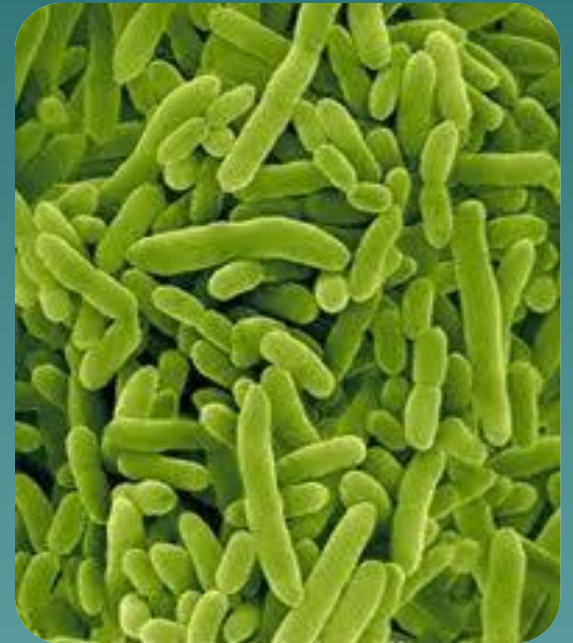


# Bioremediation (Microbial)

Elective Course: MSc (Bot), Sem-4, Paper-4  
(Biotechnological & Environmental Use of Plants)



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# Definition as per

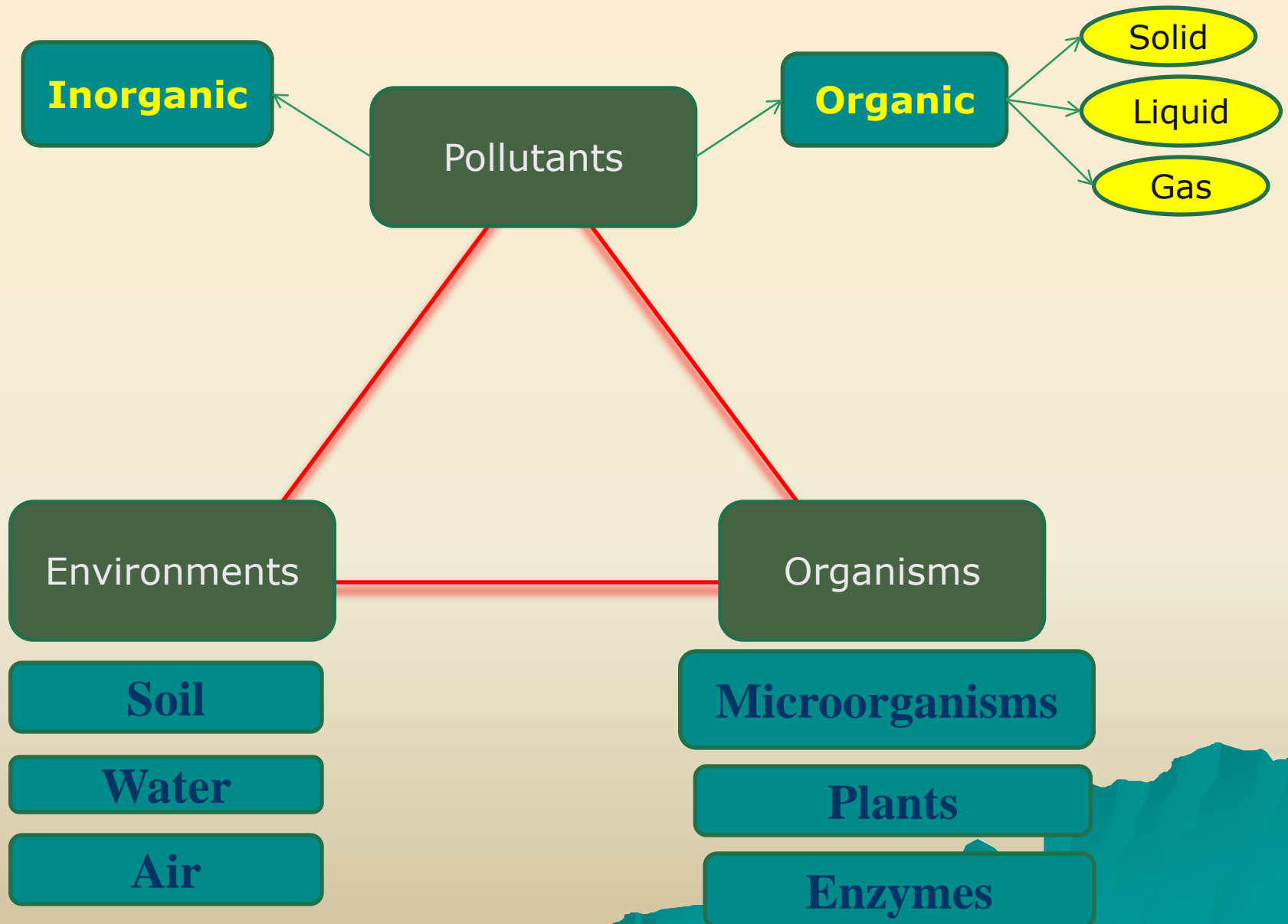
## Environmental Protection Agency (EPA)

- ◆ Bioremediation 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 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 utilize its nutrients, but by chance this enzyme can degrade a pollutant.

# ◆ Bioremediation is a triple-corners process:



# Bioremediation related topics

Environments

Organisms

Pollutants

Phytoremediation

Bioremediation of  
metals polluted  
environment

Bioremediation

Enzymes  
immobilization

Cells immobilization

Biosurfactants

Bioremediation  
techniques

Enzymes separation  
and identification

# Kinds of Bioremediation

Different kinds of bioremediation technologies are currently being used for soil treatment and many more innovative approaches involving bioremediation are being developed. Considering the similarity in their cross-media transfer potential, listed below are a few examples of bioremediation technologies and processes:

- ◆ **Natural Attenuation**
- ◆ **Aerobic/Anaerobic biodegradation**
- ◆ **Land Treatment**
- ◆ **Bioscrubbers**
- ◆ **Methanotrophic Process (in Situ)**
- ◆ **Plant Root Uptake (Phytoremediation)**
- ◆ **Solid Phase Bioremediation**
- ◆ **Bio Wall for Plume Decontamination (In Situ)**
- ◆ **Biodegradation**
- ◆ **Composting**
- ◆ **Bioreactors**
- ◆ **Dehalogenation**
- ◆ **Binding of Metals**
- ◆ **Fungi Inoculation Process**
- ◆ **Slurry Phase bioremediation**
- ◆ **Bioventing (Vapor Extraction)**

# 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.

# Key Features of *Bioremediation...contd.*

- ◆ **Field application of bioremediation may involve:**
  - **Excavation/ dig-out**
  - **Soil handling**
  - **Mixing of contaminated soils**
  - **Aeration of contaminated soils**
  - **Injection of fluid**
  - **Extraction of fluid**
  - **Introduction of nutrients and substrates**



# 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

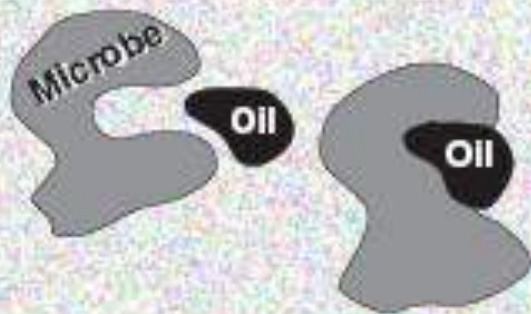
- Actinomycetes (filamentous bacteria)

# 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



Microbe eats oil



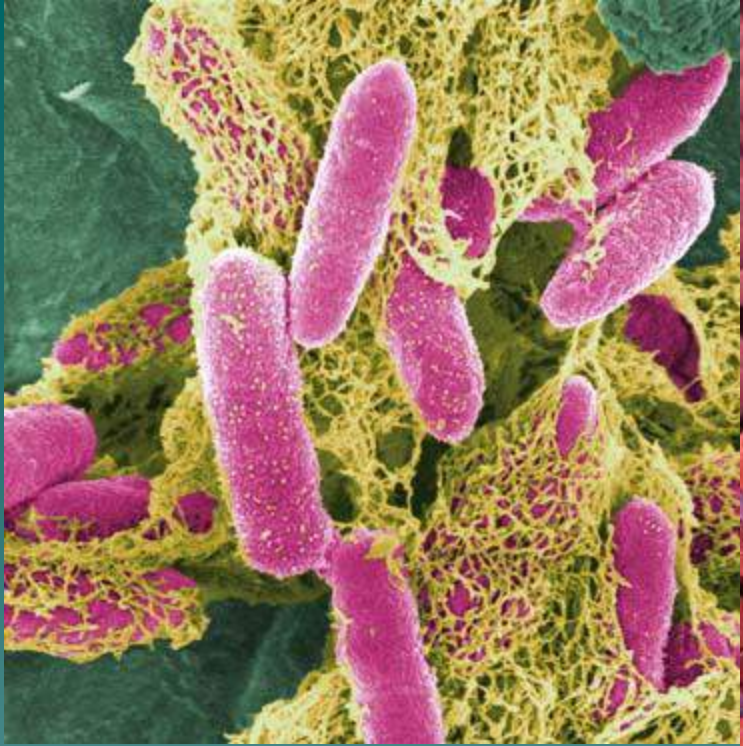
Microbe digests oil and changes it to water and harmless gases



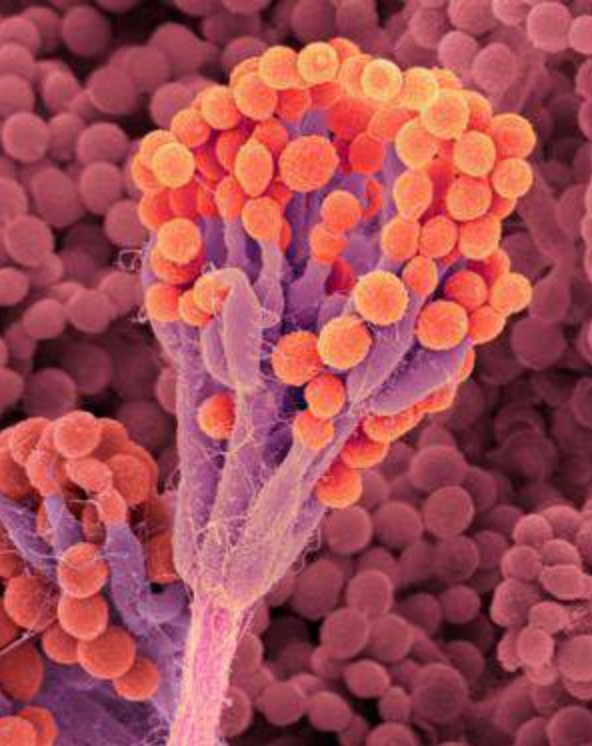
Microbe releases water and harmless gases into soil or groundwater

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





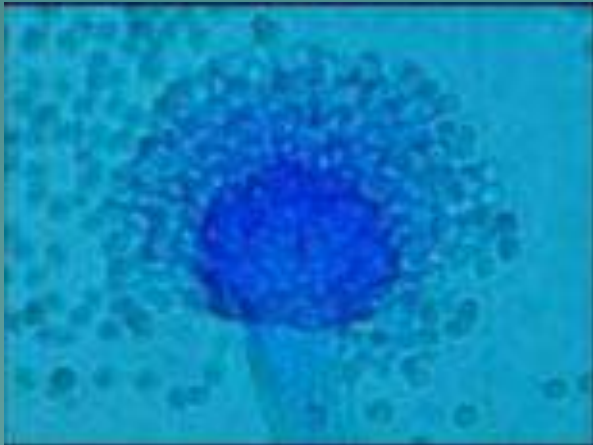
Bacteria



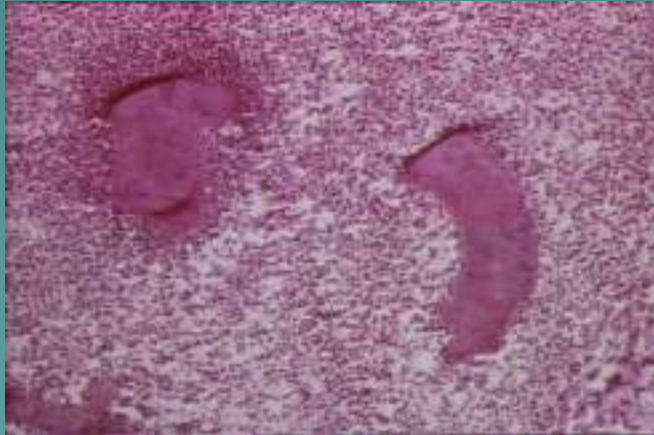
Fungi



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 environmental conditions permit microbial growth and activity
- ◆ Manipulation of environmental parameters needed for microbial growth & degradation to proceed at a faster rate.

# Stages of Microbial Purification

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.

# 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).



# 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
- ◆ **Methylophs** 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 *Methylococcus 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 (%).

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

# The Science – How Does it Work?

**Microbial Metabolism** refers to all the chemical reactions that happen in a cell or organism. All living processes are based on a complex series of chemical reactions.

**Anabolism – Building** complex molecules into simpler molecules. In anabolism, chemicals taken up by the microorganism are used to build various cell parts. Carbon and nitrogen are the basic chemicals in the proteins, sugars and nucleic acids that make up microbial cells. Microorganisms take up carbon and nitrogen from the soil, water, and air around them. In order to take up nutrients and make them into cell parts, a microorganism needs energy. This is where catabolism comes in.

**Catabolism – Breaking** complex molecules into simpler molecules. Catabolism allows microorganisms to gain energy from the chemicals available in the environment. Although most microorganisms are exposed to light and to chemical energy sources, most rely on chemicals for their energy. When chemicals break down, energy is released. Microorganisms use this energy to carry out cellular functions, such as those involved in anabolism.

# 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.



# Summary of strategies

Technology	Examples	Benefits	Limitations	Factors to consider
<i>In situ</i>	<i>In situ</i> 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
<i>Ex situ</i>	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

If appropriate biodegrading microorganisms are not present in soil or if microbial populations have been reduced because of contaminant toxicity, specific microorganisms can be added as “introduced organisms” to enhance the existing populations. This process is known as bio-augmentation. Scientist is now capable of creating ‘super bugs’\_organisms that can degrade pollutants at extremely rapid rates. Such organisms can be developed through successive adaptations under laboratory condition or can be genetically engineered.

# Advantages of bioremediation

- ◆ Bioremediation is perceived by the public as an acceptable waste treatment process. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines.
- ◆ It is safe as the residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.
- ◆ It is useful for the complete destruction of a wide variety of contaminants. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.
- ◆ Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
- ◆ It can often be carried out on site, without disruption of normal activities, no need to transport waste off site.
- ◆ It does not require too much of sophisticated equipments.
- ◆ Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

# 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.

# THANK YOU

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