Lab1/ Bioremediation

Bioremediation

Bioremediation is the use of microorganism metabolism to remove pollutants. Bioremediation can occur on its own or can be encouraged via the addition of fertilizers to increase the bioavailability within the medium (biostimulation).
Microorganisms used to perform the function of bioremediation are known as bioremediators.

It can be classified as: 1) in situ: can be used at the site of contamination

2) ex situ: contamination removed from the original site

Some of bioremediation technologies are phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

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

Lab1/ Bioremediation

Microorganisms for Bioremediation

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, these can be used to degrade or remediate environmental hazards.

These microorganisms can divided into the following groups:

1) 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 compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

2) 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.

3) 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.

4) 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 aliphatics trichloroethylene and 1,2-dichloroethane.

Bioremediation strategies

1) *In-Situ* Bioremediation: *In situ* bioremediation is the application of biological treatment to the cleanup of hazardous chemicals present in the subsurface. And can be divided:

a) 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.

b) Bioventing: is a promising new technology that stimulates the natural *in-situ* biodegradation of any aerobically degradable compounds within the soil by providing oxygen to existing soil microorganisms.

c) Bioaugmentation: Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water. At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene**,** bioaugmentation is used to ensure that the *in situ* microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic.

d) Biopiling: Biopile treatment is a full-scale technology in which excavated soils are mixed with soil amendments, placed on a treatment area, and bioremediated using forced aeration.

Biopile

2) *Ex-Situ* Bioremediation

a) Composting: is a process by which organic wastes are degraded by microorganisms, typically at elevated temperatures. Typical compost temperatures are in the range of 55° to 65° C. The increased temperatures result from heat produced by microorganisms during the degradation of the organic material in the waste.

b) Bioreactors: Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water .

Note: 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.

Bioslurry reactor

Water and gas bioremediation

Biofiltration is a process, in which, microorganisms supported on inert materials are used to degrade organic pollutants for air, gas and water bioremediation.

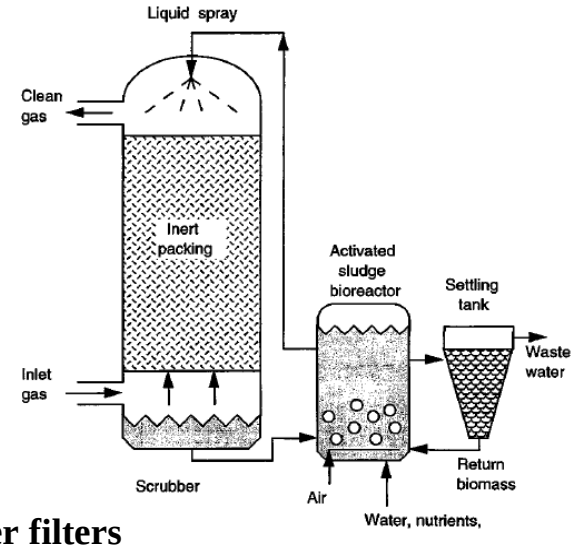
Types of biofilters:

1- Bioscrubbers.

2- Biotrickling filters.

3- Slow sand or carbon filters.

Lab1/ Bioremediation



Bioscrubber filters

Advantage of Bioremediation

1) It is a natural process

2) It is requires a very less effort and can often be carried out on site, often without causing a major disruption of normal activities.

3) It is less cost effective process than the other conventional methods

4) It is helps in complete destruction of the pollutants

5) It does not use any dangerous chemicals.

Disadvantage of Bioremediation

1) It is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.

2) It is takes longer than other treatment options, such as excavation and removal of soil or incineration.

3) There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.

4) Contaminants may be present as solids, liquids, and gases so are needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants.

Lab2/ Bioremediation

Microbial bioremediation of oil

Harmful chemical compounds are consistently introduced into the environment. Human activities, especially spills, waste, and pollution increase widespread contamination and lead to ecological damage. The presence and the concentration of these chemical compounds pose a threat to the biosphere, therefore decontamination of the environment is important.

Some of the chemicals that can contaminate the seas, groundwater, and soil are fortunately removed by naturally occurring microorganisms. These organisms prevent contaminants such as organic waste, heavy metals, and oil from entering the food chain.

Oil spills of crude petroleum pose serious threat to both flora and animals in the marine environment. Oils spills can be removed naturally from the environment through a process called bioremediation.

In the break down of the oil, the bacteria absorb oxygen and nutrients, then the hydrocarbons are spilt into fatty acids and further broken down to yield non-toxic by-products including metabolites, carbon dioxide, and water. Oxygen and nutrients are vital to oil-eating microbes or **OEMs** for proper degradation.

The hydrocarbons in oil are natural carbon compounds found in the environment. In the bioremediation of oil, the organisms involved are **oleophilic** (oil-loving) bacteria or *oil eating microbes* (OEMs). Oleophilic bacteria are

normally found in marine environments, but soil is also an excellent source for OEMs.

In order to observe the action of these microbes. You will simulate the process of bioremediation using OEMs to digest food or car oils under varying conditions (Light, Dark, Aerated, Sealed, Temperature). A dye indicator is used to detect active OEMs growing in culture containing oils. A colorless dye, tetrazolium indicator, turns red when bacteria are metabolically active in culture. You can observe the ideal optimal conditions in which OEMs are metabolically active in the breakdown of oil by simply observing the various concentrations of the red dye in the medium, rather than measuring the oil content.

Materials:

1. 100 ml Culture flask of bacteria from Rid-X
2. car engine oil (6 different types; 1 type/group)
3. (10) Test tubes (10ml) with push caps
4. (2)-10 ml disposable pipettes
5. 1ml pipette tips, pipettes
6. 5 ml of Peptone Nutrient solution (0.1, 1.0, and 2%) 1 per table.
7. Foil
8. Tetrazolium dye
9. Sterile Mineral oil
10. Parafilm
11. 15ml of Sterile Deionized water per group
12. Incubators at 4°C, 30°C, and 55°C.

Lab2/ Bioremediation

Methods

1. Work in groups, each group receives a different type of oil.

2. Label all your test tubes with the appropriate condition. You will be testing the following conditions:

a. Light (25°C)

b. Dark (25°C)

c. 4°C, 30°C, and 55°C

d. Nutrient Solution 1 (0.1% Peptone)

e. Nutrient Solution 2 (1.0% Peptone)

f. Nutrient Solution 3 (2% Peptone)

g. Aerobic

h. Anaerobic

3. For the tube labeled "Dark" use a piece of foil paper in order to fully cover the test tube.

4. Use a 1ml pipette and add 1ml of sterile deionized water into each tube.

5. Use a 1ml pipette to transfer 1 ml of the oil into each tube.

6. Use a 1ml pipette to add 1ml of OEM culture into each tube.

7. Place 1ml of nutrient solution 1 into its respective tube. Repeat this process for the other nutrient solution concentrations.

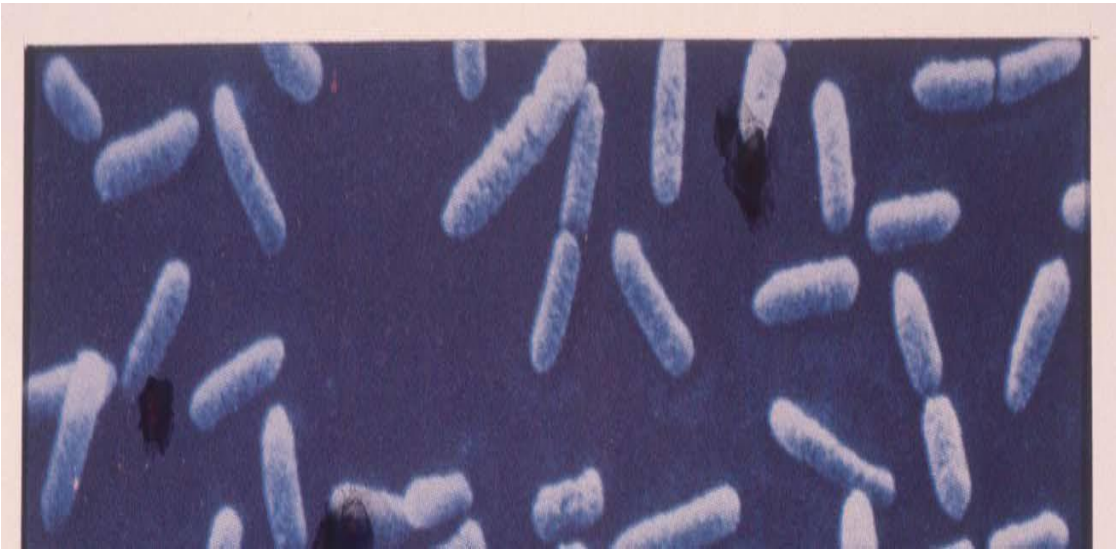
8. Add 0.1 ml of Tetrazolium dye into each tube and stir slightly.

9. Place caps over all the tubes.

10. For the anaerobic tube slowly pipette 1ml of sterile mineral oil onto the top of the liquid, cover with parafilm over and then the push cap.

11. Place each of your tubes in the correct tube racks as labeled by your TA. All other tubes unless noted are to be incubated at 30°C.

12. Next lab, observe each of your tubes and mark -, +, ++, and +++ on your data sheet according to the amount of growth present (if any) as indicated by the color of the dye.

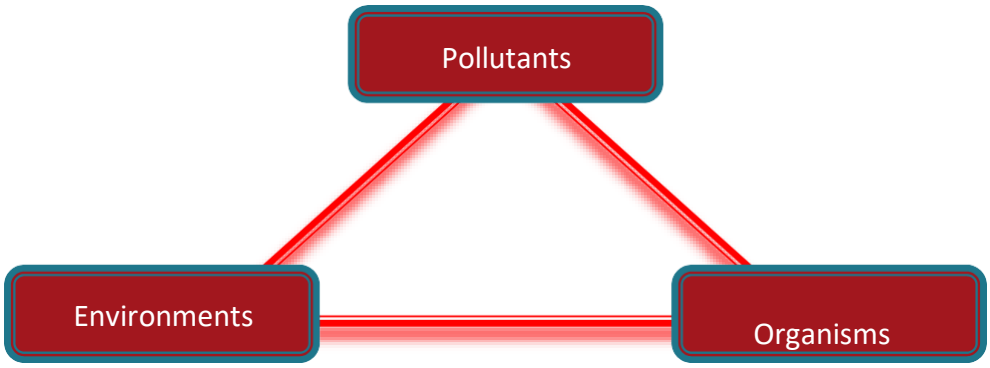


Oil-Eating Bacteria

Lab3/ Bioremediation

Methods and applications of Bioremediation

- Bioremediation is a triple-corners process:



Stages of a biodegradation:

1- Isolation of the microorganism

2- Purification of the obtained isolates

3- Identification of the microbial isolate

4- Optimization of the biodegradation conditions

5- Determination of the biodegradation efficiency

6- Identification of the biodegradation products.

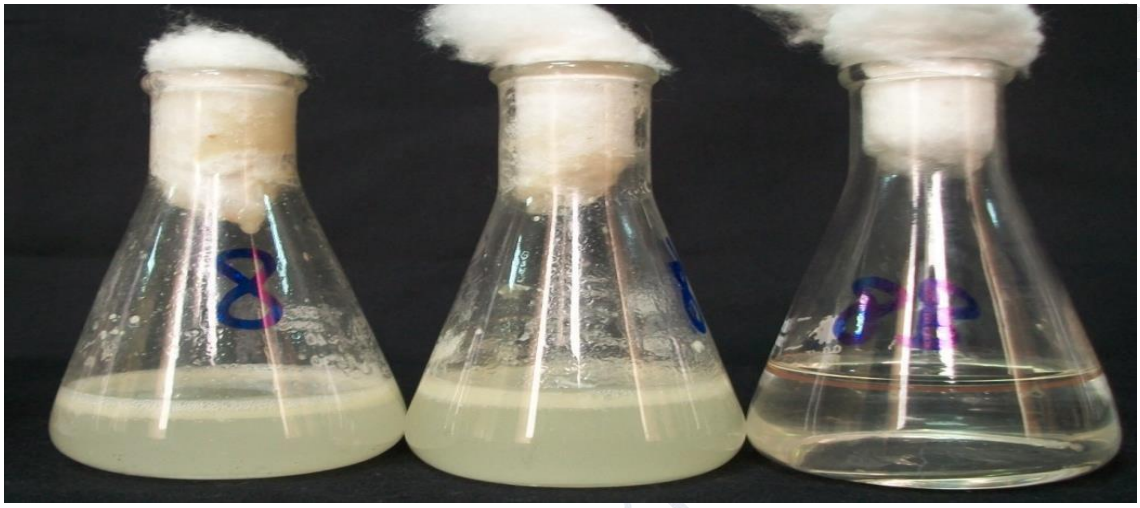
7- Cell or enzyme immobilization

8- Enzyme identification

Lab3/ Bioremediation

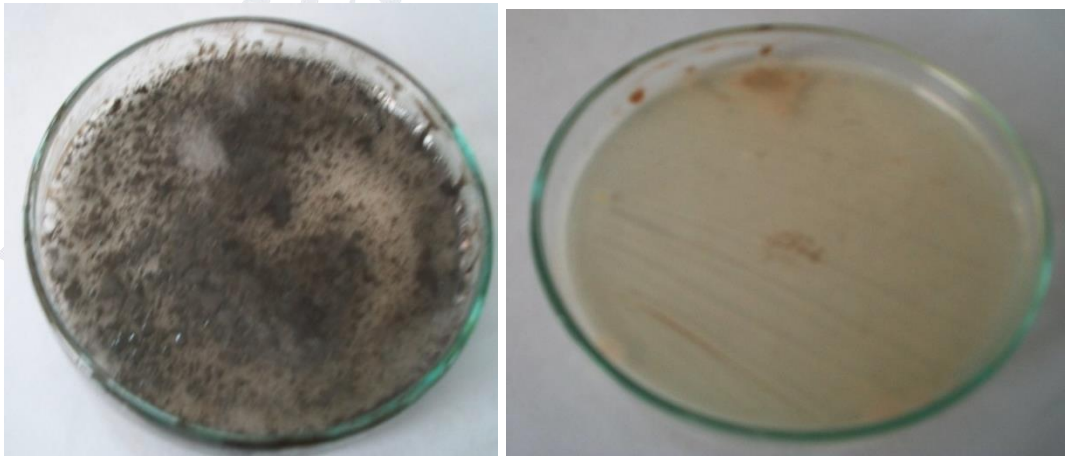
1- Isolation of the microorganism

Isolation of bacteria and fungi is performed on Mineral Salt Medium (MSM) supplemented with the pollutant to be biodegraded as a sole source of carbon (enriched technique). This medium can be used as liquid or solid.



Soil **pollutant** **Control**

Isolation of petroleum biodegrading bacteria from soil and petroleum pollution.



Isolation of fungi (A) and bacteria (B) from the pollutant on solid MSM

The medium has a pH 7 is preferred for isolation of bacteria, while pH 4.5– 5.5 for fungi.

2- Purification of the obtained isolate

This is simply performed by streaking of a lope of the MSM microbial culture or by pouring dishes technique.

The used medium is solid MSM supplemented with the pollutant as a sole source of carbon.

Purification by pouring

Purification by streaking

3- Determination of biodegradation efficiency and products:

a- Spectrophotometer.

b- HPLC

c- GC/MS

Lab3/ Bioremediation

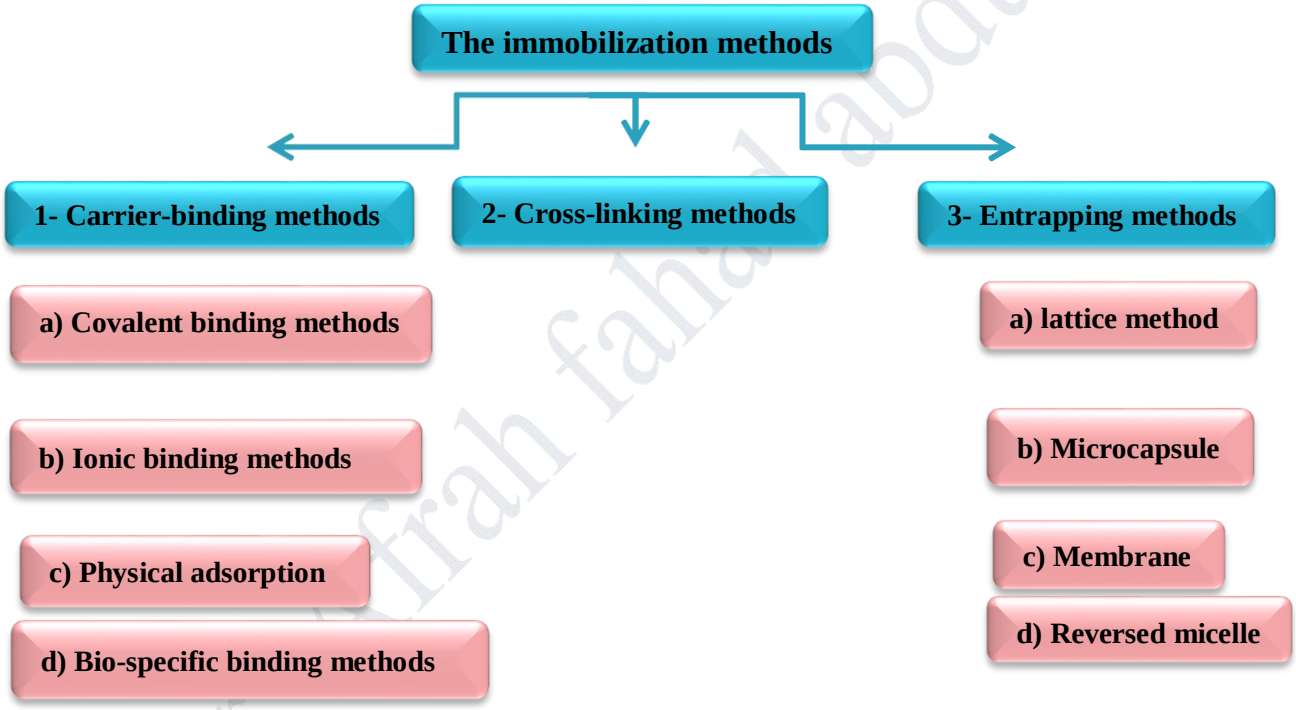
4- Using the redox indicator 2,6-dichlorophenol indophenol (DCPIP).

- The principle of this technique is that, during the microbial oxidation of the carbon source, electrons are transferred to electron acceptors such as O_2, nitrates and sulphate.

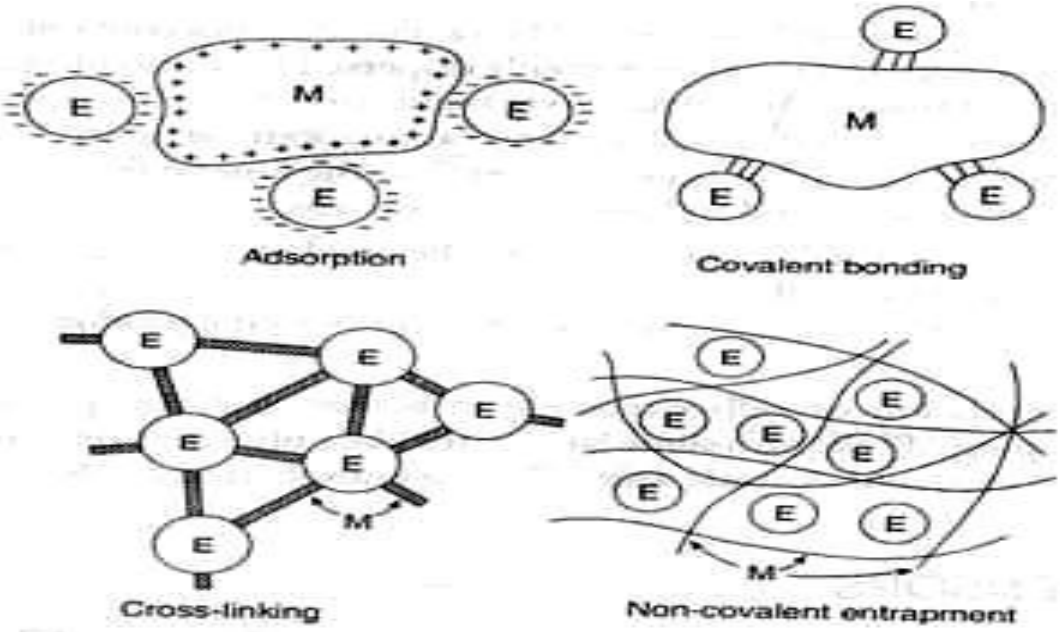
- DCPIP is an electron acceptor.

- The efficiency is determined by observing the color c hange of DCPIP from blue (oxidized) to colorless (reduced).

5- Cells and Enzymes immobilization:



Lab3/ Bioremediation



Lab4/ Bioremediation

Role of Microbial Enzymes in the Bioremediation of Pollutants

The process of bioremediation mainly depends on microorganisms which enzymatically attack the pollutants and convert them to innocuous products.
As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

A large number of enzymes from bacteria, fungi, and plants have been reported to be involved in the biodegradation of toxic organic pollutants.

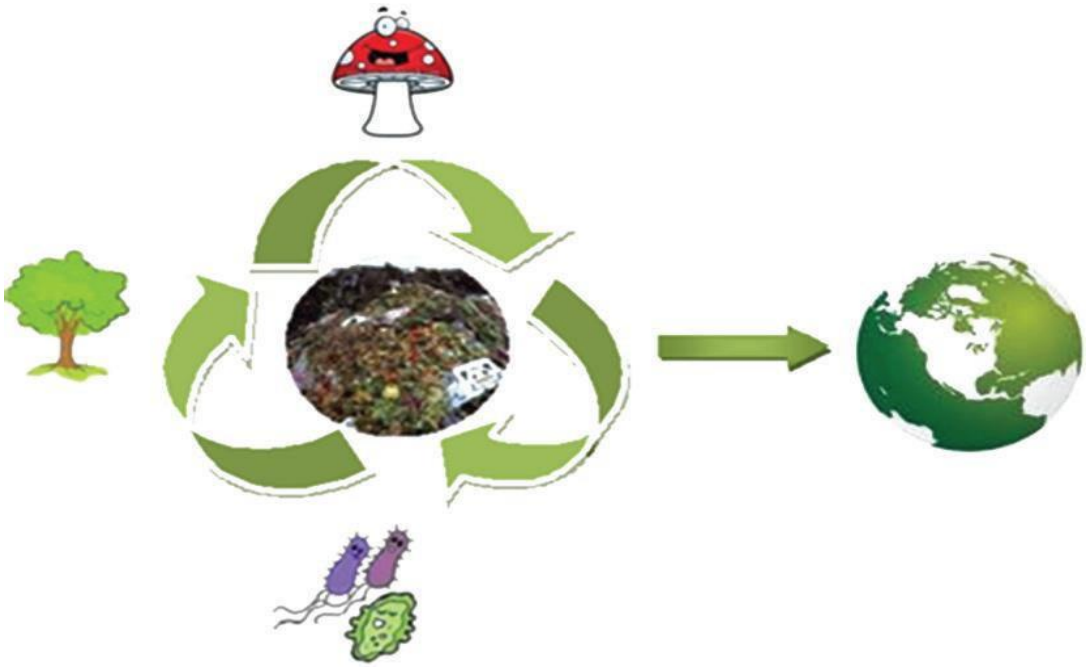


Figure 1: The process of waste bioremediation.

Lab4/ Bioremediation

Enzymes: are biological catalysts that facilitate the conversion of substrates into products by providing favorable conditions that lower the activation energy of the reaction.

An enzyme may be a protein or a glycoprotein and consists of at least one polypeptide moiety. The regions of the enzyme that are directly involved in the catalytic process are called the active sites.

An enzyme may have one or more groups that are essential for catalytic activity associated with the active sites through either covalent or noncovalent bonds; the protein or glycoprotein moiety in such an enzyme is called the apoenzyme, while the nonprotein moiety is called the prosthetic group. The combination of the apoenzyme with the prosthetic group yields the holoenzyme.

All known enzymes fall into one of these six categories. The six main divisions are (1) the oxidoreductases, (2) the transferases, (3) the hydrolases, (4) the lyases, (5) the isomerases, and (6) the ligases (synthetases).

Oxidoreductases catalyze the transfer electrons and protons from a donor to an acceptor. Transferases catalyze the transfer of a functional group from a donor to an acceptor. Hydrolases facilitate the cleavage of C–C, C–O, C–N, and other bonds by water.

Lyases catalyze the cleavage of these same bonds by elimination, leaving double bonds (or, in the reverse mode, catalyze the addition of groups across double bonds).

Isomerases facilitate geometric or structural rearrangements or isomerizations. Finally, ligases catalyze the joining of two molecules.

Microbial Enzymes in Bioremediation

1) *Microbial Oxidoreductases*:

The detoxification of toxic organic compounds by various bacteria and fungi and higher plants through oxidative coupling is mediated with oxidoreductases. Chlorinated phenolic compounds are among the most abundant recalcitrant wastes found in the effluents generated by the paper and pulp industry.

These compounds are produced upon the partial degradation of lignin during pulp bleaching process. Many fungal species are considered to be suitable for the removal of chlorinated phenolic compounds from the contaminated environments. The activity of fungi is mainly due to the action of extracellular oxidoreductase enzymes which are released from fungal mycelium into their nearby environment.

2) Microbial Laccases:

Laccases (*p*-diphenol:dioxygen oxidoreductase) constitute a family of multicopper oxidases produced by certain plants, fungi, insects, and bacteria, that catalyze the oxidation of a wide range of reduced phenolicand aromatic substrates with concomitant reduction of molecular oxygen to water. Laccases are known to occur in multiple isoenzyme forms each of which is encoded by a separate gene, and in, some cases, the genes have been expressed differently depending upon the nature of the inducer.

3) Microbial Peroxidases.

are ubiquitous enzymes that catalyze the oxidation of lignin and other phenolic compoundsat the expense of hydrogen peroxide (H2O2) in the presence of a mediator.

These peroxidases can be haem and nonhaem proteins. In mammals, they are involved in biological processes such as immune system or hormone regulation. In plants, they are involved in auxin metabolism, lignin and suberin formation, cross-linking of cell wall components, defense against pathogens, or cell elongation.

Lab5/ Bioremediation

Phytoremediation Technology in Remediation of Contaminated Soils

Phytoremediation: eco-friendly approach that utilizes the natural properties of plants to remediate contaminated soils. By growing plants in the contaminated sites, contaminants in soils will be removed, immobilized, or degraded, and the cost is much less expensive than other traditional methods.

Approximately 400 plant species have been classified as hyperaccumulators of heavy metals, such as grasses, sunflower, corn, hemp, flax, alfalfa, tobacco, willow, Indian mustard, poplar, water hyacinth, etc.

Phytoremediation of contaminated soils is generally believed to occur through the following mechanisms:

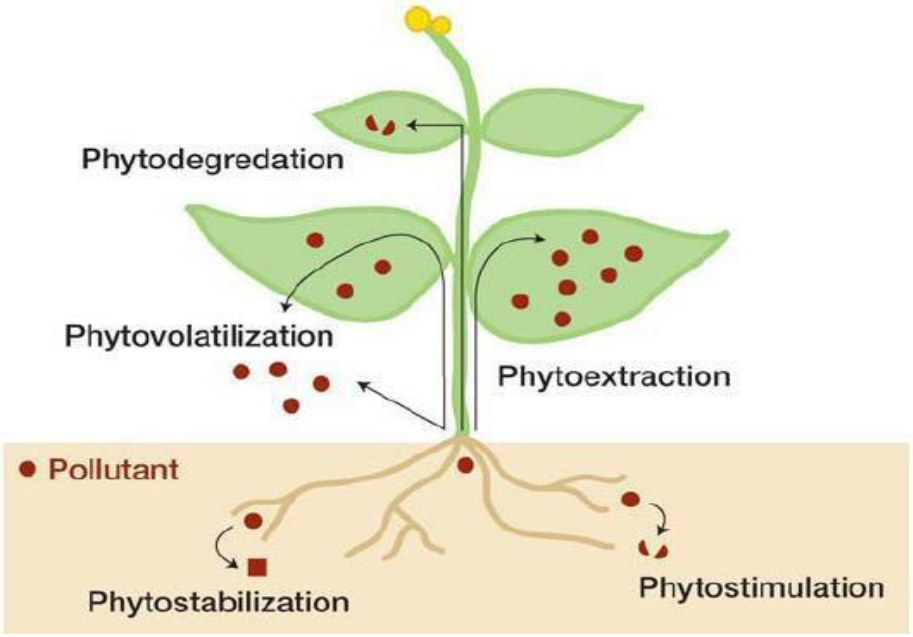
1) phytoextraction: Plants absorb contaminants and store in above-ground shoots and the harvestable parts of roots.

2) phytostabilization: Roots and their exudates immobilize contaminants through adsorption, accumulation, precipitation within the root zone, and thus prevent the spreading of contaminants.

3) phytodegradation: Plant enzymatic breakdown of organic contaminants, both internally and through secreted enzymes.

4) phytovolatilization: Contaminants taken up by the roots through the plants to the leaves and are volatized through stomata where gas exchange occurs.

5) rhizodegradation or phytostimulation: Plant roots stimulate soil microbial communities in plant root zones to break down contaminants.



Mechanisms of Phytoremediation

Phytoremediation is applicable to a broad range of contaminants, including

1) heavy metals

2) radionuclides

3) organic compounds like chlorinated solvents, polycyclic aromatic hydrocarbons, pesticides/insecticides, explosives, and surfactants.

Phytoremediation processes depend on the ability of plants to take up and metabolize pollutants to less toxic substances. The uptake, accumulation and degradation of contaminants vary from plant to plant.

Lab5/ Bioremediation

The plants used in phytoremediation are generally selected on the basis of

1) Their growth rate and biomass

2) Their ability to tolerate and accumulate contaminants

3) The depth of their root zone, and their potential to transpire groundwater

4) Plant should grow quickly in a wild range of different conditions.

The most positive characteristics of phytoremediation

1) A natural and *in situ* (does not need to remove the soil out of the Place) remediation system driven by solar and green plants.

2) It is faster than natural attenuation and can conserve the soil resources

3) It is inexpensive, and does not induce the secondary contamination

4) Reduce movement of pollutants towards groundwater

5)Sustains the soil structure, and enhance the soil quality and productivity

6) Soils followed phytoremediation are still or more suitable for its original application particularly for agricultural application, thus preventing the loss of soil resources

7) The costs are very low in comparison to current other physical or chemical methods

Phytoremediation process of inorganic contaminants, and the means for enhanced phytoremediation.

Phytoremediation of various inorganic pollutants such as Cd, Cr, Pb, Cu, Zn, Co, Ni, Se, Cs and As. This is mainly based on the use of natural hyperaccumulator plants that characterized with exceptional metal-accumulating capacity, the ability to

accumulate metals in their shoots and an exceptionally high tolerance to heavy metals.

At present, there are totally more than 400 species of hyperaccumulator plants for As, Cd, Mn, Ni, Zn etc. have been found. For example of some hyperaccumulator plants and their accumulation concentration (mg/kg dw:dry weight) for various metals are: Thlaspi caerulescens, 51600 for Zn and 18000 for Cd; Ipomea alpine, 12300 for Cu; and Pteris vitatta, 20,000 for As

Enhanced phytoremediation generally includes the following several ways:

1) Enhanced heavy metal phytoextraction with chemicals: This method is to increases mobility of metals in soil by application of chelating agents or surfactants, such as citric acid, EDTA, and NTA, so that the metals can be taken up more easily by plants

2) Utilization of Genetically engineered plants: through genetically altering the high biomass plants to extract larger amounts of metal from soils, or improving the biomass production of some hyperaccumulator plants.

3) Agricultural work techniques: this method is to enhance phytoremediation efficiency by promoting plant growth and microbial activities with suitable fertilization, carbon source addition, or cultivation systems.

4) Using plant-microbe combination systems: The rhizosphere is inoculated with new microorganism which is more effective in degrading the contaminant than the local microflora. Good plant-microbe combination can promote the activity of the effective microbes and the plant growth. It is also reported that inoculation of mycorrhizae to some plants may promote the uptake, translocation and accumulation of soil metals

Lab6/ Bioremediation

Biodegradation of Pesticides

Biodegradation: Breakdown of a substance catalyzed by enzymes *in vitro* or *in vivo*. The Biodegradation characterized depending on the purpose of hazard assessment:

1) Primary biodegradation: Alteration of the chemical structure of a substance resulting in loss of a specific property of that substance.

2) Acceptable biodegradation: remove undesirable properties of the compound. This often corresponds to primary biodegradation but it depends on the circumstances under which the products are discharged into the environment.

3) Ultimate. Complete breakdown of a compound to either fully oxidized or reduced simple molecules (such as carbon dioxide/methane, nitrate/ammonium and water).

Type of pesticides

1) Organochlorine pesticides

The organochlorine pesticides are known to be highly persistant in the environment. This class of pesticides includes the chlorinated derivatives of diphenyl ethane. for example: DDT and chlordane

DDT is the most well known pesticide from the organochlorine group. The use of organochlorine pesticides started in 1939 was an efficient insecticide, its low water solubility, its high persistence in the environment and its mode of action, unknown until that moment.

Lab6/ Bioremediation

Microbial degradation of organophosphate pesticides

The fate of pesticides in the environment is determined by both biotic and abiotic factors.

The rate at which different pesticides are biodegraded varies widely. Some pesticides suchas DDT and dieldrin remain in the environment for a long time and accumulate into food chains for decades after their application to the soil.

The degradation of organochlorine pesticides by *Pseudomonas* sp. have been studied, the bacteria was isolated from a soil sample .DDT-metabolising microbes have been isolated from a range of habitats, including soil, sewage, activated sludge, and marine and freshwater sediments.

Biodegradation of DDT residues involves co-metabolism, that is, it requires the presence of an alternative carbon source, in which microorganisms growing are able to transform DDT residues without deriving any nutrient or energy for growth from the process.

Some of the microorganisms that were able to degrade organochlorine pesticides belonging to genera *Bacillus, Pseudomonas, Arthrobacter* and *Micrococcus*.

Fungi also have the ability for biodegradation of organochlorine pesticides for example *Penicillium*, *Aspergillus*, and *Trichoderma* sp.

2) Organophosphate pesticides (OP)

The organophosphorus pesticides (**OP**) are all esters of phosphoric acid and form the major and most widely used group that accounts for more than 36% of the total world market. The most used among these is methyl parathion. Most OP compounds are degraded by microorganisms in the environment as a source of phosphorus and carbon

The **OP** possess an efficient insecticide activity, due to its characteristic of irreversibly inhibiting the enzyme acetylcholinesterase in the nervous system, which acts in both insects and in mammal.

In man, the organophosphates are absorbed through all routes, reaching high concentrations in fatty tissues, liver, kidneys, salivary glands, thyroid,
pancreas, lungs, stomach, intestines and, at smaller proportions, in the central nervous system (SNC) and muscles.

Microbial degradation of organophosphate pesticides

Methyl parathionis widely used throughout the world and its residues are regularly detected in a range of fruits and vegetables. Bacteria with the ability to degrade methyl parathion have been isolated worldwide.

The OP pesticides can be hydrolyzed and detoxified by carboxylesterase and phosphotriesterase enzymes. Two bacteria identified as *Pseudomonas putida* and *Acinetobacter rhizosphaerae,* able to rapidly degrade the organophosphate

Lab7/ Bioremediation

3) Carbamate pesticides

Carbamates were introduced as pesticides in the early 1950s and are still used extensively in pest control due to their effectiveness and broad spectrum of biological activity (insecticides, fungicides, herbicides).

Chemically, the carbamate pesticides are esters of carbamates and organic compounds derived from carbamic acid. characteristics of carbamate pesticides are high polarity and solubility in water, thermal instability and high acute toxicity.

Microbial degradation of carbamate pesticides

Carbofuran is one of the pesticides belonging to the *N*-methylcarbamate class used extensively in agriculture, a number of bacteria capable of degrading carbofuran from the environment (*Pseudomonas*, *Flavobacterium*, *Achromobacterium*, *Sphingomonas*, *Arthrobacter*).

4) Biological pesticides

biopesticides are defined as naturally occurring pest control substances. They are classified into three groups:

a) Microbial pesticides: in which a microbial living organisms (bacteria, fungi, viruses, protozoans) is the active control agent

b) Plant pesticides: pesticidal substances produced by plants from introduced genetic material (plant incorporated protectants)

c) Biochemical pesticides: naturally occurring substances that control pests by nontoxic mechanisms. These include substances that interfere with growth or mating such as pheromones.

Microbiological control is supported by beneficial interactions resulting from competition, antagonism and parasitism of microorganisms against plant pathogens, insects and weeds.

Microorganisms are able to suppress pests by producing a toxin, causing a disease or preventing the establishment of other organisms. Currently, several microorganisms involved in such processes are the active ingredient of microbial pesticides.

a) Bacteria

The majority of commercial microbial insecticides are preparations based on strains of *Bacillus thuringiensis* (Bt) that produces a crystalline inclusion body during sporulation.

The crystal proteins (Cry proteins) are toxic to many insects and are defined as endotoxins (Bt toxin) that are generally encoded by bacterial plasmids

Cry proteins are produced as protoxins that are proteolytically converted into a combination of up to four smaller toxins upon ingestion.

These proteins bind to specific receptors in the larval midgut epithelium causing the formation of large cation-selective pores that increase the water permeability of the cell membrane.

A large uptake of water then causes cell swelling and rupture of the midgut. Poisoned insects can die quickly from the toxin activity or may die within 2-3 days from septicemia due to the entering of gut contents into the bloodstream.

Bacteria belonging to other genera such as *Pseudomonas fluorescens*, *P. putida*, *P. Chlororaphis* and *Burkholderia cepacia* have also been used as biopesticides

b) Fungi

Fungi often act as important natural control agents against insects, pathogenic fungi, nematodes and as herbicide. Many fungi utilized as biopesticides are pathogenic to insect.

Fungi can act as insecticide by two ways:

a) Infection: most of the fungi species cause death to the insect through spores called conidia.

b) Mycotoxins: another fungi mode can cause death of the host by the production of mycotoxins, which can interfere in the nervous system of insects.

c) Viruses

Virus-based biopesticides have been used as insect control agents. Baculoviruses are a large virus group belonging to the family *Baculoviridae* and can infect different insect orders, particularly Lepidoptera and Diptera. The commercial formulations that have been used include Granulosis virus

d) Protozoa

Some protozoan pathogens can kill insect hosts. One important consequence of protozoan infection is the reduction in the number of offsprings by the infected insects. Species of the genera *Nosema sp*. offer the greatest biopesticide potential.