BIOREMEDIATION: A SUSTAINABLE ECO-FRIENDLY BIOTECHNOLOGICAL SOLUTION FOR ENVIRONMENTAL POLLUTION IN OIL INDUSTRIES

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Abstract

The oil industry effluents, oily sludge and oil spills on land and water cause a major threat to the environment as their constituents are toxic, mutagenic and carcinogenic. None of the available conventional methods are permanent eco-friendly disposal solution. Biological methods have been acknowledged for remediation of environments contaminated with petroleum hydrocarbons.

This paper is a report of the research study carried out by the Energy and Resources Institute (TERI). TERI developed an indigenous bacterial consortium, named Oilzapper by assembling four different bacterial species, isolated from various oil contaminated sites of India, which could degrade different fractions of total petroleum hydrocarbon (TPH) of the oily waste. The end product of bioremediation is CO_2, water and dead biomass which is environment friendly. Oilzapper technology has been applied by TERI for bioremediation of different types of oily wastes at different climatic conditions in India and abroad.

TERI has bioremediated >1, 50,000 tonnes of different types of oily waste globally and presently >60,000 tonnes of oily waste is under treatment. In >100 field case studies of different batch size on *in situ* and *ex situ* bioremediation process by TERI, the initial TPH content varying from 5% to 52% has been biodegraded to <1% in major cases in 2 – 6 months. The bioremediated soil was non-toxic and natural vegetation was grown on the same. Successful fish culturing was done in one oil contaminated lake after bioremediation. Bioremediation technology has helped various oil industries for the management of their hazardous oily wastes in environment friendly manner. Bioremediation by Oilzapper technology is an ongoing investigation whose results are highly encouraging.

Keywords: Bioremediation, Biodegradation, Oily waste, Oilzapper, Total Petroleum Hydrocarbon.

Introduction

The Hydrocarbon sector, worldwide has been undergoing radical changes leading to increased industrial activity in the area of hydrocarbon processing like exploration, drilling, processing and refining process. This increase has also led to increase in generation of oily wastes (sludge), contaminated sites and wastewater. The various types of oily wastes generated in oil refineries include crude tank bottom sludge, American Petroleum Institute (API) separator sludge, DAF (Dissolved Air Floatation) sludge, slop oil emulsion solids, cooling tower sludge, chemical and bio sludge. Besides this, industries concerned with oil exploration and drilling, storage terminals and oil depots also face the problem of sludge generation and disposal.

During the activities, contamination of wastewater stream and land occurs which is highly hazardous to the environment. Oil transportation is one of the major causes of environmental pollution by the oil industries where the land and water environment gets polluted due to oil spill, ship breakage and leakage of oil pipelines.

Recent BP oil spill at the Gulf of Mexico in April, 2010 is considered to be the largest oil spill in US history where oil were discharged in the range of 12,000 to 100,000 barrels per day. By April 25, 2010, the oil spill covered 580 square miles (1500 Km^2) which increased to the total spread of 3,850 square miles (10,000 Km^2) by April 30, 2010 (Wikipedia, 2011). According to an independent record of Shell's spills from 1982 to 1992, 1,626,000 gallons were spilt from the company's Nigerian operations in 27 separate incidences (Dabbs, 1999). In UK, the ecology of North Sea has been devastated by over 30 years due to oil spill incidents. Due to war enormous quantity of land and sea water gets contaminated by oil spill. This has severe impact on the natural environment (e.g. Gulf War 1991)(Enzler, 2006). India, US EPA (United States Environmental Protection Agency) and OECD (Organization for Economic Co-operation and Development) countries designated oily wastes as hazardous wastes (Zhu et al., 2001; Ministry of Environment and Forest, Government of India, 2000). The hazardous oily waste is composed of total petroleum hydrocarbons (TPH), water, and sediments (Dibble et al., 1979). The TPHs constitute a complex mixture of alkane; aromatic; nitrogen, sulfur, and oxygen containing compounds (NSO); and asphaltene fractions (Bhattacharya et al., 2003). Hence disposal of the same in an improper manner may cause a serious environmental problem (Yustle et al., 2000).

Environmental threats of oil contamination

Oil contamination is highly hazardous to the environment. It has severe impacts in the plant as well as animal ecosystem including human health (Mandal et al., 2007; EPA, undated).

Research articles written by Yoshida et al. (2006), Gong et al. (2001), Jong E De. (1980), and Wyszkowska et al. (2000) enumerated the following as the effects of oil contamination on soil quality

- Oil contaminated soil lose its fertility for more than 20 years. The texture and other physicochemical characteristics of the soil get affected.
- The mites and other insects can't survive in oil contaminated land leading to major imbalance in the food chain.
- Oil contamination has adverse effect on seed germination. Farmers are not able to grow agricultural crops for years.
- While Szaro et al. (1978), Agnes et al. (2003), Fernanda et al. (2010), Chen et al. (2004), Nelson-Smith et al. (1971), Environment Canada's methods for assessing oil spill treating agents (2011), Moacir et al. (2008), Samiullah (1985), Michele et al. (2000) and Peterson (2001) in their papers enumerated the following as the effects of oil contamination on Fresh water and Marine Eco-system and ecological habitat
- A large percentage of the oil spill gets spread over the surface of the stagnant aquatic system resulting anaerobic environment in the water below the surface. This leads to death of the natural flora and fauna where oxygen is the key element for their respiration.

- Physical and chemical alteration of natural habitats.
- Physical smothering effect and lethal or sub-lethal toxic effects on the aquatic life.
- Aquatic birds suffer from Hypothermia, Drowning, loss in flight, Poisoning etc.
- Crude oil exposure may cause damage to lungs, liver, kidneys, intestines and other internal organs of the aquatic birds and animals.
- Reproductive Impairment in birds, fish and reptiles
- Plants covered by the oil, unable to photosynthesize.
- Oil spill significantly reduce the density of invertebrates and taxonomic richness at least 5 km downstream.
- Some fish species has been found with altered tracheal gills impregnated with tarlike substance.
- A large percentage of the oil spill gets emulsified and solidified along with sea shore, clinging to sand, rock and stone.
- Changes in the marine ecosystem resulting from oil effects on key organisms e.g. increased abundance of intertidal algae following the death of limpets, which normally eat the algae.

Other researchers like Gomer et al. (1980), Knafla et al. (2006), Zhang et al. (1992),Carpenter et al. (1977), Lee et al. (2006), Chen et al. (2008), Lewis et al. (2008), and Rice et al. (2007) also enumerated the effects of oil contamination on human health. Such are

- Long term exposure to crude oil contamination leads to severe diseases to human and other animals.
- Polycyclic aromatic hydrocarbons (PAHs), one of the major components found in crude oil are highly health hazard like skin erythema (reddening), skin cancer, sinonasal cancer, gastrointestinal cancer, and bladder cancer.
- Inhalation of hydrocarbon vapours causes headache, nausea, dizziness, respiratory irritation.
- Benzene, Toluene, Ethylbenzene and Xylene (BTEX), the key components of crude oil causes mutations, cancers, birth defects, endocrine disruptions, nervous disorders, and liver disease, carcinogen, effect on CNS, depression, irregular heartbeats.

Conventional Methods for Remediation of Oil Contamination

Some of the conventional methods applied since early times for remediation of oily waste are as described below in Table – 1 (Vidali, 2011). It is observed that none of the conventional methods is environment friendly solution (Sood et al., 2009).

It is an established that virtually all types of hydrocarbons are susceptible to microbial degradation and hence the relevance of using the biotechnological approach using the microbial capability for bioremediation of the hazardous waste is justified (Atlas, 1991; Head, 1998). Bioremediation has emerged as one of the most promising treatment options for oil contamination (Bragg et al., 1994 and Prince et al., 1994). Bioremediation has been defined as “the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes”

(Office of Technology Assessment, 1991). This technology is based on the premise that a large percentage of oil components are readily biodegradable in nature (Atlas, 1984, atlas, 1981and Prince, 1993).

Table – 1: Conventional methods for remediation of Oily waste (Vidali, 2011).

Method	Description	Merits	Demerits
Land filling	Sludge is stored in secured land fill sites with measures taken to avoid leakage and leaching of the toxic constituents to the underground.	The oil contaminated site gets cleaned up.	Confines the pollution while doing little to reduce or remove it. Cost-intensive due to transportation and leading to limitation of land availability. Sabotage or negligence might lead to groundwater contamination as well as green house gas emission.
Incineration	In this process, the sludge is burnt in large incinerators and their volume is decreased considerably.	Contaminated sludge is removed to a greater extent. Very quick method.	Direct contribution to the air pollution in terms of emission of hazardous gases like NOx, CO, SOx & VOCs etc. to air and environment. After incineration, equal amount of fly ash will be generated and will be sprayed to the environment, which will be poisonous to human health. Due to incineration, the PAHs (polycyclic aromatic hydrocarbons) will be generated and contributed to the environment. These PAH are highly toxic and rather mutagenic to human health. Transportation of sludge might generate secondary pollutants as further contamination. The method is highly cost-intensive
Air Spurging	Remove VOCs (volatile organic compounds) by spurging of air. Mainly applied in aquifer water.	Quick method. Almost all VOCs get removed from the system	Increase air pollution by adding VOCs to air Health hazardous and not environment-friendly Confined to a particular system High cost investment Only VOCs are removed and not total oil contamination
Surfactants	Remove contaminated oils by solubilizing or making dispersions using chemical surfactants and cleaning up of the site.	Site gets cleaned	Increase water pollution by dumping material in sea Health hazardous and not environment-friendly Confined to a particular system High cost investment Adds extra pollution load to the environment by applying chemical surfactants.

Bioremediation - An environment friendly approach

Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to nontoxic compounds (Lal et al.,1996). There are innumerable strains of microbes under basic categories of bacteria, yeast or fungi, which degrade oily sludge and waste water effluent sludge through digestion of harmful chemicals and compounds present in oily sludge and waste water effluent sludge into simpler, less toxic or non-toxic substances (bartha et al., 1984). Many species of soil bacteria, for example, use petroleum hydrocarbons as a food/energy source, transforming them into harmless substances consisting mainly of carbon dioxide, water and fatty acids (Bartha et al., 1984 an Sarma et al.,2004).

Bioremediation exploits this natural process by promoting the growth of microbes that can effectively degrade specific contaminants and convert them to nontoxic by-products. There are two basic types of bioremediation:

(a) "Biostimulation" provides nutrients to the indigenous microbial populations and promotes growth and increases metabolic activity that is used to degrade contaminants.

(b) "Bioaugmentation" introduces specific blends of microorganisms into a contaminated environment or into a bioreactor to initiate the bioremediation process, which increases the population of the fit to handle the biodegradative process in the contaminated area (Vidali, 2011).

Laboratory studies and field tests have shown that bioremediation can enhance oil biodegradation on contaminated shorelines (Prince, 1993 and Swannell et al., 1996). The success of bioremediation depends on having the appropriate microorganisms in place under suitable environmental conditions. Its operational use can be limited by the composition of the contaminant.

Advantages of Bioremediation:

- An ecologically sound, natural process; existing microorganisms can increase in numbers when oily sludge and waste water effluent sludge (the contaminants) is present. When the contaminants are degraded, the microbial population naturally declines. The residues from the biological treatment are usually harmless products (such as carbon dioxide, water and fatty acids). The carbon dioxide generated is used up by plants for photosynthesis; hence carbon sequestration would take place.
- Instead of merely transferring contaminants from one environmental medium to another (e.g., from water to the air or to land) bioremediation destroys the target chemicals.
- Bioremediation is usually less expensive than other technologies that are often used to clean up hazardous waste. For example, during the cleanup of the Exxon Valdez spill, the cost of bioremediating 120 km of shoreline was less than one day's costs for physical washing (Atlas et al., 1995).
- Bioremediation can often be accomplished where the problem is located ("*in situ*"). This eliminates the need to transfer large quantities of contaminated waste off site, and the potential threats to human health and the environment that can arise during such transportation.

Oilzapper- Biotechnology Intervention for Remediation

Olizapper is a biotechnological intervention for remediation of oily sludge, oil contaminated soil and oil contaminated drill cuttings. Since oily sludge is a complex molecule, there is no single bacterial strain found to biodegrade all its components. Hence the approach of selecting specific bacterial strain for biodegradation of specific component of oily waste and make a consortium of the optimum strains has given success in field bioremediation study (Mishra et al., 2004; 2001; 2001a). The Energy and Resources Institute (TERI), New Delhi, under the sponsorship from Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India, had initiated research to find out a suitable biotechnological solution for disposal of oily sludge / oil contaminated soil.

OILZAPPER, a consortium of crude oil / drill cutting / oily sludge degrading bacteria, deriving from various bacterial cultures existing in the natural environment has been developed through an extensive research at TERI. At the same time TERI jointly with IOCL R&D centre, Faridabad, developed Oilivorous – S and Oilivorous – A for application to the specific quality of oily sludge. Oilivorous – S has been found to be effective against the oily sludge with high sulfur content whereas Oilivorous – A has been developed specifically for oily sludge which are highly acidic in nature.

Advantages of the Oilzapper Technology In Bioremediation

- Oilzapper microbes are indigenous, natural occurring efficient microbes which exist in normal soil and were isolated from different geoclimatic regions of India. The selected microbes are the most adapted to degrade toxic hydrocarbons at local environmental conditions.
- In the bioremediation process a specially designed nutrient formulation is used to increase the efficiency of microbes.
- The microbes can biodegrade at temperature ranging from 15°C to 60°C and even in high saline soil and up to 1.5 depth of surface.
- In the process a consortium of four microbes is used which could degrade alkane, aromatic, NSO (Nitrogen, Sulphur, Oxygen containing compound) and asphaltane fractions of crude oil / oily sludge.
- An ecologically sound, natural process; sludge eating bacteria can increase in numbers when an oily sludge (contaminant) is available. When the contaminant is bioremediated, the microbial population naturally declines. The residues from the biological treatment are usually harmless products (carbon dioxide, water and fatty acids).
- Usually less expensive than other technologies that are often used to clean up hazardous waste.
- The organisms used in the consortium are not pathogenic and easy to handle.

Although extensive research has been conducted on oil bioremediation, most existing studies have concentrated on either evaluating the feasibility of bioremediation for dealing with oil contamination, or testing favored products and methods (Mearns et al., 1997). Only limited numbers of pilot-scale and field trials, which may provide the most convincing demonstrations of this technology, have been carried out. The paper describes TERI's experience on field case studies on bioremediation of oily waste using OILZAPPER technology in India and Abroad.

Methodology

Selection and Preparation of Bioremediation Sites

100 field studies were carried out on bioremediation of petroleum hydrocarbon contaminated waste in India and Abroad. The type of contamination includes waste oily sludge, emulsified oily sludge, accidental oil spill on land and water, oil contaminated drill cuttings, acidic oily sludge, high sulfur containing oily sludge, synthetic oil based mud waste etc. as described in Table- 2 (Mandal et al., 2007, 2010, 2009, 2007a, 2009a; Lal et al., 2007 and Sarma et al., 2007, 2006).

The bioremediation job was carried out *in situ* as well as *ex situ*. In case of *in situ* bioremediation the process was executed on site of the hydrocarbon contaminated area. Whereas in case of *ex situ* bioremediation process, a secured HDPE (high density poly ethylene) lined bioremediation site was prepared near the contaminated area and the contaminated soil / sludge was transported to the secured bioremediation site where the bioremediation process was executed. Where required, one bore well was installed near the secured bioremediation site for monitoring the ground water quality during the bioremediation process.

Selection of microbial consortium

TERI has isolated 324 indigenous microbial species, from different geoclimatic conditions of India and Abroad, which can biodegrade the oil content present in the oily waste. Depending on the climatic condition of the bioremediation site as well as type of hydrocarbon contamination a microbial consortium is selected for effective biodegradation of the hydrocarbon contaminated waste. For each sites the microbial consortium was selected for application on the oily waste. The microbial consortium named “Oilzapper” has been applied for bioremediation of oily wastes like crude oily sludge, emulsified oily sludge, oil contaminated land and water in various oil installations of different climatic conditions in India, “Oilivorous – A” for bioremediation of acidic oily waste and “Oilivorous – S” for bioremediation of high sulfur containing oily waste, “Oilzapper- D” for bioremediation of oil contaminated drill cuttings and oil based muds, etc. TERI has also developed indigenous microbial consortia for bioremediation of oil contaminated land at Kuwait and oil contaminated drill cuttings at Abu Dhabi.

Application of microbial consortium on oily waste

The microbial consortium was produced in 1500 liter bioreactor at TERI, New Delhi, India. The consortium was immobilized with a suitable carrier material, packed in sterilized polybags (packing size 5 - 20 kg) and transported to the respective sites for its application on oily waste. The consortium was applied on oily waste by manual spreading at regular intervals of one month. Specially designed nutrient formulation was dissolved in water and spread uniformly to the bioremediation site with the help of water sprinkler. This is done to enhance the population of the microbial consortium and also to mitigate the initial toxic shock due to the oil contamination while application on the oily sludge in the field. Mixing of oily sludge and microbes was done by tilling of bioremediation sites. In the control site, microbial consortium was not added, however rest of the other activities like tilling, watering etc. were carried out in the same manner as the experimental bioremediation site.

Tilling and watering

Tilling of the bioremediation sites was done at a regular interval of once in a week. This was done with the help of a tractor attached with cultivator or soil excavator like JCB/ Hitachi. Regular tilling was done to maintain aeration for the microbial consortium at the bioremediation sites. Regular watering of the bioremediation sites was done at every fourth day using sweet water collected in a water tanker and by sprinkling the same with the help of water sprinkler. During monsoon season, watering was done depending on the frequency of rain.

Sampling

Sludge / soil samples were collected from the bioremediation sites at zero day i.e. before application of microbes on the bioremediation site and at every 30 days interval after application of the microbial consortium. The bioremediation site was divided in four equal blocks, which were further divided in four sub-blocks. Equal quantity of samples were collected randomly from each sub-block i.e. total 16 samples were collected from one site. Samples were collected using a hollow stainless steel pipe of 3 inch diameter and 50 cm. in length and by inserting the same vertically on the bioremediation site from the surface till the bottom in one particular point. This was done to collect uniform samples from each depth of the bioremediation site. The samples were collected in sterile plastic containers. The sixteen samples were mixed uniformly to get a homogenized composite mixture, which was considered as the representative sample from the site. Mixing was done in a large container by hand with hand gloves. $\sqrt{N}+1$ statistical technique was applied for analyzing the composite sample in replicates in the laboratory for monitoring of the bioremediation job. The bore well water samples were collected in sterile plastic bottles from each bore wells installed at the nearby area of the bioremediation site. The bore wells were flushed thoroughly before collecting the samples.

Table – 2: Location of bioremediation sites in the field case studies by TERI, India.

Name of the company where bioremediation job has been carried out	Types of oil contamination treated	Quantity of oil contamination (Tonnes)
BG Exploration & Production India Limited, India	Synthetic oil based mud (SOBM) waste	2185.00
Bharat Petroleum Corporation Limited, India	Tank bottom oily sludge/ oil contaminated soil	5000.00
Cairn Energy Pty. Ltd., India	Oil contaminated Drill cuttings	510.00
Chennai Petroleum Corporation Limited, India	Tank bottom / ETP oily sludge	4000.00
Chandrapur Super Thermal Power Station, India	Thermal power station sludge	20.00
Hindustan Petroleum Corporation Limited, India	Tank bottom / ETP oily sludge	1500.00
Indian Oil Corporation Limited, India	High sulfur containing / Acidic oily sludge	55850.00
Indian Petrochemicals Corporation Limited, India	Tank bottom / ETP oily sludge	150.00
Mangalore Refinery and Petrochemicals Limited, India	Tank bottom / ETP oily sludge	2150.00

Oil and Natural Gas Corporation Limited, India	Tank bottom / ETP / emulsified oily sludge/ oil contaminated land/ oily sludge pit	74589.00
Oil India Limited, India	Tank bottom / ETP oily sludge / oil contaminated water	6670.00
Reliance Petroleum Limited, India	Tank bottom / ETP oily sludge	300.00
Tata Power Company Limited, India	Thermal power station sludge	10.00
KOC, Kuwait	Oil contaminated land	700.00
ADNOC, Abu Dhabi	Oil contaminated drill cuttings	200.00
Total quantity of oil contaminated waste bioremediated by TERI, New Delhi, India		**153834.00**

Equipments Used

The following equipments were utilized for the bioremediation operation in the field:

- JCB Backhoe Loader – for loading oily sludge to the dumper truck for transportation to the bioremediation site from the storage location. Also used for spreading of oily sludge and leveling of the bioremediation site.
- Dumper Truck – for transportation of the oily sludge.
- Light Commercial Vehicle (LCV) – for transportation of the microbial consortium from laboratory to the bioremediation site.
- Bioreactor assembly – for bulk production of the microbial consortium.
- Tractor with cultivator / JCB – used for tilling of the oily sludge at the bioremediation site.
- Water Tanker with sprinkler – used for spraying of sweet water to the soil at the bioremediation site.
- Bore wells – used for collection of ground water for regular monitoring.
- Small hollow pipe – used for collection of soil samples from the bioremediation site.

Monitoring of Bioremediation Process

Samples of oily waste from the bioremediation site and bore well water from the bore wells situated near the bioremediation site were collected at zero day i.e. during initiation of the bioremediation job and after regular interval till the completion of the job. The samples were analysed for the selected parameters as mentioned below

Characterization of Oily Sludge

Total petroleum hydrocarbon (TPH) was extracted from a known quantity of oily sludge by solvent extraction method (USEPA 1664 & 8100) by Soxhlet extractor using various solvents like hexane, methylene chloride and chloroform consecutively. The extracts were pooled and dried at room temperature by evaporation of solvents under gentle nitrogen stream in a fume hood. The amount of TPH recovered from the oily waste was quantified by gravimetric method. The sediments/ash content in the residual oily sludge was measured by heating the sludge, after TPH extraction, at 600°C for five hours using a crucible and subsequent cooling to room temperature. The amount of ash recovered from the residual sludge was quantified gravimetrically (Mishra et al., 2001).

The TPH extracted from the oily sludge was further fractionated for various fractions like alkane, aromatic, NSO and asphaltene fractions. A known quantity of TPH was dissolved in n-pentane. The insoluble fraction (asphaltene) was quantified. The soluble fraction was further loaded on silica gel column and eluted with different solvents (Walker et al., 1975). The alkane fraction was eluted with hexane, followed by the aromatic fraction that was eluted with benzene. The NSO fraction was eluted with chloroform and methanol. Alkane and aromatic fractions were concentrated by evaporation of solvents and then 0.2 μl of each was analyzed by gas chromatography (GC Hewlett Packard, 5890 Series II) to identify all the compounds present in the alkane and aromatic fractions by matching the retention time with authentic standards (Mishra et al., 2001).

Determination of Microbial Count

Total Bacterial Count (TBC) was determined by standard spread plate method with serial dilutions of the oily sludge samples. Standard Luria Bertini agar plate (Himedia catalog no. M 557) was used for determining TBC. (Mishra et al., 2004)

Determination of Ph, Moisture Content and Selected Heavy Metals

20% (w/w) solution of the sludge sample was prepared in distilled water and mixed thoroughly using magnetic stirrer. After settling down, the liquid layer was separated by filtration. The pH of the filtrate was measured using standard pH meter (Orion Expandable Ion Analyzer model no. EA – 940). The pH of the ground water samples was measured directly by the above pH meter. Moisture content of the oily sludge was determined by the standard method IS – 2720 – P2. Selected heavy metals (Lead, Arsenic, Manganese, Chromium, Molybdenum, Cobalt, Cadmium, Selenium, Zinc and Nickel) were analysed as per USEPA - 846 method using Atomic Absorption Spectrophotometer AAS- TJA (Unicam, USA) SOLAAR M Series Model. Oil and grease in the ground water samples were determined as per the standard method IS 3025 (P 39): 1991.

Biodegradation of TPH in the Oily Sludge

The oily sludge samples were collected from the bioremediation site at the zero day i.e. before initiation of the bioremediation job and after regular interval of one month till the completion of the bioremediation job. The TPH and its various fractions were analyzed for every sample. The decrease in the TPH content and its fractions with time and the percent biodegradation was calculated. Simultaneously, biodegradation of alkane and aromatic fractions was assessed by quantitative measurement of the peaks from the GC chromatogram with the help of standard calibration curve of each compound of alkane and aromatic fractions (Mishra et al., 2001a).

Toxicity Studies

The bioremediated soil was studied for soil characteristics with respect to agricultural quality (i.e. analysis of nitrogen, phosphorous, potassium, texture, pH, electrical conductivity, soil water holding capacity, etc. by IS standard methods) as well as soil toxicity like fish toxicity (by IS method no 6582 : P – II : 2001), presence of selected heavy metals, benzene, toluene, ethylbenzene, xylene, polycylic aromatic hydrocarbon(PAH), Polychlorinated biphenyls (PCBs) etc. (by USEPA methods).

Results and Discussion

Composition of Oily Sludge

In initial oil content in terms of solvent extractable TPH in the case studies varied from 5% to 52% in the field case studies. The remaining part was moisture and residual soil. The steam extractable TPH in the oily sludge was found to be nil. TPH extracted from the oily sludge contained 40 - 70% alkane fraction, 15 - 30% aromatic fraction, 5 - 15% heavy fractions like NSO asphaltene, resins etc. Table – 3 below describes the detailed composition of the oily waste undertaken in one of the case studies in India.

Biodegradation

After complete application of the microbial consortium to the bioremediation sites, it was observed that within 2 - 6 months period the TPH content of the oily waste has been biodegraded to less than 1% indicating more than 95% biodegradation in almost all the case studies. Whereas the degradation of oily waste in the control sites were hardly 5 – 15% in the same time period. Figure – 1 & 2 below describes the trend of biodegradation in two of the case studies. Figure – 3 below describes the GC chromatograms indicating the biodegradation of the alkane and aromatic fraction of the oily waste in one of the case studies. This indicates that the bioremediation process by using the microbial consortium is an efficient process for degradation of oil contamination.

Table- 3 : Composition of Oily waste undertaken for the bioremediation job at CPF, Gandhar, ONGC Ankleshwar Asset, India:

Constituents of crude oil/ oily sludge	Composition (%)
Steam extractable total petroleum hydrocarbon (TPH) in crude oil/oily sludge	Nil
Solvent extractable TPH in crude oil/oily sludge	44.65
Water content in crude oil/oily sludge	27.12
Heavy fraction of hydrocarbons	22.14
Sediments/inorganic	6.09
Constituents of TPH	
Alkane fraction	68
Aromatic fraction	20
NSO & Asphaltene fraction	12

PH And Microbial Count of the Oily Sludge Samples at the Bioremediation Site

Throughout the bioremediation treatment process, pH of the samples was within 6.5 to 8.8 in all the cases, except in the case of acidic oily sludge. The microbial counts were maintained in the range of 10^7 to 10^9 CFU per gram of sample in the experimental bioremediation site. However, in the control site the microbial count was found to be in the range of 10^2 to 10^4 CFU per gram of sample.

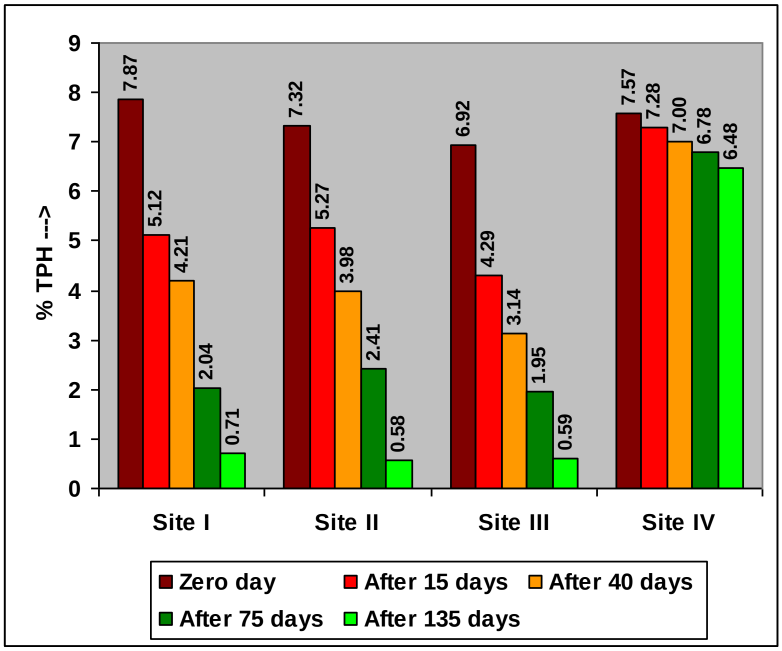

Figure – 1: ***Biodegradation of TPH of oil contaminated soil at South Santhal CTF, ONGC, Mehsana Asset, India. Site – I, II & III are experimental sites and Site – IV is control site where no Oilzapper was applied.***

Ground Water Quality

The pHs in all the ground water samples were within 7.5 to 8.5. In all the samples the oil and grease was found to be nil. This indicated that there was no leaching of oil to the underground water.

Heavy metal analysis

All the heavy metals before and after bioremediation were within the permissible limit as per Hazardous waste (management & handling) rules, amendment 2008, of India. However, there was no sign of biodegradation of heavy metals.

Soil Toxicity

The bioremediated soil was tested for soil toxicity as per the method described above and found to be non-toxic. There was no death of fish in the fish toxicity test in 10% leachate of the bioremediated soil. Also natural vegetation was found to be grown on the site after bioremediation. In one case study various vegetable and fruit species was grown successfully on the oil contaminated site after bioremediation (Figure – 4). In one oil contaminated lake fish culturing was done after bioremediation. Various fish species was found to be grown healthily and survived for long time. The fish species were tested for bioaccumulation of toxic component of hydrocarbons in the fish tissues. There was no traces of accumulation of petroleum hydrocarbon component was observed in the grown fish species (Figure – 5). Hence bioremediation by Oilzapper helps in ecorestoration of the hydrocarbon contaminated sites.

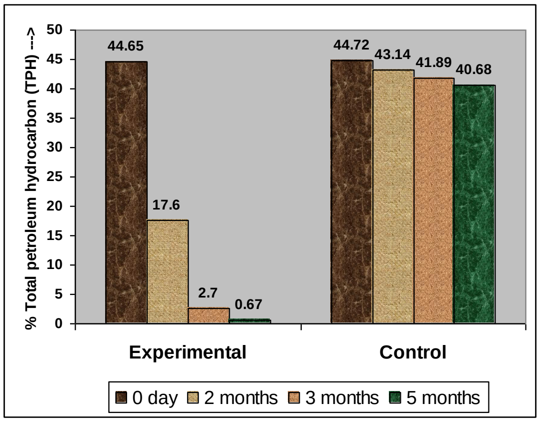

Figure – 2: ***Biodegradation of TPH of oil contaminated soil at CPF Gandhar, ONGC, Ankleshwar Asset, India.***

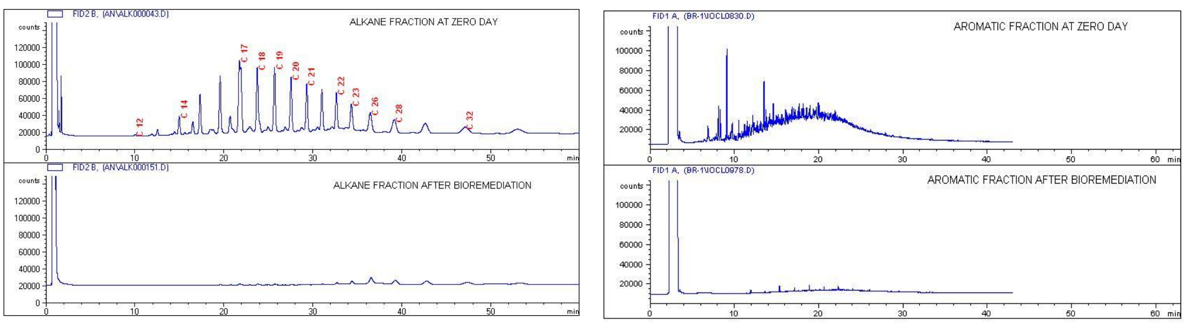

Figure 3 : ***GC chromatogram indicating the biodegradation of alkaneand aromatic fraction of TPH at CPF Gandhar, ONGC, Ankleshwar Asset, India.***

SUMMARY

Oil and gas industries contribute to major industrial pollution. Various preventive measures are taken care by the industries to minimize the environmental pollution. Bioremediation has been found to be the most environment friendly method for treatment of oil contamination generated due to various petroleum industries. It is the most cost effective technology. Using bioremediation technology TERI, India, has treated more than 1,50,000 metric tonnes of oil contamination at various oil installations in India and abroad and more than 60,000 tonnes of oil contamination is under treatment by bioremediation. Bioremediated soil has been found containing TPH content to the extent of <1%. Bioremediation technology has helped the oil industries in ecorestoration of the hydrocarbon contaminated sites.

Fruits and vegetables grown and Insects & animals survived on bioremediated soil

Figure – 4 ***: Bioremediation site near GC-2 at Burhan, South Kuwait Oil Field, Kuwait Oil Company (KOC), Kuwait.***

Oil contaminated lake before bioremediation

Oil contaminated lake after bioremediation

Natural vegetation on oil contaminated lake after bioremediation

Fish grown on oil contaminated lake after bioremediation

Figure – 5 : ***Eco-restoration of oil contaminated lake by bioremediation technology at North Kadi, Mehsana Asset, of Oil and Natural gas Corporation Limited (ONGC), India.***

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