**Bioremediation of Contaminated Soil**

Soil bioremediation is defined as use of biological processes to degrade, break down, transform, and/or essentially remove contaminants from soil. Bioremediation is a natural process which relies on bacteria, fungi, and plants to alter contaminants as these organisms carry out their normal life functions. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source.

Many substances known to have toxic properties have been introduced into the environment through human activity. These substances range in degree of toxicity and danger to human health. Many of these substances either immediately or ultimately come in contact with and are sequestered by soil. Conventional methods to remove, reduce, or mitigate toxic substances introduced into soil or ground water via anthropogenic activities and processes include pump and treat systems, soil vapor extraction etc. Utility of each of these conventional methods of treatment of contaminated soil suffers from recognizable drawbacks and may involve some level of risk.

On the other hand, bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, biostimulation, and bioaugmentation. Bioremediation which occurs without human intervention other than monitoring is often called natural attenuation. This natural attenuation relies on natural conditions and behavior of soil microorganisms that are indigenous to soil. Biostimulation also utilizes indigenous microbial populations to remediate contaminated soils. It is a process by which the microbial activity can be enhanced by increased supply of nutrients or by addition of certain stimulating agents to soil to catalyze natural attenuation processes. Bioaugmentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms.

During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and, through oxidation-reduction reactions, metabolize the target contaminant into useable energy for microbes. By-products (metabolites) released back into the environment are typically in a less toxic form than the parent contaminants.

Due to industrialization and extensive use of insecticides, herbicides and pesticides, the solids and waste lands world over are getting polluted. The most common pollutants are hydrocarbons, chlorinated solvents, polychlorobiphenyls and metals.

**Bio-stimulation in soil bioremediation:** It can be done by many ways:

- Addition of nutrients such as nitrogen and phosphorus.
- Supplementation with co-substrates e.g. methane added to degrade trichloroethylene.
- Addition of surfactants to disperse the hydrophobic compounds in water.
Addition of nutrient and co-substrates promote microbial growth while surfactants expose the hydrophobic molecules. In all these situations, the result is that there occurs bio-stimulation by effective bioremediation of polluted soil or waste land.

**Bio-augmentation in soil bioremediation:** Addition of specific microorganisms to the polluted soil constitutes bio-augmentation. The pollutants are very complex molecules and the native soil microorganisms alone may not be capable of degrading them effectively. The examples of such pollutants include polychlorobiphenyls (PCBs), trinitrotoluene (TNT), polyaromatic hydrocarbons (PAHs) and certain pesticides.

Based on the research findings at the laboratory level, it is now possible to add a combination of microorganisms referred to as consortium or cocktail of microorganisms, to achieve bio-augmentation.

With the development of genetically engineered microorganisms (GEMs), they can be also used to bio-augment soils for very efficient bioremediation. But the direct use of GEMs in the soils is associated with several risks and health hazards.

**Techniques of Soil Bioremediation:**

The most commonly used methods for the bioremediation of soils are - bioventing and phytoremediation (in situ bioremediation) and land farming and slurry phase bioreactors (ex situ bioremediation).

- **In Situ Bioremediation of Soils:**

  It involves a direct approach for the microbial degradation of pollutants at the sites of contamination. Addition of adequate quantities of nutrients at the sites promotes microbial growth. This technique is used for the bioremediation of sub-surfaces of soils, buildings and road ways that are polluted. Sometimes, water (oxygenated) is cycled through the sub-surfaces for increasing the efficiency of microbial degradation. Two types of in situ soil bioremediation techniques are explained here- bioventing and phytoremediation.

  - **Bioventing:**
    This is very efficient and cost-effective technique for the bioremediation of petroleum contaminated soils. Bioventing involves aerobic biodegradation of pollutants by circulating air through sub-surfaces of soil. Although, it takes some years, bioventing can be used for the degradation of soluble paraffin’s and polyaromatic hydrocarbons. The major limitation of this technique is air circulation which is not always practicable.
- **Phytoremediation:**
  Bioremediation by use of plants constitutes phytoremediation. Specific plants are cultivated at the sites of polluted soil. These plants are capable of stimulating the biodegradation of pollutants in the soil adjacent to roots (rhizosphere) although phytoremediation is a cheap and environmental friendly clean-up process for the biodegradation of soil pollutants, it takes several years. Commonly used plants are - Indian mustard (*Brassica juncea*), Willow (*Salix* species), Poplar tree (*Populus deltoides*), Sunflower (*Helianthus Annuus*) etc.

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

- **Land farming**
  Land farming is well proven ex-situ, an above-ground bioremediation technique for the bioremediation of hydrocarbon contaminated soils. A diagrammatic representation of land farming system (also referred to as solid phase soil reactor) is shown here.
  This technology usually involves spreading of excavated contaminated soils on a thin layer of sand on the ground surface having microorganisms and nutrients. The soil has to be regularly ploughed for good mixing and aeration. If the soil is mixed with compost and/or temperature is increased the efficiency of biodegradation increases.

  The enhanced microbial activity results in degradation of adsorbed petroleum product constituents through microbial respiration. If contaminated soils are shallow (i.e., < 3 feet below ground surface), it may be possible to effectively stimulate microbial activity without excavating the soils. If petroleum contaminated soil is deeper than 5 feet, the soils should be excavated and reapplied on the ground surface.

  Land farming has been successfully used for the bioremediation of soils polluted with chloroethane benzene, toluene and xylene. The last three compounds are often referred to as BTX aromatics.
- **Slurry-phase Bioreactors** -

Slurry bioreactors (SB) are one of the most important types of ex situ technique. Treatment of soils and sediments in slurry bioreactors has become one of the best options for the bioremediation of soils polluted by recalcitrant pollutants under controlled environmental conditions. In SB, soil is excavated and conditioned and loaded into bioreactors. A main feature of SB is that soil inside reactor is kept in aqueous suspension by some type of mixing in a way that biological treatment is carried out under saturated conditions and nearly homogeneous suspension.

Main operating modes of SB include batch and semi-continuous. Polluted soil is first crushed and screened. The coarser fractions of soils (pebbles and sands, 0.85 to 4 mm) are then discarded and sent to direct disposal, whereas fine fractions (clay and organic matter, < 0.85 mm) are retained and loaded into bioreactors. It is generally recognized that pollutants concentrate in fine particles of soil.

Polluted, fine fractions of soils are mixed with water or wastewater to form slurry with a concentration in the range between 15 to 60% w/v (as shown below). Continuous mixing is done to keep solid particles in suspension with proper aeration with microorganisms. Due to a close contact between the pollutants and the microorganisms, and the optimal conditions (nutrient supply, temperature, aeration etc.), the degradation is very rapid and efficient. Slurry-phase bioreactors, however, are not suitable for widespread use due to high cost.
| Microorganisms and Hydrocarbon
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microorganisms</strong></td>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>Monocyclic aromatic hydrocarbons, benzene, toluene, ethyl benzene and xylene, phenol compounds</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>Monocyclic aromatic hydrocarbons, e.g. benzene and xylene.</td>
</tr>
<tr>
<td><em>A. niger, A. fumigatus, F. solani and P. funiculosum</em></td>
<td>Hydrocarbon</td>
</tr>
</tbody>
</table>

| Microorganisms and heavy metals
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microorganisms</strong></td>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Heavy metals, lead, mercury and nickel</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> and <em>Pseudomonas aeruginosa</em></td>
<td>$Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}$ and $Cu^{2+}$</td>
</tr>
</tbody>
</table>

| Microorganisms and pesticides
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microorganisms</strong></td>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td><em>Bacillus, Staphylococcus</em></td>
<td>Endosulfan</td>
</tr>
<tr>
<td><em>Acinetobactor sp.</em>, <em>Pseudomonas sp.</em>, <em>Enterobacter sp. and Photobacterium sp.</em></td>
<td>chlorpyrifos and methyl parathion</td>
</tr>
</tbody>
</table>