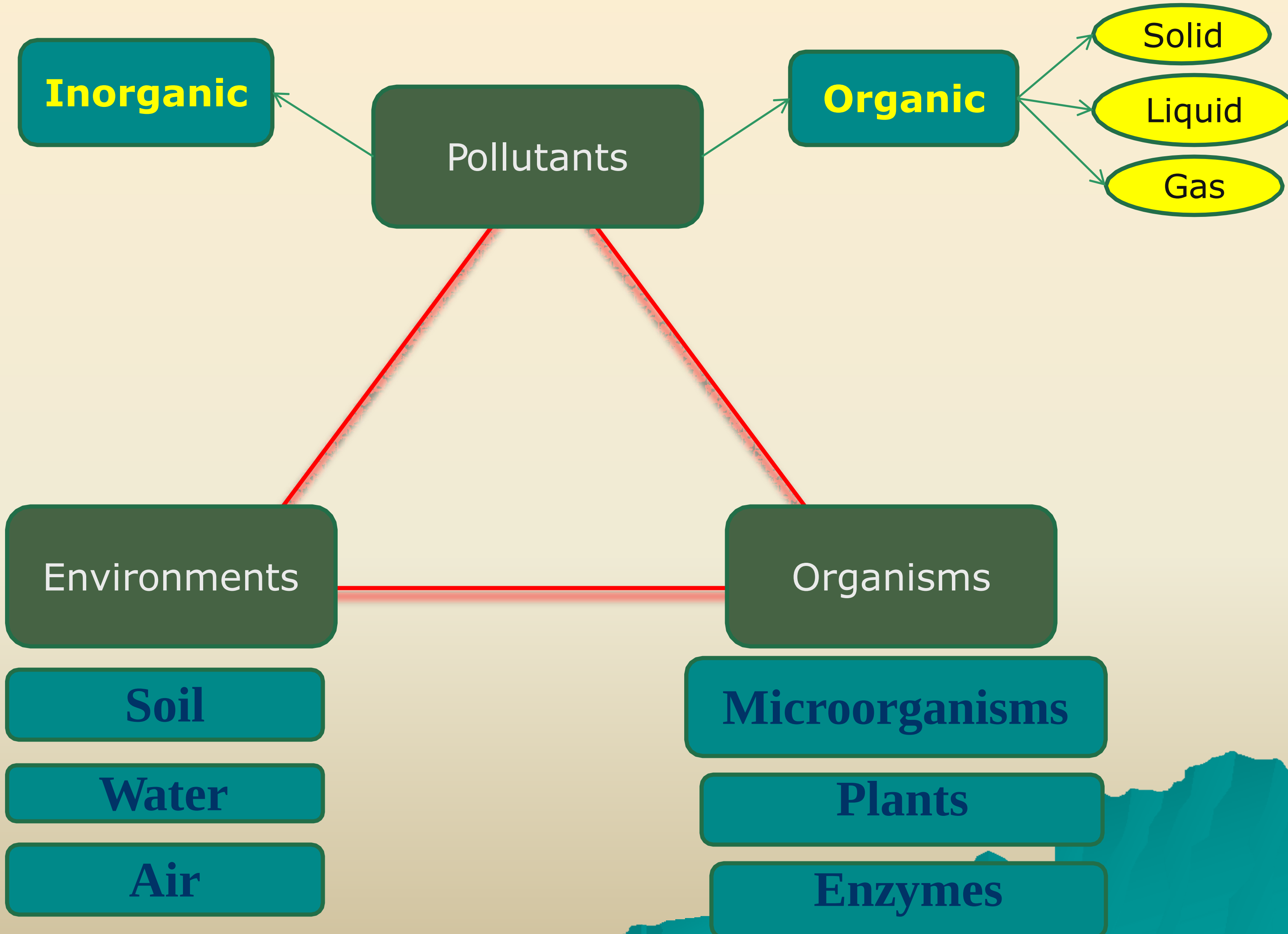
Definition as per Environmental Protection Agency (EPA)

- Biodegradation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities.
- It uses naturally occurring microorganisms like bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment.

Definitions

- Bioremediation is any process that uses organisms (microorganism, algae and plant) or their enzymes to remove the desired pollutant and to return the polluted environment to its original condition.
- Biodegradation is the use of these organisms in the degradation of different pollutants.
- Xenobiotic compounds are chemical compounds found in an organism but it is not normally produced or expected to be present in it.
- Cometabolism: in this process the microorganism produces an enzyme to utilizes its nutrients, but by chance this enzyme can degrade a pollutant.

Bioremediation is a triple-corners process:



Types of Biodegradation

Different kinds of remediation technologies are currently being used for soil treatment and many more innovative approaches involving bioremediation are being developed. few examples

- **Natural Attenuation**
- **Aerobic biodegradation**
- **Anaerobic biodegradation**

Natural attenuation refers to **processes that naturally transform contaminants to less harmful forms or immobilize contaminants so that they are less of a threat to the environment**

These processes include aerobic and anaerobic biodegradation, sorption, volatilization, and chemical or biological stabilization, transformation of contaminants.

For ex. *Cycloclasticus, Pseudomonas, Pseudoalteromonas, Halomonas, Marinomonas* and *Dietzia* play the most important role in PAH mineralization in the deep-sea sediments of Artic Ocean.

Aerobic Degradation

Aerobic biodegradation is the breakdown of organic pollutants by microorganisms when oxygen is present.

Aerobic bacteria (aerobe) have an oxygen grounded metabolism.

Oxygen is used in the chemical responses that break down the pollutant molecules into water and carbon dioxide.

For ex. *Pseudomonas putida is a gram-negative soil bacterium that is involved in the bioremediation of toluene, a component of paint thinner. It is also capable of degrading naphthalene, a product of petroleum refining, in contaminated soils.*

Anaerobic Biodegradation

Itis a series of processes in which microorganisms break down biodegradable material in the absence of oxygen.

It's extensively used to treat waste water sludge and biodegradable waste because it provides volume and mass reduction of the input material.

For ex. *Dechloromonas aromatica is a rod-shaped bacterium which can oxidize aromatics including benzoate, chlorobenzoate, and toluene, coupling the reaction with the reduction of oxygen, chlorate, or nitrate. It is the only organism able to oxidize benzene anaerobically.*

Factors of Bioremediation

The control and optimization of bioremediation process is a complex system of many factors:

- existence of a microbial population
- 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).

Pollutant Quality

The rate of decomposition depends on the structural and chemical properties of litter. For eg., the litter of bryophytes are decomposed at a slower rate due to the presence of lignin like complex chemicals.

Temperature

Temperature regulates the growth and activity of microorganisms. The temperature is different at different elevations. The species diversity and the microorganism count is affected by environmental changes.

Aeration

The oxygen present in the pores of the soil helps in the growth of microorganisms. In the water-logged soils, the aerobic microorganisms are absent. Here only anaerobic microorganisms can grow and initiate decomposition.

Soil pH

The presence of cations and anions governs the pH of the soil, which in turn affects microbial growth.

Inorganic Chemicals

After decomposition, the elements like potassium, sodium, calcium, magnesium are released into the soil. Some of these are used by the microorganisms for their growth. Thus, it affects the rate of decomposition.

Moisture

The water present in the soil is responsible for various physiological processes of microorganisms present in the soil. The growth of microorganisms is thus governed by the presence of moisture in the soil.

Key Features of Bioremediation

- **Most bioremediation treatment technologies destroy the contaminants in the soil matrix.**
- **These treatment technologies are generally designed to reduce toxicity either by destruction or by transforming toxic organic compounds into less toxic compounds.**
- **Indigenous micro-organisms, including bacteria and fungi, are most commonly used. In some cases, wastes may be inoculated with specific bacteria or fungi known to biodegrade the contaminants in question. Plants may also be used to enhance biodegradation & stabilize the soil.**
- **The addition of nutrients or electron acceptors (such as hydrogen peroxide or ozone) to enhance growth and reproduction of indigenous organisms may be required.**

Bioremediation - Technology description

Bioremediation involves the use of micro-organisms to chemically degrade organic contaminants. Aerobic processes use organisms that require oxygen to be able to degrade contaminants. In some cases, additional nutrients such as nitrogen and phosphorous are also needed to encourage the growth of biodegrading organisms. A biomass of organisms – which may include entrained constituents of the waste, partially degraded constituents, and intermediate biodegradation products – is formed during the treatment process (United States Environmental Protection Agency, 1990)

Although bioremediation is applied in many different ways, the description of typical solid phase bioremediation, composting, bio-venting, and traditional in situ biodegradation is provided here, besides the description of a few common bioremediation technologies.

Bioremediation

Bioremediation is the use of microorganisms to destroy or immobilize waste materials

Microorganisms include:

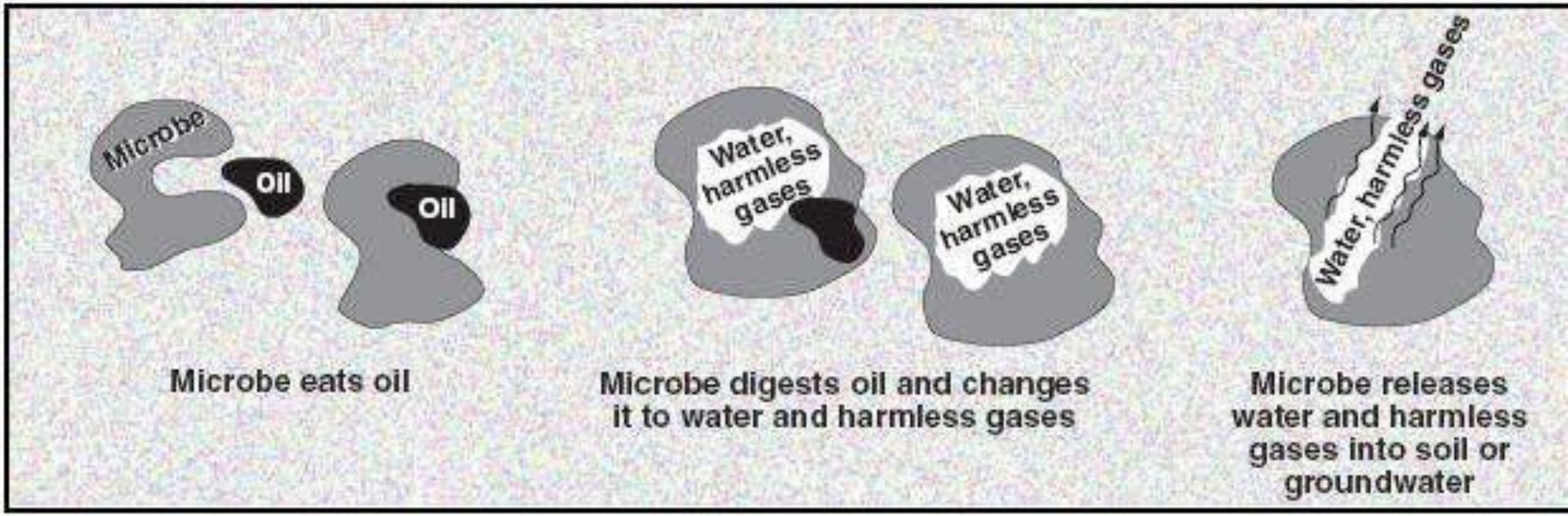
- Bacteria (aerobic and anaerobic)
- Fungi
- Actinomycetes (filamentous bacteria)

Bioremediation – Concept (Contd..)

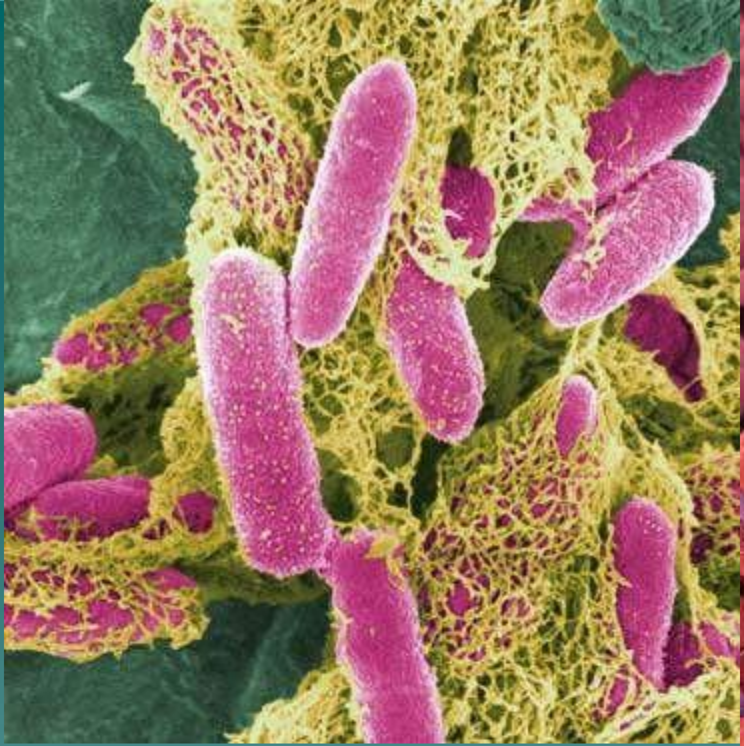
Recent studies in molecular biology and ecology offer opportunities for more efficient biological processes to clean-up of polluted water and land areas

- Bioremediation allows natural processes to clean up harmful chemicals in the environment.
- Microscopic "bugs" or *microbes* that live in soil and groundwater like to eat certain harmful chemicals.
- When microbes completely digest these chemicals, they change them into water and harmless gases such as carbon dioxide.

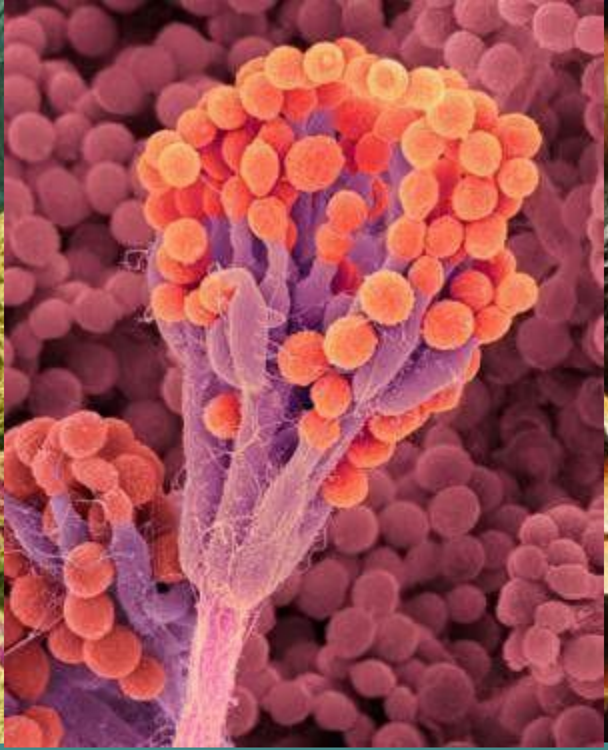
Schematic



Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity.

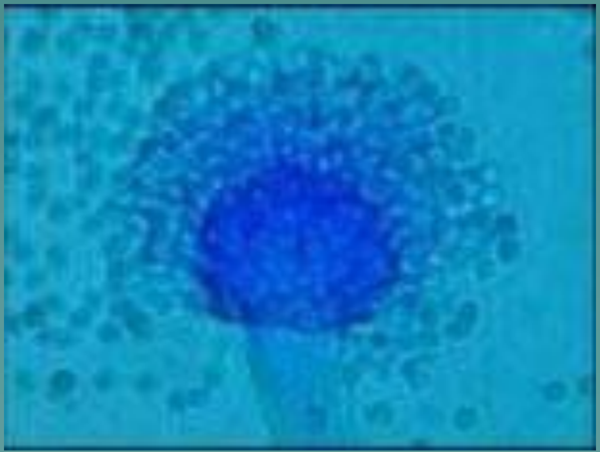


Bacteria

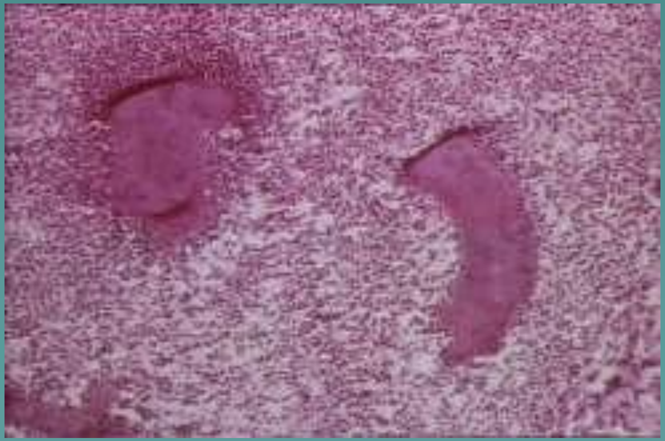


Fungi

Fungi



Actinomycetes



Actinomycetes

Actinomycetes

Bioremediation - Basic facts

- The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site
- Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes.
- Biodegradation of a compound is often a result of the actions of multiple organisms.
- Microorganisms must enzymatically attack the pollutants
- Bioremediation can be effective only where environ-mental conditions permit microbial growth and activity
- Manipulation of environmental parameters needed for microbial growth & degradation to proceed at a faster rate.

Microbial Populations

- 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. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards.

Types of microorganisms

- **Aerobic** Grows in presence of oxygen, degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy. Examples: *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium*.
- **Anaerobic** Grows in absence of oxygen. are not as frequently as aerobic, degrade polychlorinated biphenyls (PCBs), dechlorination of the solvent trichloroethylene (TCE), and chloroform. Examples: *Escherichia coli, Staphylococcus genus, Clostridium genus*
- **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. Examples: *Bacillus methanicus, Pseudomonas methanica, Methanomonas methanooxidans and M ethylococcus capsulatus*

Bioremediation mechanism

Microorganisms destroy organic contaminants in the course of using the chemicals for their own growth and reproduction

Organic chemicals provide:

- carbon, source of cell building material
- electrons, source of energy

Cells catalyze oxidation of organic chemicals (electron donors), causing transfer of electrons from organic chemicals to some electron acceptor

Composition of a microbial cell (%).

Carbon	50	Sodium	1.0
Nitrogen	14	Calcium	0.5
Oxygen	20	Magnesium	0.5
Hydrogen	8.0	Chloride	0.5
Phosphorous	3.0	Iron	0.2
Sulfur	1.0	All others	0.3
Potassium	1.0		

Bio-stimulation

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 & carbon.
- Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells.
- Phosphorous and sulfur contribute with 70% of the remainders. The nutritional requirement of carbon to nitrogen ratio is 10:1, and carbon to phosphorous is 30:1.

Biostimulation (Contd..)

- For degradation it is necessary that bacteria and the contaminants be in contact. This is not easily achieved, as neither the microbes nor contaminants are uniformly spread in the soil.
- Some bacteria are mobile and exhibit a chemotactic response, sensing the contaminant and moving toward it.
- Other microbes such as fungi grow in a filamentous form toward the contaminant.
- It is possible to enhance the mobilization of the contaminant utilizing some surfactants such as sodium dodecyl sulphate

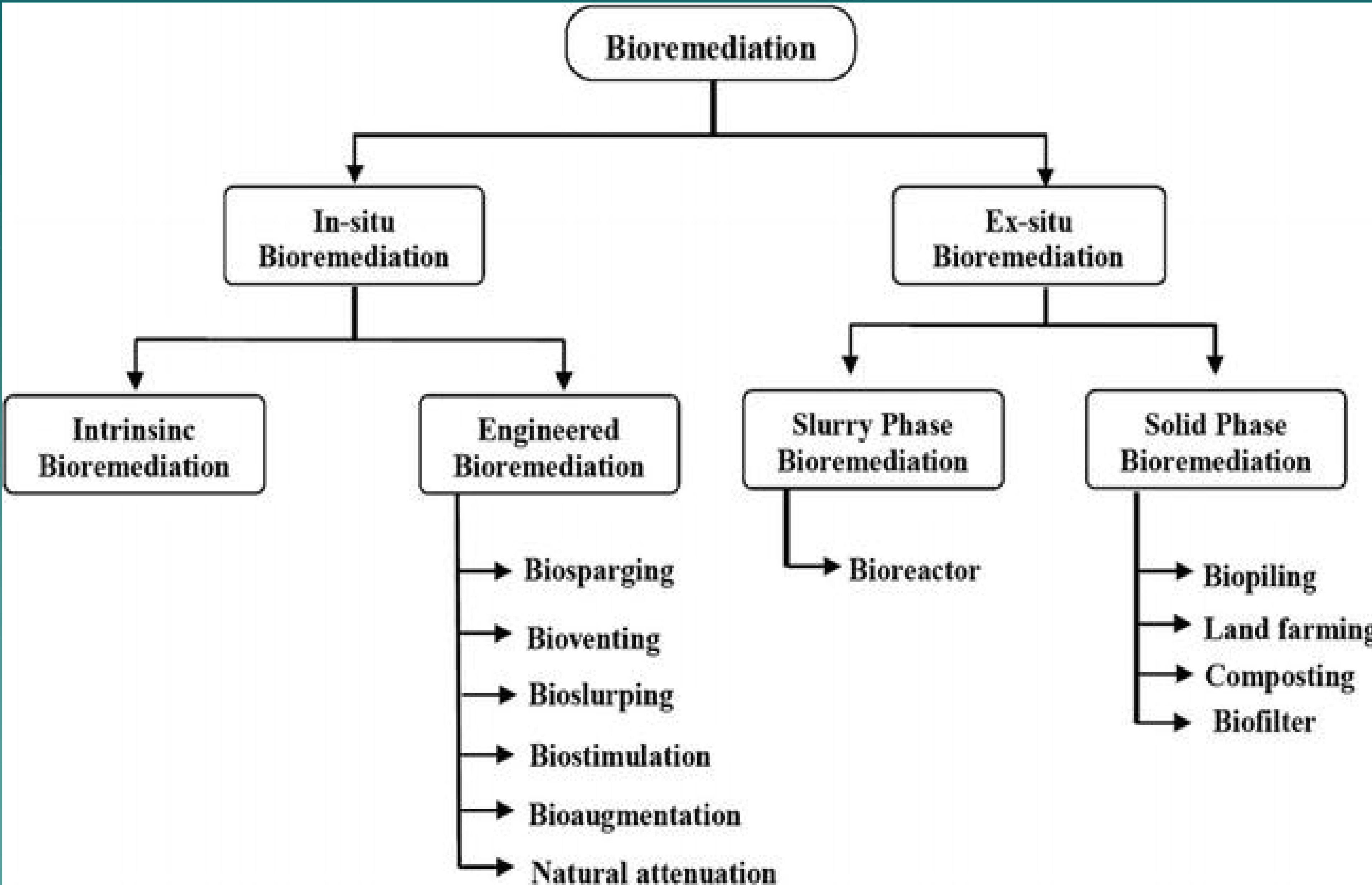
Methods of Bioremediation:

There are two broad classes of bioremediation-

1) In-situ bioremediation – Onsite treatment for detoxification

2) Ex-situ bioremediation- Of site treatment toxic materials

3) Sometimes bioremediation takes place by natural ways & means called Intrinsic bioremediation or natural attenuation.


Bioremediation
In-situ Bioremediation
Ex-situ Bioremediation
Intrinsinc Bioremediation
Engineered Bioremediation
Slurry Phase Bioremediation
Solid Phase Bioremediation
Biosparging
Bioventing
Bioslurping
Biostimulation
Bioaugmentation
Natural attenuation
Bioreactor
Biopiling
Land farming
Composting
Biofilter

In situ Bioremediation Techniques

In situ bioremediation refers to the use of decontamination procedures on-site to clean polluted soil or groundwater with minimal damage to the soil structure.

These bioremediation approaches are cost-effective because excavation processes are avoided. However, the cost of designing and installing complex equipment to increase biotic activity in bioremediation is a major worry.

In situ bioremediation approaches have been employed to detoxify chlorinated solvents, dyes, nutrients, heavy metals, and organic waste sites.

Bioventing, Biosparging and Bioaugmentation are all included in in situ bioremediation techniques.

Bioventing

Bioventing is a form of in situ bioremediation technology that encourages aerobic decomposition.

By delivering oxygen into an unsaturated zone, it improves the innate capacity of indigenous microorganisms to break down organic pollutants adsorbed to soil.

Through vertical and horizontal wells, air is injected directly into the contaminated zone.

Only the amount of air needed for degradation is used in this procedure. It also reduces pollutant volatilization and discharge into the environment.

In the 1990s, bioventing was one of the first large-scale technologies to be implemented, and it is now widely employed in commercial applications.

Biosparging

Biosparging is the process of **pumping pressurized air or gas** into a polluted area to stimulate in-situ aerobic biological activity.

This technology targets chemical substances such as mineral oils and benzene, toluene, ethylbenzene, xylene, and naphthalene (BTEXN) that can be biodegraded under **aerobic conditions** and are used to treat soluble and residual contaminants in the saturated zone.

By giving oxygen to the microorganisms and increasing the interactions between air, water, and the aquifer, the injection of air (and gaseous nutrients if needed) **promotes** the development of the **aerobic microbial population** and thereby enhances the bioavailability of pollutants.

The **goal** of a sparging system is to increase pollutant biodegradation while minimizing volatile and semi-volatile organic compound volatilization.

The air injection flow rate is designed to give the amount of oxygen needed to improve bacterial contamination degradation. However, some volatilization may occur, necessitating air capture and treatment, depending on the operation mode and design chosen.

Bioaugmentation

Bioaugmentation is a type of in-situ bioremediation. It involves researching the local indigenous varieties to see if biostimulation is viable.

Bioaugmentation is the **addition of extra archaea or bacterial cultures to boost pollutant breakdown**, whereas biostimulation is the addition of nutritional supplements to boost bacterial metabolism.

If the indigenous bacteria discovered in the area can metabolize the contaminants, more **indigenous bacterial cultures** will be introduced into the area to speed up the breakdown of the contaminants.

Exogenous microbes with such advanced pathways are introduced if the indigenous variety lacks the metabolic aptitude to undertake the repair procedure.
A number of synthetic and natural organic chemicals and compounds, such as acetone, acrylic acid, ammonia, nitrite, furfural, or hazardous **substances** that can be **handled with bioaugmentation products**

Bioslurping

combines elements of **bioventing and vacuum-enhanced pumping** of free-product to recover free-product from the groundwater and soil, and to bioremediate soils. The bioslurper system uses a "slurp" tube that extends into the free-product layer. Much like a straw in a glass draws liquid, the pump draws liquid (including free-product) and soil gas up the tube in the same process stream. Pumping lifts light non-aqueous phase liquids (LNAPLs), such as oil, off the top of the water table and from the capillary fringe and brought to the surface, where it is separated from water and air.

Intrinsic bioremediation

Intrinsic bioremediation also known as natural reduction is an in-situ bioremediation technique, which involves passive remediation of polluted sites, without any external force (human intervention). This process deals with stimulation of indigenous or naturally occurring microbial population. The process based on both microbial aerobic and anaerobic processes to biodegrade polluting constituents containing those that are recalcitrant. The absence of external force implies that the technique is less expensive compared to other in-situ techniques.

Advantages of in-situ bioremediation

-In-situ bioremediation methods do not required excavation of the contaminated soil.
-This method provides volumetric treatment, treating both dissolved and solid contaminants.
-The time required to treat sub-surface pollution using accelerated in-situ bioremediation can often be faster than pump and treat processes.
-It may be possible to completely transform organic contaminants to innocuous substances like carbon dioxide, water and ethane.
-It is a cost effective method because there is minimal site disruption.

Limitation of in-situ bioremediation

-Depending on specific site, some contaminants may not be absolutely transformed to harmless products.
-If transformation stops at an intermediate compound, the intermediate may be more toxic and/or mobile than parent compound some are recalcitrant contaminants cannot be biodegradable.
-When incorrectly applied, injection wells may become blocked by profuse microbial growth due to addition of nutrients, electron donor and electron acceptor.
-Heavy metals and organic compounds concentration inhibit activity of indigenous microorganisms.
-In-situ bioremediation usually required microorganism's acclimatization, which may not develop for spills and recalcitrant compounds.

Ex-situ Bioremediation Techniques

Ex-situ bioremediation is a biological procedure in which excavated soil is placed in a lined above-ground treatment area and aerated after processing to help the indigenous microbial population degrade organic pollutants.

Organic pollutants such as petroleum hydrocarbon mixtures, polycyclic aromatic hydrocarbons (PAH), phenols, cresols, and some pesticides can be used as a **source of carbon and energy** by specific microorganisms under aerobic circumstances, and then degraded to carbon dioxide and water.

It's rare to have to add microbial populations, but it's common to need to assess nutrient requirements and **supplement** the soil's basic nutrients and organic substrate if any of these elements are insufficient or absent.

To allow the microbial population to grow cultures capable of sustaining deterioration, **oxygen** (through the introduction of air) is required.

Landfarming

-Land farming is the most basic method of bioremediation.

-Contaminated soils are **blended** with soil amendments like bulking agents and fertilizers before being tilled into the ground.They are **excavated** and **spread out** in layers of around 0.3m thickness on a lined treatment area in land farming.

-Periodic flipping of the bed and the addition of nutrients can help with bioremediation.

-Microbiological and oxidative mechanisms degrade, convert, and immobilize contaminants.

-The rate of pollutant degradation is optimized by controlling soil conditions. Moisture content, aeration frequency, and pH are all variables that can be modified.

-Landfarming techniques **require large areas** and are not generally viable for small sites due to the limiting thickness of soil layers (0.3m), but they can be the **cheapest** kind of bioremediation.

Biopiles

A biopile is a type of ex situ treatment that uses biological processes to transform pollutants into low-toxic byproducts.

It is often used to lower petroleum component concentrations in soils. Biopiles are a type of remediation system that is used for a short period of time.

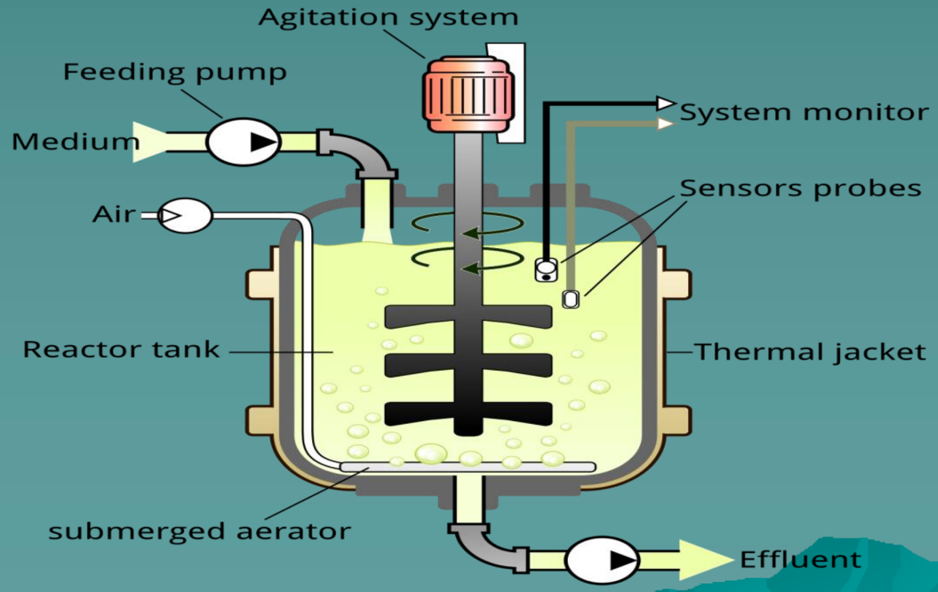
To maximize and manage the pace of biodegradation, excavated soil or silt is piled over an impermeable base or pad with aeration.

Pads are often designed with a cover and sufficient drainage to manage precipitation exposure, as well as probes to monitor temperature, moisture content, and pollutant concentrations.

Depending on the site's qualities and regulatory requirements, optional equipment may include a moisture addition system, leachate collection system, and off-gas treatment.

Slurry-phase bioremediation (Bioreactor)

Slurry-phase bioremediation is a relative more rapid process compared to the other treatment processes. Contaminated soil is combined with water, nutrient and oxygen in the bioreactor to create the optimum environment for the microorganisms to degrade the contaminants which are present in soil. This processing involves the separation of stones and rubbles from the contaminated soil. The added water concentration depends on the concentration of pollutants, the biodegradation process rate and the physicochemical properties of the soil. After completion of this process the soil is removed and dried up by using vacuum filters, pressure filters and centrifuges. The subsequent procedure is soil disposition and advance treatment of the resultant fluids.



Advantages of ex-situ bioremediation

-Suitable for a wide range of contaminants

-Suitability relatively simple to assess from site investigation data

-Biodegradation are greater in a bioreactor system than or in solid-phase systems because the contaminated environment is more manageable and more controllable and predictable.

Disadvantages

-Not applicable to heavy metal contamination or chlorinated hydrocarbons such as trichloroethylene.

-Non-permeable soil requires additional processing.

-The contaminant can be stripped from soil via soil washing or physical extraction before being placed in bioreactor.

Bioremediation – Advantages

Positive Impact On The Environment: The most significant advantage of adopting bioremediation technologies is the positive impact on the environment. Nature is used to fix nature in bioremediation.

Safest And Least Invasive: This is the safest and least invasive soil and groundwater treatment available when properly done by skilled workers using specialised bioremediation equipment.

Highly Treatable: Organic pathogens, arsenic, fluoride, nitrate, volatile organic compounds, metals, and a variety of other pollutants such as ammonia and phosphates can all be treated by bioremediation.

Removal of Pesticides And Herbicides: It works well to remove pesticides and herbicides from aquifers, as well as seawater intrusion.

No Risk of Transportation: For the most part, work is done on-site, avoiding the risks of transportation.

Less Requirement Of Equipment: Except for specific parts, very little equipment is required.

Low Maintenance Cost: Maintenance costs are low, and input costs are low.

Reduction Of Liability: Liability is reduced since toxins are less likely to escape.

Low Energy Consumption: In comparison to incineration and landfilling, there is very little energy consumed.

Disadvantages of bioremediation

- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- Research is needed to develop and engineer bioremediation technologies for complex mixtures of contaminants that are not evenly dispersed in the environment.
- Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration.

Summary of strategies

Technology	Examples	Benefits	Limitations	Factors to consider
In situ	*In situ* bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	Biodegradative abilities of indigenous microorganisms Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Landfarming Composting Biopiles	Cost efficient Low cost Can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation	See above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relatively high cost capital Relatively high operating cost	See above Bioaugmentation Toxicity of amendments Toxic concentrations of contaminants