

Draft Guidelines for Bioremediation of Oily Sludge Contaminated Sites

1. Introduction

Crude oil is naturally derived organic material that is composed of extremely complex and a variable mixture of compounds that are primarily hydrocarbons. It contains approximately 84% carbon, 14% hydrogen, 1-3% sulfur and less than 1% each of nitrogen, oxygen, metals and salts. The activities associated with crude oil handling such as drilling, transportation, refining etc. may generate various forms of oily wastes such as crude oily sludge, residual oily sludge, contaminated drill cuttings /synthetic oil based mud waste etc. Most of which may fall into category of 'hazardous waste' of the Hazardous and Other Wastes (Management and Transboundary Movement) Rules, 2016 (HOWM Rules). Petroleum hydrocarbons (a component of crude oil) contamination represents one of the most toxic compositions of oily sludge contaminated sites.

The various types of oily wastes generated in oil refineries includes crude tank bottom sludge, oil-water separator sludge, DAF (Dissolved Air Flootation) sludge, slop oil emulsion solids, cooling tower sludge, chemical and bio sludge. Though the composition of oily sludge from oil Industry may vary, however, a typical organic composition of the same is given in Table – 1, and heavy metal composition in Table - 2. Besides this, industries concerned with oil exploration and drilling, storage terminals and oil depots also meet with the problem of oily sludge and oil contaminated drill cuttings generation. Disposal of this waste is a major environmental concern. Improper handling and accidental spillage of such wastes may contaminate land and water streams. Possibilities also exist of accidental oil spill due to leakage of pipeline during transportation of crude oil that may lead to contamination of soil and water to a greater extent.

The contamination in soil and groundwater by oily substances exists in many forms; namely, oil adsorbed to the soil particles, vapors in soil, dissolved in groundwater and as a free product. Free product lighter than water that floats on water is called light non-aqueous phase liquid (LNAPL), and heavier than water that sinks is called dense non-aqueous phase liquid (DNAPL).

Table-1: Typical organic composition of oily sludge from an oil refinery

Constituent of oily sludge (%)	Composition (%)
Solvent Extractable Total Petroleum Hydrocarbons (TPH)	25 – 60
Water content	15 - 40
Residues including organic	15 – 30
Composition of TPH (in total solvent extractable TPH)	
Saturated hydrocarbon fraction (alkanes)	30 – 60
Aromatic hydrocarbons (BTEX, PAHs)	25 – 45
NSO (nitrogen, sulphur, oxygen) & Asphaltene fraction	10 - 25

(TPH Concentration Limit for waste to be hazardous: 5,000 mg/kg as per HOWM Rules, 2016, GoI)

Source: The Energy Resources Institute (TERI), New Delhi

Disposal of such wastes must be carried out adopting suitable treatment and/or disposal techniques like incineration, secured landfilling, biodegradation using land farming, surfactant washing chemical oxidation, and other conventional methods. Biodegradation of petroleum hydrocarbon typically occurs under aerobic conditions. In biodegradation based remedies, oxygen has to be present, and microorganisms have the capability to degrade the majority of natural hydrocarbon constituents, especially the dominant saturated and unsaturated alkanes, monoaromatic hydrocarbons and low-weight polycyclic aromatic hydrocarbons (PAH). The higher-weight PAHs, resins and asphaltenes are more recalcitrant to biodegradation.

The rates of oil degradation in the environment are generally determined by the populations of indigenous hydrocarbon degrading microorganisms, availability of oxygen (for example, air, dissolved oxygen in water), and nutrients (such as nitrogen and phosphorous) that are required for bacterial growth, and any inhibiting conditions that influence growth rates of those hydrocarbon degrading microbial populations. Microbial communities exposed to hydrocarbons become adapted, exhibiting selective enrichment resulting in increased proportions of hydrocarbon degrading bacteria. In unpolluted environments it is estimated that hydrocarbon degraders generally constitute less than 1% of the microbial community, whereas in oil polluted environment the hydrocarbon degraders often represent 1-10% of microorganisms.

Thus, aerobic bioremediation is one of the effective options which may be suitable in treating such wastes. Major factors in addition to oxygen availability and nutrients that influence the rate of biodegradation of hydrocarbons are temperature, pH and moisture content. Nutrients such as nitrogen and phosphorous are required for the microbial cell growth and their activity. Water is essential for the survival and growth of microbes. For bioremediation of oil impacted soils, water is also required for solubilizing nutrients present in the soil which can then diffuse in and out of microorganism cells. Excess moisture content can slowdown biodegradation as it hinders the diffusion of oxygen into soil and decreases the bioavailable oxygen required for aerobic respiration. The optimum moisture content for the petroleum hydrocarbon degradation is 45% to 85% of the water holding capacity of the soil or 12% to 30% by weight of the soil. The rate of biodegradation is a function of pH as microbial species survive and are active within a certain pH range. The bioavailability of nutrients also is a pH dependent. As such soil pH is important and should be maintained near neutral (pH 7) but pH between 6 and 8 is acceptable. The temperature, which influences the rate of enzymatic reaction and hence the rate of biodegradation, should be between 25°C to 45°C. Elevated heavy metals concentrations and elevated contaminant concentrations (for example free product) can be toxic to microorganisms, and can inhibit the growth, and thereby inhibit biodegradation of the petroleum compounds. All these factors together affect the microbial growth and their efficiency to degrade the various constituents of crude oil. It is therefore, essential to closely monitor and manage these factors during the period of bioremediation.

Table – 2: Heavy Metal Concentration in Oily Sludge and Oily Sludge Leachate

Heavy Metals	Oily sludge (mg/kg)	Oily sludge leachate (mg/L)
Zinc	2.5 -3.5	BD
Manganese	0.15 -3.11	BD
Copper	BD – 1.22	BD
Nickel	0.45 – 3.0	0.001 – 0.02
Lead	0.2 – 0.5	BD
Cobalt	BD – 0.6	BD
Chromium (total)	0.5 – 2.4	0.002 – 0.16
Arsenic	0.1 – 0.5	0.01 -0.04
Cadmium	BD – 0.06	BD – 0.05
Selenium	0.1 – 0.3	BD – 0.01

(Source: The Energy Resources Institute, New Delhi), BD - Below Detectable

2. Bioremediation Concepts

Bioremediation uses indigenous microorganisms to treat petroleum contamination. Despite its broad definition, bioremediation usually refers specifically to the use of microorganisms that naturally exist in contaminated environments. Bioremediation is a process that uses microorganisms to return the environment altered by contaminants to its original condition. Bioremediation may be employed for specific contaminants, such as chlorinated solvents and some pesticides that are amenable to biodegradation under anaerobic conditions, aromatic hydrocarbons that are generally amenable to biodegradation under aerobic conditions, or a more general approach may be taken, such as oil spills that are biodegraded using microbial consortia (multiple microorganisms) under aerobic environment. 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.

There are a number of advantages in bioremediation, for example, in hydrocarbon spills (more specifically gasoline), remediation can be achieved at much deeper depths that cannot be reached easily without excavation. This is much less expensive than excavation followed by treatment elsewhere or incineration, and reduces or eliminates the need for pump and treat, a common practice at sites where hydrocarbons have contaminated groundwater. Under appropriate conditions, these pollutants can be treated on site, thus reducing exposure risks for clean-up personnel and transportation accidents. Bioremediation may subsequently enable appropriate reuse of treated soil and minimizing disposal of waste to landfill thereby providing sufficient protection of human health and the environment.

Generally, bioremediation technologies can be classified as *in-situ* or *ex-situ*. *In-situ* bioremediation involves treating the contaminated material without removing it from its original place at the site (no excavation of soils or pumping of groundwater). *Ex-situ* involves the removal of the contaminated material from the original place to be treated above-ground or elsewhere. In-situ bioremediation typically costs less and causes minimal disturbance to the site as the contaminated material is treated in place without removal from the original site. Bioventing, Bioslurping and biosparging are examples of commonly used in—situ bioremediation technology for remediating hydrocarbon contamination. Bioventing is used for remediating hydrocarbon contamination from vadose zone (above the groundwater table). In this technology, contaminated soils having low

concentration are treated by supplying oxygen through air injection wells. Oxygen can also be supplied through vacuum extraction wells where vacuum draw air through the subsurface. In case of injection wells, the injection rate is adjusted such that the injected air does not result in the release of volatile organic compounds to the atmosphere through stripping. Oxygen levels of 2 to 5% in soil vapor are generally sufficient to promote biodegradation. Bioslurping is combination of bioventing and vacuum enhanced free product recovery technology and is used when there is a need for multiphase extraction technology. Bisparging is used for remediating saturated zone and groundwater. Like in bioventing, air and sometime nutrient are injected into the saturated zone (groundwater) to promote biodegradation.

Examples of ex-situ bioremediation include bioreactors, land-farming, and biopiles. In bioreactor process, contaminated material and water are mixed together and agitated to enhance biodegradation. Land-farming involves spreading of contaminated material (typically over a collection system to collect any leachate) to allow for good aeration. Biopiles involve forming piles of contaminated material, and pumping air into the piles to stimulate microbial activity.

Depending on the type of manipulation of the condition, bioremediation can also be classified as bioaugmentation, biostimulation or intrinsic bioremediation.

- **Biostimulation** - Addition of nutrients and/or oxygen to contaminated water or soil to facilitate the growth and activity of indigenous bacteria already existing in the soil or water. The degradation of contaminants is monitored to ensure the effectiveness of the remediation process.
- **Bioaugmentation** - Microorganisms that can degrade a particular contaminant are added to the contaminated soil or water.
- **Intrinsic Bioremediation** - Also known as natural attenuation, this type of bioremediation occurs naturally in contaminated soil or water. This natural bioremediation is the work of microorganisms and is seen in petroleum contaminated sites. Researchers are studying whether intrinsic bioremediation happens in areas with other types of chemical contamination. Application of this technique requires close monitoring of contaminant degradation to ensure that environmental and human health are protected.

All three types of bioremediation can be used at the site of contamination (*in-situ*) or on contamination removed from the original site (*ex-situ*). In the case of contaminated soil, sediments, and sludge, it can involve land tilling in order to make the nutrients and oxygen more available to the microorganisms.

However, like all other technologies, bioremediation has its limitations. Total Petroleum Hydrocarbon (TPH) concentration less than 8% (by weight of soil) can be readily treated. As TPH concentration increases above 8%, the rate of biodegradation decreases. Long and high molecular hydrocarbons having 20 or more carbon atoms are not readily biodegradable and hence may take a long time to achieve remediation goals. Asphaltenes, polar resins and tar compounds are non-biodegradable. Some of the contaminants such as chlorinated organics are resistant to microbial degradation by aerobic pathway (generally amenable to anaerobic biodegradation). Bioremediation, when used under suitable conditions and managed properly, is environmentally friendly and cost-effective method of treating soils containing organic contaminants compared to traditional methods such as incineration.

3. Process for bioremediation of oil contaminated sites

Bioremediation of oil contaminated sites initially require the following before the actual process is commenced.

- (i) Should have proven scientific data on biodegradability of the contaminants/compounds present at the contaminated site and the ability of the indigenous microbes to be able to degrade them.
- (ii) Data should be available on compounds not suitable for bioremediation by the proposed degradation pathway and microbes/microbial consortia (aerobic or anaerobic).

3.1 Types of hazardous oily wastes that can be bioremediated by aerobic pathway include:

- (i) Case - 1: Oil contamination on land / stagnant water due to accidental oil spill
- (ii) Case - 2: Residual oily sludge lying in old dumping pits.

- (iii) Case - 3: Crude oily sludge generated during processing.
- (iv) Case - 4: Oil contaminated drill cuttings / synthetic oil based mud waste
- (v) Case - 5: Crude oily sludge from marketing installation / depots / tap off points / retail outlets of petroleum refineries.
- (vi) Case - 6: Oil contaminated wastes from other allied industries

3.2 Bioremediation of hazardous waste shall be carried out after obtaining authorization from the concerned State Pollution Control Board / Pollution Control Committee in compliance with the provisions laid down under the Hazardous and Other Wastes (Management and Transboundary Movement) Rules, 2016. The operator shall also fulfill other requirements, if necessary, from other regulatory bodies/authority.

3.2.1 Pre-treatment Activities

- (i) Identification of oily sludge contaminated site and its isolation from other areas/surroundings. The contaminated areas should be truly barricaded/ fenced properly so as to prevent unauthorized entry of human beings and other domestic animals.
- (ii) Removal of recoverable free-product or oil to the extent practicable. The recovered oil shall be disposed appropriately.
- (iii) Collection of samples from the contaminated site for analysis of Total petroleum hydrocarbon (TPH) and other necessary parameters such as volatile organic compounds (by USEPA method 8260 or equivalent that can detect lower molecular weight volatile organics including petroleum and chlorinated organic), semi-volatile organic compounds (by USEPA method 8270 or equivalent that can detect high aromatic hydrocarbons), heavy metals etc. (to be identified on the basis of contamination). Analysis of soil pH, dissolved oxygen and oxidation reduction potential in groundwater.
- (iv) Depending upon above analysis result, feasibility of adopting bio-remediation technique shall be evaluated.

3.2.2 Selection of in-site & ex-situ remediation option

A suitable bioremediation technique shall be selected for *In-Situ* / *Ex-Situ* bioremediation depending upon the nature of contamination. The *in-situ* bioremediation shall be considered in following cases:

- (i) where contamination has occurred deep below the ground level and excavation of such contaminated soil becomes extremely costly and difficult but the geological data supports that surrounding environment/population is not affected because of possible migration of pollutants till the desired level of bio-remediation is achieved
- (ii) residual oily sludge already lying in old dumping pits and their excavation is extremely costly and difficult but the geological data supports that there shall not be further impact on surrounding environment/population
- (iii) stagnant water contaminated due to oil spillage

In rest of the cases, *Ex-Situ* bioremediation shall be considered.

3.3 Treatment Activities

3.3.1 Bioremediation Site Preparation

For Ex-situ bioremediation site can be prepared as follows

The contaminated site needs to be properly identified and barricaded/fenced adequately before initiation of the bioremediation job. Precautions need to be taken for preventing the unauthorized entry of human being and birds/animals to the bioremediation site that can result in exposure to contamination.

For Ex-situ bioremediation site can be prepared as follows

- (i) Select an open land near to the oil spilled contaminated site for bioremediation.

- (ii) Prepare one containment boundary (can be made of concrete or wall) of 2 ft. below the surface and 2 ft above the surface (total height 4 ft). The width of the boundary can be of 1 to 1.5 ft.
- (iii) Dig out soil up to 2 ft depth (at minimum or one foot deeper than the depth of contamination) and make the bottom with slope.
- (iv) Lay down 1.5 mm thick High Density Poly Ethylene (HDPE) liner at the bottom. Prepare suitable layers below and top of the liner so that it does not rupture/crack.
- (v) Put the soil back inside the containment boundary.
- (vi) Prepare two leachate collection pits of (1m x 1m x 1m) at two opposite corners at the bottom of the slope within the bioremediation site along with the HDPE liner. The leachate, if any, shall be drained through the surface of the liner and collected in the pit and sent for further treatment.
- (vii) Purified air for effective bioremediation, if necessary, may be applied using a grid of tubes for providing oxygen for the contaminated area which can assist the indigenous microorganisms or the applied microorganisms for efficient biodegradation/bioremediation. Alternatively, manual mixing with spade to provide sufficient oxygen for aeration during bioremediation may be considered. During manual mixing hands should be covered with safety gloves and gum boots should be put on. JCB machine may also be used for proper mixing; however, caution must be exercised to not rupture the liner underneath.
- (viii) In case of bioremediation of water contaminated by oil spill, a continuous aeration system can be arranged by installing suitable devices for providing sufficient oxygen.
- (ix) Ensure that there are piezometric monitoring wells intersecting shallow groundwater are installed at the downstream of the bioremediation site within 100 m. However, if there is any existing shallow bore well lying within 100 m in the down gradient side of the bioremediation site, the same can also be used for groundwater

monitoring in addition to the new monitoring wells. Groundwater flow direction shall be assessed and understood by installing at least three shallow monitoring wells in a triangular fashion and measuring groundwater elevations.

- (x) When new contamination is identified due to accidental spill on land, *in-situ* remediation shall not be carried, instead the contaminated portion should be transferred to a secured site immediately (on war-footing) so as to avoid further contamination of soil and groundwater and perform *ex-situ* bioremediation. After removal of the contamination material from the spill location, soil sampling should be done to confirm that all contaminated material is removed and the remaining soils are clean.
- (v) Loamy/silt or sandy types of soil show better microbial activity for removal of TPH. In case of contaminated clay soil, some coarse or sandy materials may be added for exposure of bacterial mass with contaminants and to create good air ventilati Identification of oily sludge contaminated site and its isolation from other areas/surroundings. The contaminated areas should be truly barricaded/ fenced properly so as to prevent unauthorized entry of human beings and other domestic animals.
- (xi) on in the medium. In the case of very sandy soils, wood chips sawdust, straw hay or animal manure can be added to increase moisture holding capacity of sands. Optimum conditions required for bacterial degradation of oil is:
- | | |
|----------------|-------------------------------|
| Soil moisture: | 30 – 90%; |
| Soil pH: | 6.5-8.0; |
| CNP ratio: | 100:1:1; |
| Soil type: | low clay or silt content; and |
| HC: | 5-10% dry wt. of soil |
- (xii) The leachate collected in the leachate collection pits shall be treated/discharged as per the conditions stipulated by the concerned SPCBs/PCCs.

3.3.2 Transfer of oily waste to the bioremediation site

- (i) The bioremediation site should be as nearer as possible from the oil contaminated site. This will help in avoiding environmental pollution by

- spillage during transportation of oily sludge / drill cuttings from the contaminated site to the remediation site.
- (ii) Oily sludge / drill cuttings should be transported in closed packed drums or containers. The drums/containers can directly be transported by the dumper and unloaded at the bioremediation site.
 - (iii) Where the oily sludge is liquid in nature and the storage pit is near the site, under such cases the same can be transported to the bioremediation site using high pressure transfer pump/gully sucker.
 - (iv) In cases where the oily sludge is required to be stored, the oily sludge received at the remediation site should be stored in drums or containers under a shed with proper flooring and secondary containment to prevent leaks and spills.

3.3.3 Activities during Bioremediation

- (i) Before application of the microbes and nutrients for bioremediation (as required), the pH of the contaminated oily sludge should be tested and if necessary adjusted to the comfort level of the applied microbes.
- (ii) The lab tested microbe/microbial consortium developed to remediate oily sludge contaminated sites shall to be applied by spraying on to the contaminated oily sludge. The microbe/consortium may be immobilized if necessary on a carrier material, however, the carrier material must be inert and non-toxic.
- (iii) The quantity of microbe/consortium applied should be documented every time it is applied.
- (iv) If necessary, nutrients (micro and macro) may be applied to the site after the application of microbial consortium. The nature, type, quantity of nutrients applied must be documented and provided to the regulatory authorities.
- (v) Microbes and nutrients may be reapplied from time to time depending on the quantum of soil contamination and the extent of remediation goals.

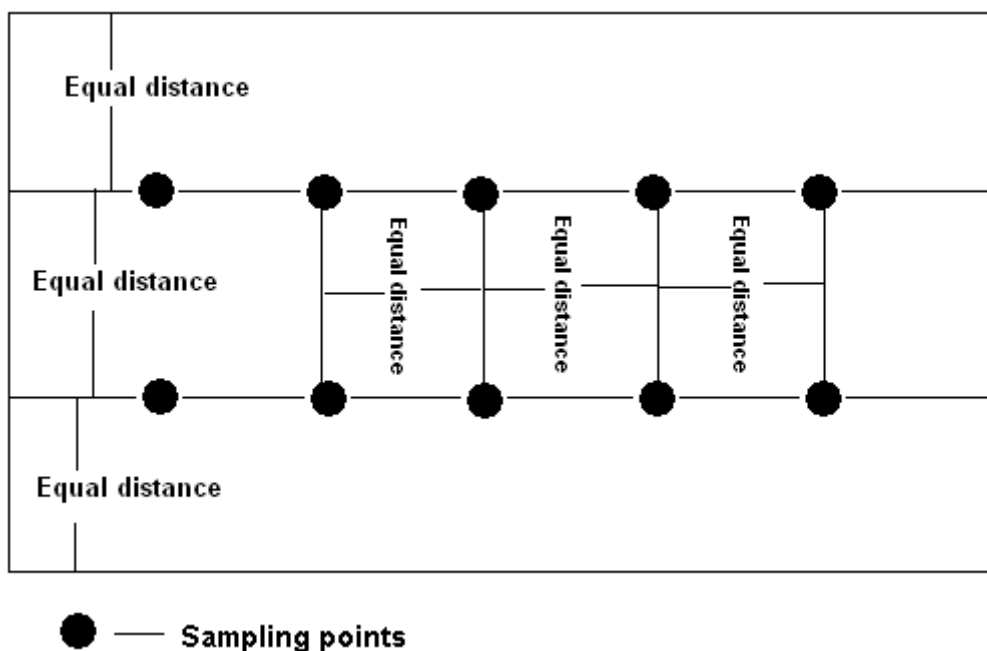
- (vi) The application of microbes and nutrients may be done manually using personnel protective equipments like hand gloves, mask, coverall etc.
- (vii) To maintain sufficient moisture content, spraying of water on the bioremediation may be done, which helps the microbes to grow in a healthy environment for rapid biodegradation process. The moisture content should be measured and documented.
- (viii) Proper arrangement and engineering controls shall be made to prevent releases or adverse impact due to rain.

3.3.4 Sampling of the contaminated site/oily waste

To monitor the progress and performance of bioremediation, samples of the waste material from the bioremediation pit shall be collected at regular intervals. Oil contaminated soil samples from the bioremediation pit would be collected as follows

- (i) The sampling is done at grid points in a statistical manner covering the whole site as per the diagram as described in **Figure - 1**. For adequate monitoring, samples must be collected from the same point for comparison of analysis results.

Figure – 1: Schematic diagram of sampling points from bioremediation site

**Note:**

- a. Divide the whole site in rows of equal distance between each rows (approx. 10 – 15 meter distance for larger site and approx. 3 – 5-meter distance for smaller site)
 - b. Collect sample from sampling points which are of equal distance with each other. (approx. 10 – 15 meter distance for larger site and approx. 3 – 5 meter distance for smaller site).
- (ii) To compare the efficiency of bioremediation, a baseline data may be generated by taking representative samples of contaminated soils as a reference to which the data of the soil under remediation could be compared.
 - (iii) Sample must be collected from different depths of the sludge i.e. from surface, 10 cm depth, 20 cm depth etc. Approximately 200 grams of sample to be collected from each depth.
 - (iv) The samples would be collected in sterile polyethylene bags/glass bottles using suitable personal protective equipment.
 - (v) For collection of sample from different depths, one hollow metallic pipe or spade may be used.

- (vi) Each collected sample should be transported to specific lab for analysis of relevant parameters.
- (vii) Samples would be collected at regular time intervals (normally on monthly basis or even shorter intervals depending upon the biodegradation/bioremediation efficiency) for monitoring the performance of the bioremediation job i.e. at Zero day (before the first application of microbes to the bioremediation site), after 1 month, after 2 months, till the completion of the bioremediation job.
- (viii) Groundwater monitoring should be done depending upon the groundwater flow direction.
- (ix) Install monitoring wells, one upstream and two downstream of the secured bioremediation site as per the groundwater flow direction.
- (x) Before sampling, the monitoring well must be purged until water quality parameters stabilize over three consecutive readings to ensure that the collected sample is representative of groundwater conditions. The stabilization criteria are as follows (per USEPA):
- | | |
|-------------|---|
| pH | ± 0.1 pH units |
| EC | ± 3% ($\mu\text{S}/\text{cm}$ or mS/cm) |
| Temperature | ± 3% |
| ORP | ± 10 mV |
| DO | ± 10% |
- (xi) Then collect approx. 500 ml sample from each bore well in sterilized amber coloured glass bottles and transported to lab for analysis of relevant parameters using proper sampling handling and preservation procedures.
- (xii) Sample of oil spilled contaminated water should be collected from selected points covering the total depth of the contaminated area. Approximately 500 ml of water sample should be collected.
- (xiii) Samples would be collected before, during and after the bioremediation work on monthly basis or the time frame for sample collection should be decided based on laboratory trials.

3.3.5 Sampling for baseline data

- (i) Analysis of soil samples outside the contaminated area is essential/necessary to find out the oil contamination in the outside soil by overflow / rain. Also to compare the quality of the bioremediated soil with the outside soil (as a reference background sample).
- (ii) The samples are collected from the surface, 10 cm, 20cm, 50 cm and 100 cm depth of the site. Samples are collected using a hollow pipeline by inserting the pipelines vertically to the ground. Approximately 200 gm of sample to be collected from each depth. All the soil samples should be collected in polyethylene bags.
- (iii) Samples are collected from random points one each in four directions and mixed to get a composite sample.
- (iv) Each collected sample should be transported to specific lab for analysis of relevant parameters using proper sampling handling and preservation procedures.
- (v) Samples would be collected before and after the bioremediation work.

3.3.6 Parameters for analysis

The oil contaminated soil samples would be analysed for the following parameters

- (i) Total petroleum hydrocarbon (TPH) content for soil and groundwater samples
- (ii) 10% of the soil and groundwater samples shall be analysed for volatile organic compounds (by USEPA method 8260 or equivalent), and semi-volatile organic compounds (by USEPA method 8270 or equivalent)
- (iii) 25% of the soil and groundwater samples shall be analysed for Heavy metals: Nickel (Ni), Vanadium (V), Zinc (Zn), Manganese (Mn),

Copper (Cu), Lead (Pb), Cobalt (Co), Arsenic (As), cadmium (Cd), total Chromium (Cr) and Selenium (Se).

3.3.7 Post-Treatment Activities: (Responsibility of occupier)

- (i) The bioremediated soil/sludge, once confirmed treated would be suitable for disposal for green belt development or road filling or composting or growing non-crop plants if the total petroleum hydrocarbon (TPH) content in the bioremediated product is less than 5 gm/kg of TPH.
- (ii) Submit report to regulatory authorities describing the bioremediation activities, documentation and the result achieved after bioremediation along with possible safe disposal/usage options for the bioremediated soil.

3.4 Bioremediation by Occupier/Agency

- (i) Bioremediation can be carried out by the occupier/agency who has the professional experience, knowledge base and proven technology to treat hazardous oily wastes or oil contaminated soils to acceptable levels using biodegradation. Bioremediation is permitted using indigenous microbes within the purview of '***The manufacturer, use, import, Export and storage of hazardous microorganism, genetically engineered organism or cells rules, 1989***'. Microbes can be isolated from oily sludge contaminated sites for use in bioremediation.
- (ii) The occupier/agency shall either develop the suitable microbes on their own or may procure such microbes/take services from other organizations to carry out bioremediation commercially. However, permission must be obtained from Department of Biotechnology, Ministry of Science & Technology, GoI for using a microbial consortium in bioremediation.

- (iii) A bioremediation report should be prepared for the contaminated site including all the process parameters, concentrations of the relevant compounds/substances, process technique, methodology and post remediation monitoring/management plan. The complete report should be submitted to the regulatory authorities.
- (iv) The agency for carrying out the bioremediation job should have the following requisite:
- The identified consortium should completely be characterized using appropriate microbial finger printing (for example, phospholipid fatty acid (PLFA) analysis, DNA or RNA analysis, or microarrays).
 - The usefulness of the isolated microbial consortium in bioremediation of oily sludge contaminated sites, may if necessary be published in peer-reviewed national and international journals, however publications may not be of constrain.
 - The microbial consortium being used should have potent live microbes for biodegradation of oily wastes or able to stimulate the indigenous microbes using suitable technique developed by the occupier/agency.
 - The use of genetically modified microbe/microbial consortium should never be used for the purpose of bioremediation.
 - The agency must have undertaken and demonstrated experience in carrying out small/large scale bioremediation of oily wastes in actual field conditions or on a pilot scale initially in the laboratory.
 - The microbes/agents to be applied for the bioremediation process must be easy to handle and must not be harmful to the environment and human health.
 - The agency must have sufficient/dedicated manpower, which has experience in carrying on-field bioremediation.
