Decrease in pH value) …..(4-10)
$H_2CO_3 + Ca^{+2} \rightarrow CaCO_3 + H_2O + CO_2$ (Increase the pH value and $CaCO_3$ precipitate) …… (4-11).

If algal species can be found out which would tolerate high pH and carbonate, the photosynthesis by such algae may led to precipitation of $CaCO_3$.

Wastewater treatment (Algal photosynthesis):

Wastewaters resulting from municipal and industrial activities mainly contain organic and inorganic substances, pathogenic organisms and various toxic materials like heavy metals.

Aerobic oxidation ponds or stabilization ponds (Facultative ponds) are used for such treatment which is also called secondary stage of treatment.

Facultative ponds:

These are the most common types of stabilization ponds, which are naturally treatment system. They normally receive raw sewage or that which has receive only preliminary treatment. Facultative refers to a mixture of aerobic and anaerobic conditions. In facultative pond aerobic conditions are maintained in the upper layers, while anaerobic condition exists towards the bottom. The range of pond depth most commonly used is from 1-1.5m.

Principle of treatment: depend mainly to the symbiotic relationship between algae and bacteria, which are naturally occurring in the pond:

- Simple micro-algae which grow naturally in the pond can serve as rich source of oxygen, which supplied by the photosynthetic activity of algae.
- The algal system supply the oxygen needed by the bacteria and protozoa in the process to oxidize and degradation of organic content of the waste.
- One of the major end products of the bacterial metabolism is carbon dioxide which is readily utilized by the algae during photosynthesis.
- The pH of the pond contents also follows a daily cycle increasing with photosynthesis to a maximum which, maybe as high as 10. this happens because at peak demand algae remove CO_2 from the media more rapidly than it is replaced by bacterial respiration, as a result the bicarbonate (HCO^-) ions present dissociate to provide not only more CO_2 but also the alkaline hydroxyl ion which increase the pH value:
  $HCO^-_3 \rightarrow CO_2 + OH^-$.

Algal genera:

The species of different algal genera which are best known in wastewater treatment are members of green algae, some of them are: *Chlorella, Scenedesmus, Euglena, Chlamidomonas, Hydrodictyon, Ulothrix, Tribonema, Oscillatoria and Anabaena.*

<u>The combined treatment of wastewater by algae and bacteria are useful in various ways:</u>

1. The oxygen production with algal photosynthesis is valuable for meeting the BOD requirement by bacterial flora (decomposer) to oxidize organic matter. Oxygen factor, which is ratio of the quantity of oxygen produced by algae and the quantity of oxygen required by the decomposing bacteria, is maintained at 1.5 – 1.6 (mg/l) high level of BOD removal, about 80-90% can occur.
2. Algal treatments precipitate some of the toxic substances like heavy metals from waste.
3. Algal cell rich in protein could serve as food and feed or manure.
4. Possible reuse of treated water.
5. Reduce the risk of large number of pathogenic microbes either become dead and get settled at the bottom of the pond or removed with algal biomass.

Eutrofication:

Eutrophic water refers to the stage at which it is enriched with undesirable organic and inorganic nutrients containing phosphorous and nitrogen coming from; natural sources of nitrogen and phosphorous and man made sources like; agricultural and municipal waste containing fertilizer and detergents. Undesirable amount of these elements in wastewater favors excessive algal growth and thus lead to eutrophication.

Eutrophication causes:

1. Depletion of oxygen.
2. Foul smell through generation of sulphides.
3. Death of non resistant organisms.
4. Still there is a problem, that the dead algal cells may release toxins in water, as will as depleted the oxygen level.

Control of algae in eutrophic waste can be done:

1. Chemically: the use of algaecides like copper sulphate, sodium arsenate, 2, 4-D (dichloro phenoxy acetic acid) and 2, 3 dichloro naphtha quinon.
2. Biologically: use of cyanophages (viruses which kill these algae cell) such as viruses Lpp-1, A-1, N-1, etc.

Biodegradation:

Biological degradation or simply biodegradation:

Is generally considered as a phenomenon of biological transformation of organic compounds by living organisms, particularly microbes, also defined as:

Natural process changes in the molecular structure and completely broken of a compounds yielding simpler (mineralization) and comparatively harmless (non-toxic) products like CO_2, H_2O, NH_3, CH_4, H_2s and PO_3. Such changes are brought about by the catabolic activities of bacteria or fungi by their intracellular or extracellular enzymes secreted in the medium.

- When the compound is not fully broken, it is termed biotransformation.
- Many of the recalcitrant (resistant or non biodegradable) substances produced by transformation may sometimes be more toxic than the original compound.

Biological fate of xenobiotic (novel to microbial system) compounds in the environment can be indicated as shown in Fig. (5-1).

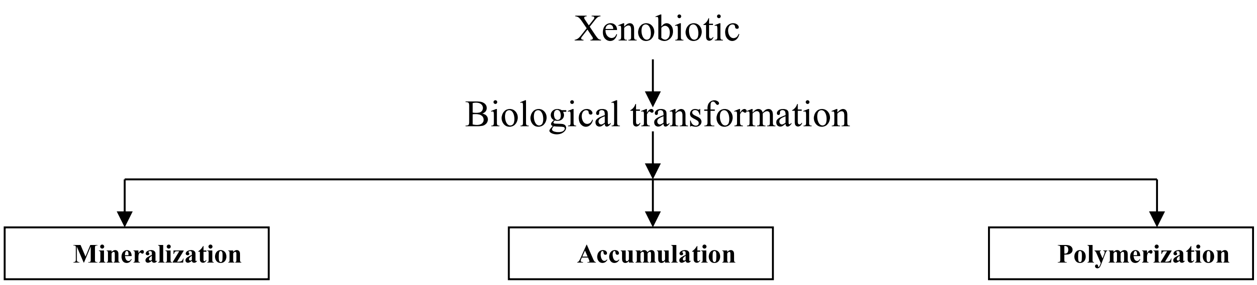


Figure (5-1): Biological fate of xenobiotic compounds in the environment.

Under suitable conditions: all natural organics get decomposed and are not deposited in the environment with the exception of natural polymers like lignin and soil humus getting degraded very slowly.

The general scheme of biodegradation may be represented as shown in Figure 5-2.

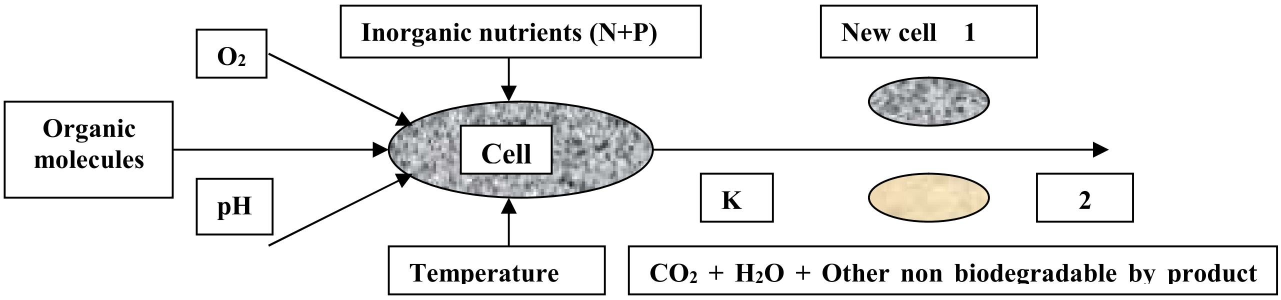


Figure (5-2): Biodegradation process of organic molecules.
K: is the rate of coefficient and is a function of biodegradability of organics.

Many of the wastes are generally complex in nature and the capability of microbes to degrade such compounds may occur in different ways:

- Particular strain of a microbe may degrade only one type of compound or its related groups.
- Some chemicals substances the synergistic action of microbial communities or polyculture, displaying wide range of degradative abilites rather than a monoculture, is desirable.
- Some times when the degrading material dose not serve as a sole source of carbon and energy (non-growth substances) for the organisms, but is associated with another growth substrate, then also it gets biotransformed and the phenomenon is termed as *Co-metabolism*. In another meaning, microorganisms which are grown on a particular substrate can oxidize other substrate (co-substrate) and the co-substrate does not assimilate, while the oxidation product utilized by microorganisms. For example, toluene is primary substrate for *Pseudomonas putida* in breaking trichloroethylene.

Factors that effect in biodegradation are:
Temperature, pH, availability of nutrients, O_2 supply, biomass of the degrader, competition among microbial communities and the nature and concentration of the substrate as well.

The chemical nature of the compounds has also great influence in the biodegradation process. In general the hydrocarbons degraded as follows order:

1. Aliphatic compounds are degraded more easily than aromatic ones. Algae and fungi cannot cleave aromatic rings, where as bacteria can.

2. Recalcitrance of a compound for degradation increase with increased branching, polymerization, and presence of polycyclic and heterocyclic residues.
3. Water soluble compound s are easier to degrade than insoluble forms.
4. Alkenes are easier to degrade than alkanes, while alkanes are more amenable than aromatics.
5. For aromatics, degradability may be influenced by molecular orientation, e.g. *ortho* > *para* > *meta*.
6. Halogen, nitrogen and solfonate substitutions group inhibit biodegradation.

Examples: 1. Aliphatic compounds like
2. Aromatic 3. Polyaromatic
4. hetrocyclic

Environmental protection agency (EPA) list of some organic priority pollutants injected to the environment by human activities, which are highly hazardous and poising and causes serious problems to the environment.

1. Acenaphthalene	2. benzidine	3. carbon tetrachloride CCl_4
4. chlorinated phenol	5. dichlorobenzene	6. hexachloroethane
7. naphthalene	8. toluene, benzopyrene	9.polychlorinated biphenyl's PCB_s
10.hexachlorocyclohexane	11. pesticides – aldrin, DDT, endrin, etc.	

The degrading capacity of soil microorganisms are being isolated from toxic waste site. <u>The biodegradation capacity can be enhanced (augmented) through</u>:

- Genetic upgrading of the degradative genes, understanding their catabolic pathways.
- Interaction between microbial strains to eliminate the pollutants.

About 40 – 50 microbial strains with suitable degradative potentials for a variety of complex compounds have been isolated. Some of the microbes which can degrade various chemicals are:

Chemicals	**Microbes**
1. Hydrocarbons	*Pseudomonas, Nocardia, Arthrobcter, Mycobacterium*
2. PCB_s	*Pseudomonas, Candida, Alcaligenes*

3. Phenolics	*Pseudomonas, Flavobacterium, Trichosporon, Bacillus, Candida, Aspergillus*
4. Polycyclic aromatics	*Arthrobacter, Nocardia, Alcaligenes, Pseudomonas*
5. Naphthalene	*Pseudomonas, Nocardia*
6. Benzene	*Mycobacterium, Alcaligenes, Pesudomonas*

Aerobic and anaerobic degradation:

Microbial degradation or transformation of organic compounds may involve either of the two process of aerobic (oxygen dependant) or anaerobic situation, while in some cases it may need both the condition to detoxify some xenobiotic compounds (sequential degradation).

Aerobic degradation:

In the conventional aerobic system, the substrate is used as source of carbon and energy. It serves as an electron donor resulting in bacterial growth. The extent of degradation is correlated with the (1) rate of O_2 consumption, (2)as also previous acclimation of the organisms in the same substrate.

Two enzymes primarily involved in the process:

- Dioxygenase enzyme: can act on both aromatic and aliphatic compounds, with incorporation of two molecular oxygen atoms in the reaction.
- Monoxygenase enzyme: can act on aliphatic and aromatic compounds also, with incorporation of one oxygen atom in the reaction.
- Another class enzymes involved in aerobic condition is peroxidases, which, act on lignin compounds degradation.

Anaerobic degradation:

This process is of widespread occurrence and relies on the metabolic activity of mixed microbial populations present in soils or sediments when O_2 supply is limited.

The overall process of anaerobic degradation of complex wastes is shown in Fig. 5-3.

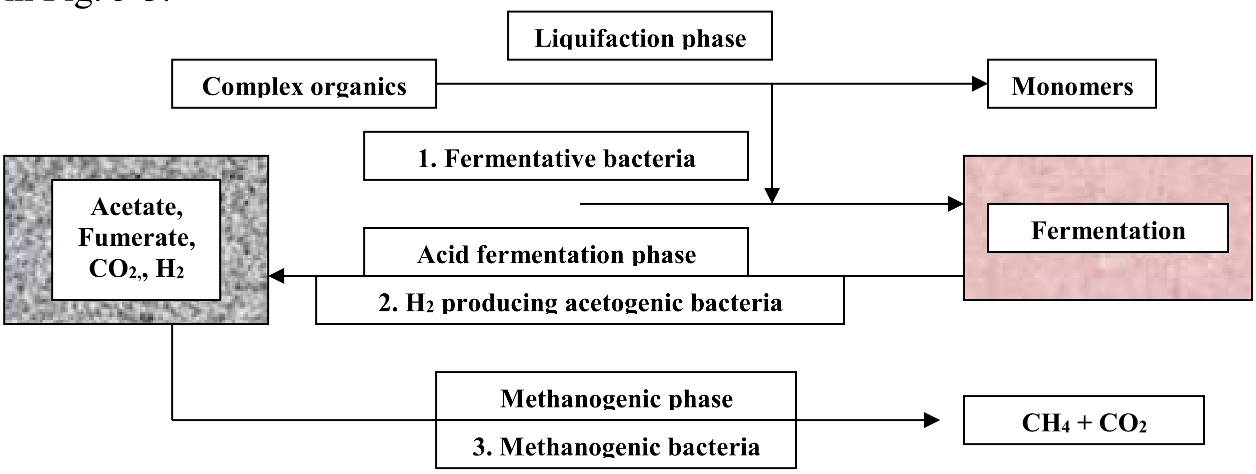


Figure (5-3): Anaerobic degradation of complex wastes.

Three temperatures ranges are used in anaerobic digestion:

- Cold digestion at about 20˚C.
- Mesophilic digestion at about 20˚C – 40˚C.
- Themophilic digestion at about 40˚C - 55˚C.

Some anaerobic process which, may occur in some soils or sediments: 1. Denitrification 2. sulphate reduction 3. dehalogenation and 4. fermentation coupled to methanogeneses.

Some anaerobic microbial transformation reactions of organic compounds are:

Reaction type	Examples
1. hydro-/ dehydrogenations	Phenol, catechol, benzoate, fatty acid, unsaturated hydrocarbons
2. carboxy-/ decarboxylation	Cresol, toluene, benzoate, short hydrocarbons
3. reductive dehalogenation	Polychlorinated aromatic compounds
4. dechlorination	PCB_s, phenols
5. Methylation	Heavy metals

<u>The disadvantages of anaerobic process are:</u>

- Slow.
- Growth yield of anaerobic bacteria is extremely low due to low energy yield.
- Needs long retention time and produce H_2S gas.

<u>More advantages than the aerobic one due to:</u>

- Its non-dependence of O_2 supply.
- Save the cost of energy for O_2 transfer.
- Safe, since few toxic chemicals can be stripped into the ambient air.
- Chlorinated xenobiotics need to be dehalogented to make them harmless; they mostly need anaerobic situation for dehalogenation.
- Materials like cellulose and fats, which remain unaffected by the aerobic process, breakdown under this situation.
- Unlike aerobic condition, in an anaerobic degradation the chlorinated molecules are used as a direct source of electron. See the example below:

$$\text{3- Chlorobenzoate} \xrightarrow{\boxed{\text{Dechlorination}}} \text{Benzoat} \xrightarrow{\boxed{\text{Metabolized}}} \text{Acetat} \xrightarrow{\boxed{\text{Transformed}}} CH_4 + CO_2$$

Sequential degradation:

Sequential degradation for some xenobiotic compounds takes place by:

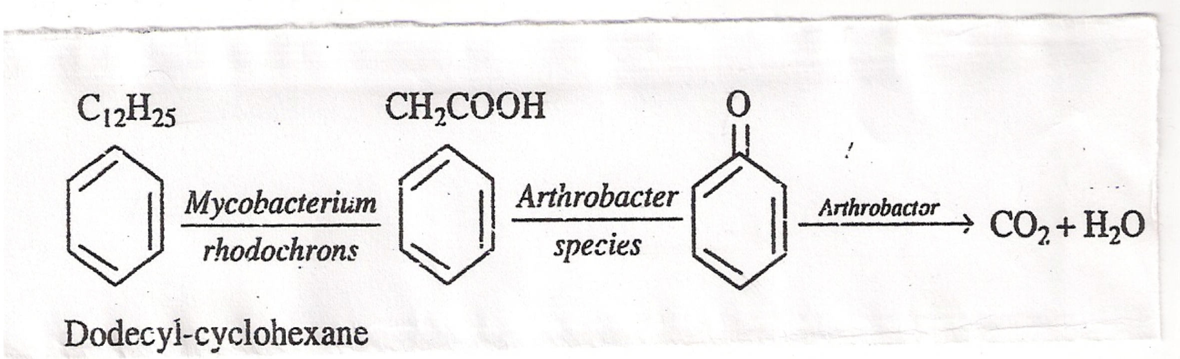
1. Aerobic and anaerobic degradation

In many cases, both anaerobic and aerobic sequences are combined. This helps in the reduction of toxicity and mineralization of compounds which are otherwise recalcitrant. For example, tetrachloroethylene and tetrachloromethane may be mineralized in sequential steps of anaerobic and aerobic conditions, so that initially TCE and chloroform are formed, which, later in anaerobic methanogenic stage, are converted into CO_2 and H_2O. In such sequential stages, the BOD reduction is also taken care of, as is being done in case of wastewater from pulp and paper industries.

Example:

2. Synergistic action of two or more microbes (microbial consortia) for sequential degradation for some xenobiotic chemicals:

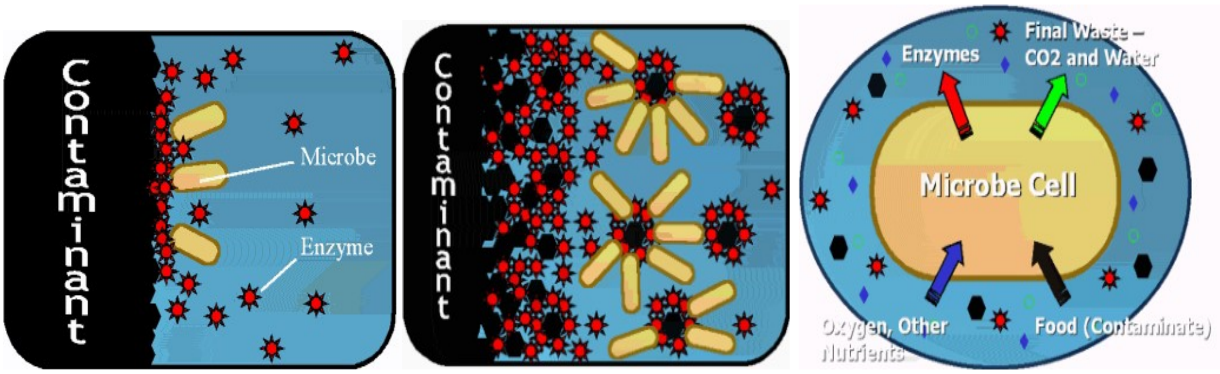
- A single bacterium may transform it to another form, but can not complete the breakdown.
- Then second group of microbe may act in a complementary total breakdown of the compound through a (team work). This sort of synergistic action also prevents the build-up of toxic intermediates in the environment. As in example below:



Dr. Nadhem H.H.

Bioremediation of Environmental Pollutants
Principles of Bioremediation

- Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site.
- By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site.



Factors of Bioremediation

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

Characteristics of Microbial Populations for Bioremediation Processes:

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are an energy source and a carbon source.

We can subdivide these microorganisms into the following groups:

- *Aerobic*. In the presence of oxygen. Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.
- *Anaerobic*. In the absence of oxygen. Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform.
- *Ligninolytic fungi*. Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs.
- *Methylotrophs*. Aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1,2-dichloroethane.

Mechanisms of oxidation:

It is evidenced that the initial step in aerobic catabolism of a monoaromatic or PAH molecule by bacteria occurs via:

- Oxidation of a single ring to a dihydrodiol. These dihydroxylated intermediates may further be metabolized via ring cleavage, resulting in intermediates that are further converted to carbon dioxide.
- In general, HMW PAHs are slowly degraded by indigenous microorganisms and may persist is soils and sediments. The recalcitrance of these pollutants is due in part to a strong adsorption of HMW PAHs to soil organic matter and low solubility, which results in decreased bioavailability for microbial degradation. Microorganisms show fundamental differences in the mechanisms of aromatic metabolism used. Bacteria initiate the oxidation of aromatics by incorporating both atoms of molecular oxygen into aromatic ring to produce a *cis*-dihydrodiol, which is then dehydrogenated to give catechols.
- Some bacteria also have been reported to attack PAHs with a methane monooxygenase.

- Similarly, aliphatic hydrocarbons are also attacked with oxygen as a reactant. The oxygen is inserted into the end carbon by a monooxygenase enzyme leading to production of an acid. The fatty acid is then degraded piecemeal through a process termed *beta* oxidation resulting in two-carbon fatty acids being released.

Environmental Factors
Nutrients

Although the microorganisms are present in contaminated soil, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated.

Bio - stimulation: usually involves the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon. Carbon is the most basic element of living forms and is needed in greater quantities than other elements.

Environmental requirements

Optimum environmental conditions for the degradation of contaminants are reported in Table 3.

Table 3: Environmental conditions, for bioremediation of contaminants.

Parameters	Condition required for microbial activity	Optimum value for an oil degradation
Soil moisture	25–28% of water holding capacity	30–90%
Soil pH	5.5–8.8	6.5–8.0
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10–40%
Nutrient content	N and p for microbial growth	C:N:P = 100:10:1
Temperature (°C)	15–45	20–30
Contaminants	Not too	toxic Hydrocarbon 5–10% of dry weight of soil
Heavy metals	Total content 2000 ppm	700 ppm
Type of soil	Low clay or silt content	

Bioremediation Strategies:

Different techniques are employed depending on the degree of saturation and aeration of an area:

- *In situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance.
- *Ex situ* techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water).

***In situ* bioremediation:**

These techniques are generally the most desirable options due to: lower cost and fewer disturbances since they provide the treatment in place avoiding excavation and transport of contaminants. *In situ* treatment is limited by the depth of the soil that can be effectively treated. In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases.

The most important land treatments using In Situ techniques:

1. ***Bioventing*** is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface
2. ***Biosparging.*** Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater.
3. ***Bio-augmentation.*** Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit: 1) nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and 2) most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

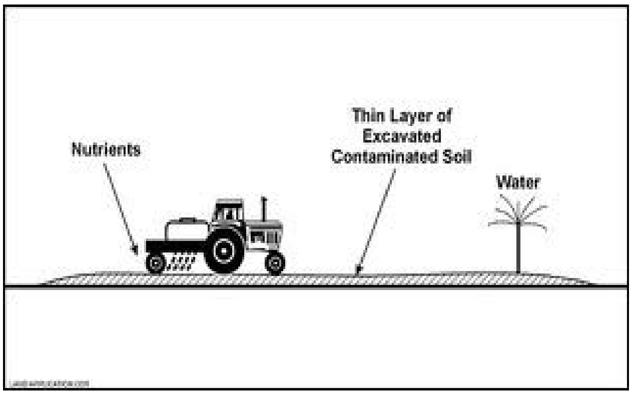
***Ex situ* bioremediation**

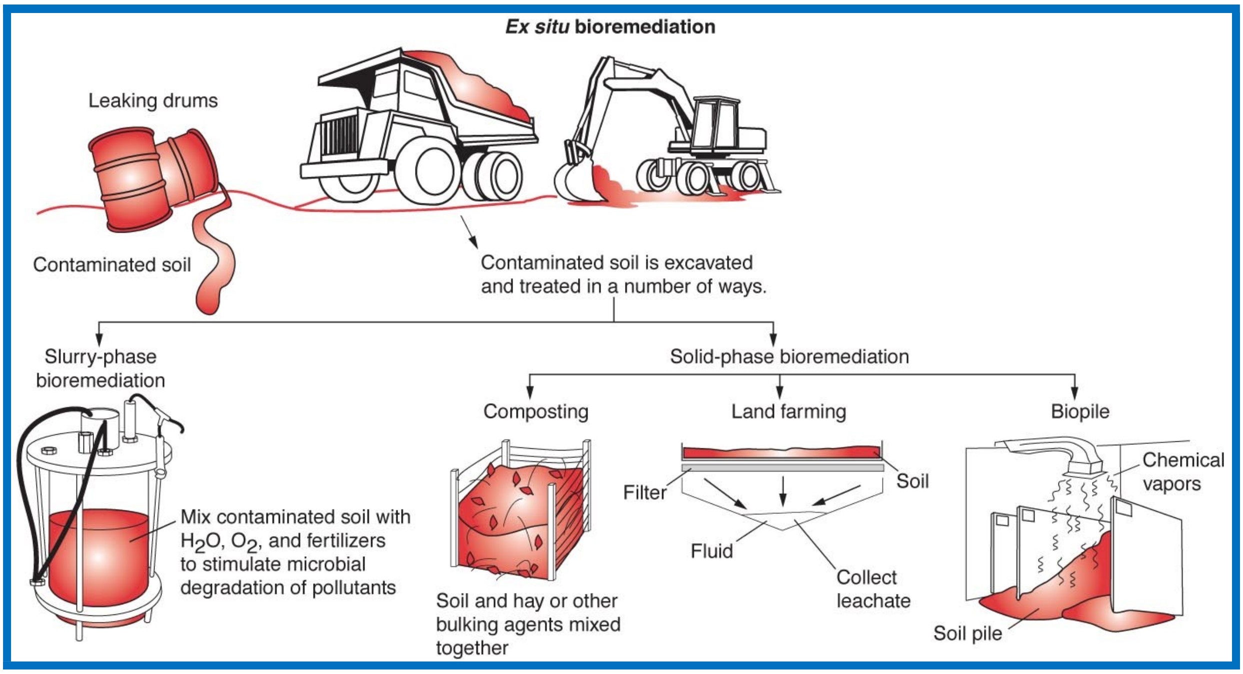
These techniques involve the excavation or removal of contaminated soil from ground.

1. ***Landfarming*** is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded (The soil is spread in thin lifts up to 1/2-inch thick). The goal is to stimulate indigenous bio-degradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil.
2. ***Composting*** is a technique that involves combining contaminated soil with nonhazardous organic amendants such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting.
3. ***Bioreactors:*** Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas)

mixing condition to increase the bioremediation rate of soil bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants.

In general, the rate and extent of biodegradation are greater in a bioreactor system than in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable.





Advantages of bioremediation

Bioremediation has the following advantages:

- It may result in complete degradation of organic compounds to nontoxic byproducts.
- There are minimum mechanical equipment requirements

- It can be implemented as in-situ or ex-situ process. In-situ bioremediation is safer since it does not require excavation of contaminated soils. Also, it does not disturb the natural surroundings of the site.
- Low cost compared to other remediation technologies.

Disadvantages of bioremediation

Bioremediation has the following disadvantages:

- There is a potential for partial degradation to metabolites that are still toxic and/or potentially more highly mobile in the environment.
- The process is highly sensitive to toxins and environmental conditions.
- Extensive monitoring is required to determine biodegradation rates.
- It may be difficult to control volatile organic compounds during ex-situ bioremediation process
- Generally requires longer treatment time as compared to other remediation technologies.