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LARGE SCALE BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED WASTE AT INDIAN OIL REFINERIES: CASE STUDIES

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ABSTRACT

The petroleum industry effluents, oily sludge and oil spills cause a serious threat to the environment as their constituents are toxic, mutagenic and carcinogenic. Safe disposal of these wastes is serious problem. None of the available conventional disposal methods are environment friendly. Biological methods have been well reviewed and acknowledged for remediation of petroleum hydrocarbon contaminated waste (oily waste). An indigenous microbial consortium was developed by assemble of four species of bacteria, isolated from various oil contaminated sites of India, which could biodegrade different fractions of total petroleum hydrocarbon (TPH) of the oily waste to environment friendly end products. The said consortium was applied on field scale at different oil refineries in India and successfully bioremediated 48,914 tons of different types of oily waste. In 44 field case studies of different batch size of *ex situ* bioremediation process, the initial TPH content varying from 83.50 to 531.30 gm/kg of oily waste, has been biodegraded to < 10 gm/kg of oily waste in major cases in 2 – 12 months. In one refinery due to coastal climate, the bioremediation time was > 20 months. The bioremediated soil was non-toxic and natural vegetation was found to be grown on the same. Bioremediation technology has helped various oil industries for the management of their hazardous oily wastes in environment friendly manner.

KEYWORDS: Bioremediation, Biodegradation, Oily waste, Microbial consortium, Total Petroleum Hydrocarbon.

INTRODUCTION

Petroleum refineries unavoidably generate enormous quantity of waste oily sludge, in terms of tank bottom and effluent treatment plant (ETP) oily sludge, and oil contaminated soil in their day to day refining process. These waste oily sludge and oil contaminated soil (termed as "oily waste") are stored in sludge pits inside the refinery premises and due to stringent regulatory norms, the disposal of these oily waste in an environment friendly manner is a serious problem. 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 TPH constitutes a complex mixture of alkane; aromatic; nitrogen, sulfur, and oxygen containing compounds

(NSO); and asphaltene fractions (Bhattacharya et al., 2003). Oil contamination has severe impacts in the plant and animal ecosystem including human health (Mandal et al., 2007; EPA, undated). Crude oil exposure may cause damage to lungs, liver, kidneys, intestines and other internal organs. Polycyclic aromatic hydrocarbons (PAH) may lead to cancer, Inhalation leads to headache, nausea, dizziness, respiratory irritation, BTEX (Benzene, Toluene, Eethyl benzene & Xylene) cause mutations, cancers, birth defects, nervous disorders, and liver disease, depression, irregular heartbeats etc. (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). Oil contaminated soil loose its fertility and have impact on seed germination. (Yoshida et al., 2006 and Gong et al., 2001). Hence disposal of the oily waste in an improper manner may cause a serious environmental problem (Yustle et al., 2000). Various conventional methods like land filling, incineration, air spurging, etc. have been applied since early times for remediation of oily waste (Vidali, 2011 and Mandal et. al., 2007). It is observed that none of the conventional methods is environment friendly solution (Sood et al., 2009). The common drawback is that they are not the permanent solution for the environmental pollution and sometimes they are not cost effective (Mandal et. al., 2007, and Ouyang et. al., 2005)

It is 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 applied as a cost effective, ecologically friendly and efficient treatment technology for the contamination of hydrocarbon polluted soils (Chikere et. al., 2009). Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to nontoxic compounds (Lal et al.,1996 and Bartha et al., 1984). 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 and composition of the contaminant. Although extensive laboratory research has been conducted on oil bioremediation, only limited numbers of pilot-scale and field trials with small quantity of oily sludge, which may provide the most convincing demonstrations of this technology, have been carried out in India and abroad. (Raghavan et. al., 1999; Mishra et. al., 2001; B K Gogoi et. al., 2003, Ouyang et. al., 2005, Chikere et. al., 2009 and Liu et. al., 2009). The present paper describes our experience on field case studies on bioremediation of oily waste at various oil refineries in India using indigenously developed microbial consortium.

MATERIALS AND METHODS

3.1 Isolation and Identification of Microbial Strains

The crude oil contaminated soil samples were collected from different oil refineries and oil exploration sites of India. The solvent extractable TPH in the crude oil contaminated soil samples was estimated (Mishra et al. 2001[a]). For enrichment, 5 g samples of soil were inoculated into 100 mL of minimal salt medium (MSM) (Lal and Khanna 1996) containing steam sterilized crude oil (1%, w/v) as carbon source and incubated at 37°C on a rotary shaker (200 rpm) for 7 days. 5 mL of enriched culture was reinoculated in fresh medium under similar conditions and five such cycles were repeated. After five cycles of enrichment, 1 mL of culture was diluted up to 10^8 fold, and 100µL of all dilutions were plated on MSM agar plates with crude oil (1 % w/v). The bacterial colonies obtained were further purified on the MSM agar plates (with crude oil 1 % w/v). The isolates were routinely sub cultured and frozen stock cultures were stored in 25 % glycerol at -70°C.

Identification of the isolated bacterial strains was done by sequencing of the 16S rDNA gene with the Microseq 16S DNA sequencing Kit ™ (PE Applied Biosystems, Inc, USA) (Sarma et al, 2004). The sequences were analyzed with an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems, Inc,

USA) as per manufacturer's instructions. The sequences of 16S rDNA genes were subjected to BLAST searches of the NCBI GenBank database for identification.

3.2 Degradation of Crude Oil by Microbial Strains

Degradation of crude oil by the bacterial isolates was monitored in 250 mL flasks in triplicates containing 100 mL MSM with 1 % (w/v) of crude oil (steam sterilized) as sole carbon source and incubation on a rotary shaker (200 rpm) at 37°C. The isolates were grown previously in MSM for 24 h with 1 % (w/v) crude oil to a cell density of 10^8 cells mL^{-1} and were inoculated into the medium with 5 % (v/v) as inoculum. Uninoculated controls were kept to monitor natural weathering of crude oil. Residual crude oil was extracted from the cultures by using solvents (Sarma et al. 2004). For quantitative analysis, the residual crude oil was fractionated by silica gel column (Walker et al. 1975). The different fractions were analyzed by gas liquid chromatography (Hewlett Packard 5890 series II) fitted with flame ionization detectors (Mishra et al. 2001[a]). The profile of the different fractions of petroleum hydrocarbons extracted from inoculated flasks was compared with that of the uninoculated controls to determine the extent of degradation.

3.3 Selection of Microbial Consortium

Based on the efficiency to degrade different fractions of total petroleum hydrocarbons (aliphatic, aromatic, asphaltanes, NSO compounds) and also based on the environmental parameters from where these bacterial strain have been isolated, few bacterial consortium have been developed for application on the actual field (Mishra et. al. 2001, 2001a, 2004; Lal et al. 1996; Sood et. al. 2009, 2009a and Prasad et. al. 2005). The crude oil degrading efficiency (qualitative and quantitative) of individual bacterial isolates was screened on minimal salt medium using crude oil as sole carbon and energy source as per the methods described above.

3.4 Selection and Preparation of Bioremediation Sites

In total 44 field studies were carried out in batches on bioremediation of oily waste in Indian oil refineries mainly in Indian Oil Corporation Limited (IOCL), Chennai Petroleum Corporation Limited (CPCL) and Mangalore Refinery and Petrochemicals Limited (MRPL). The type of contamination included acidic oily sludge (only at Digboi refinery of IOCL) and non-acidic waste oily sludge (at other refineries) as described in Table- 1 (Mandal et al., 2011, Lal et al., 2007 and Sarma et. al., 2006). The bioremediation jobs were carried out *ex situ* in different batches, where a secured HDPE (high density poly ethylene) lined bioremediation site was prepared near the sludge storage pit inside the refinery premises. The oily waste was excavated by using excavator and transported to the secured bioremediation site using dumper / trailer where the bioremediation process was executed. The bioremediation sites were located at different climatic zones spread all over India. In some places like Gujarat refinery, IOCL, the climate was semi-arid with average maximum temperature of 43^0C and average yearly rainfall of 930 mm. IOCL Panipat refinery was located in fully arid zone. The temperature at IOCL Mathura refinery reaches upto 46^0C at peak summer. At Haldia refinery, IOCL, average temperature is 7 – 22 ^{0}C and there is heavy rainfall during monsoon. At Mangalore refinery the average temperature is 27 – 34^0C and frequently heavy rainfall of average 42418 mm and coastal and fully terrain zone. The average temperature at CPCL refinery is 16 – 45 ^{0}C and heavy rainfall of average 1400 mm being a coastal zone. At Assam region (Guwahati and Digboi refinery) the climate was of high humidity with heavy rainfall and average temperature was 6 – 8 ^{0}C in winter and 35 - 38^0C in summer season. In few case the main complexity was due to heavy rain during the bioremediation process which used to delay the performance of the bioremediation job as the movement of equipments like excavator etc. was very difficult in rainy season.

Indian Oil Corporation Limited receives crude oil for refining process from various sources both indigenous as well as outside India. Due to the variation of source of crude, the physicochemical properties of the crude vary to a greater extent. Data collected from different refineries of IOCL, indicates that the density @ 15 ^{0}C of crude oil varies in the range from 0.8390 – 0.8745, specific gravity in the range from 0.8280 - 0.8750, API

gravity in the range from 16.0 - 48.1, Basic sediment and water content (BS&W) in the range from 0.06 % - 0.23 % and Sulphur content in the range from 0.02% - 4.30%. This indicates the variation in the chemical composition of the oily sludge undertaken for bioremediation at different Indian oil refineries.

3.5 Application of Microbial Consortium on Oily Waste

The microbial consortium was produced in 1500 liter bioreactor (purchased from M/s Bioengineering AG, Switzerland) 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, containing Nitrogen (N), Phosphorous (P) and Potassium (K) compounds, was dissolved in water and spread uniformly to the bioremediation site with the help of water sprinkler. This was 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 waste in the field. Mixing of oily waste 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.

Table – 1: ***Details of field case studies on the bioremediation jobs carried out at various oil refineries in India.***

Particulars of oil refinery	pH of oily waste	Quantity (Ton) of oily waste bioremediated	No. of batches of bioremediation
Chennai Petroleum Corporation Limited (CPCL), Chennai, Tamilnadu state, India (**CPCL, Chennai**)	6.5 - 8.5	4,100	4
IOCL, Barauni refinery, Barauni, Bihar state, India (**IOCL, Barauni**)	6.5 - 8.5	5,250	3
IOCL, Digboi refinery, Digboi, Assam state, India (**IOCL,Digboi**)	1.0 –3.0 (Acidic)	9,258	12
IOCL, Gujarat refinery, Baroda, Gujarat state, India (**IOCL, Gujarat**)	6.5 - 8.5	11,500	7
IOCL, Haldia refinery, Haldia, West Bengal state, India (**IOCL, Haldia**)	6.5 - 8.5	10,500	6
IOCL, Mathura refinery, Mathura, Uttar Pradesh state, India (**IOCL, Mathura)**	6.5 - 8.5	2,850	3
IOCL, Panipat refinery, Panipat, Haryana state, India (**IOCL, Panipat**)	6.5 - 8.5	3,333	7
Mangalore Refinery and Petrochemicals Limited (MRPL), Karnataka state, India (**MRPL, Mangalore**)	6.5 - 8.5	2,150	2
Total / Range		**48,941**	**44**

3.6 Tilling and Watering

Tilling of the bioremediation sites was done at a regular interval of once in a week to maintain aeration for the microbial consortium at the bioremediation sites. This was done with the help of a tractor attached with cultivator or soil excavator like JCB/ Hitachi. Watering of the bioremediation sites was done as per the requirement to maintain the moisture content of the soil for quicker biodegradation.

3.7 Sampling

Oily waste 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 (Cline, 1994). 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.

3.8 Monitoring of Bioremediation Process

Samples of oily waste from the bioremediation site were collected at zero day and after regular interval till the completion of the job. The samples were analysed for the selected parameters as mentioned below:

3.8.1 Characterization of oily waste

Total petroleum hydrocarbon (TPH) was extracted from a known quantity of oily waste by solvent extraction method by Soxhlet extractor using various solvents like hexane, methylene chloride and chloroform consecutively. The extracts were pooled and dried at room temperature after distillation of solvents in a fume hood. The amount of TPH recovered 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 was quantified gravimetrically (Mishra et al., 2001). The extracted TPH 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).

3.8.2 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)

3.8.3 Determination of pH, moisture content and selected heavy metals

pH of 20% (w/w) solution of the oily waste sample was measured using standard pH meter (Orion Expandable Ion Analyzer model no. EA – 940). The pH of the ground water samples was measured directly. Moisture content of the oily waste 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.

3.8.4 Biodegradation of TPH in the oily waste

The decrease in the TPH content and its fractions with time and the percent biodegradation was calculated from the TPH data of the samples. 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).

3.8.5 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).

Seed germination studies with the soil before and after bioremediation were also carried out to compare the soil toxicity against seed germination. A filter paper disk (Whatman no. 1) was placed in the lid of each triplicate 9 cm glass petri dishes and soaked with 7 ml. water extracts, with dilutions of 2%, 4%, 10% and 20% (w/v) in deionized water, of the oil contaminated soil before and after bioremediation. One triplicate set of control petri dish was also prepared where only deionized water was used for soaking the filter paper disk. The deionized water was produced in the laboratory using deionizer unit model no. "RO – DI Ultra" supplied by M/s Rions India. The water extracts were prepared by mixing the required quantity of soil in deionized water using magnetic stirrer for 30 minutes, allowing the soil water mixture settle down at ambient temperature and filtering the liquid layer gravimetrically through filter paper (Whatman no. 1). In each petri dish 20 seeds of cereal crop *Hordeum vulgare* (barley seeds) were disposed on the surface of filter paper and incubated in darkness at 20 ± 1^0 C for 5 days. Moisture in each petri dish was maintained by adding 5 ml deionized water on daily basis. Seed germination and the length of the root produced by the seeds were measured after incubation period in all the extracts and the same were compared with those of the control. The germination index (GI) was calculated by multiplying percent germination (G) and relative root growth (RRG), both expressed as percentage (%) of control values as described in the formula below:

Germination Index (GI) = (% G × % RRG) / 100, where,

Germination = %G = (number of seeds germinated in experimental sample / number of seeds germinated in control) × 100 and

Relative root growth = %RRG = (mean root length in experimental sample / mean root length in control) x 100.

The germination index (GI) has been proven to be the most sensitive parameter, capable of detecting low levels of toxicity which affect root growth, as well as high toxicity levels which affect the seed germination. (Tehmina & Rukhsana, 2005; Delgado, 2010; Graciela & Painceira, 2005).

RESULTS AND DISCUSSIONS

4.1 Isolation and Identification of Hydrocarbon Degrading Microbial Strains

A total of 324 culturable bacterial strains were isolated from crude oily sludge and oil contaminated soil samples collected from 15 oil installations located at different geoclimatic regions of India (Table - 2). The culturable bacterial strains isolated from all the sampling locations were purified and preserved as frozen stock cultures in 25 % glycerol at –70 ^{O}C for further experiments. All the bacterial strains were given an accession number and identified by the method mentioned above. It was found that out of 324 isolates only 110 different species of bacterial strains were obtained (Table - 2). All the bacterial strains that have been isolated were analyzed for its efficiency to degrade the total petroleum hydrocarbon (TPH) and its different fractions of the crude oil and oily waste.

4.2 Development of Microbial Consortium

All the bacterial strains that have been isolated were analyzed for its efficiency to degrade the total petroleum hydrocarbon (TPH) and its different fractions of the crude oil and oily sludge. A consortium was developed containing five different most efficient strains to biodegrade different fractions TPH of the oily waste at Indian oil refineries and the same was applied for biodegradation in the field scale (Mishra et. al. 2001, 2001a, 2004; Lal et al. 1996 and Mandal et. al., 2011). For IOCL Digboi refinery a separate consortium was developed and applied for biodegradation of acidic oily sludge on field scale (Sood et. al. 2009, 2009a and Prasad et. al. 2005). The microbial consortium was grown in bulk scale as described above and applied on the oily waste for bioremediation along with the nutrient formulation. The quantity of microbes was decided so as to maintain the microbial count in the range of 10^7 to 10^9 colony forming unit (CFU) per gm of oily waste in the bioremediation site. Average quantity of nutrient formulation applied for bioremediation job was in the range of 0.01 – 0.07 kg per Ton of oily waste respectively (Table – 1).

4.3 Composition of Oily Waste

The average initial oil content in terms of solvent extractable TPH in the field case studies varied from 160.30 – 372.50 gm/kg of oily waste. The remaining part of the oily waste was moisture (191.90 – 371.30 gm/kg) and residual soil including inorganics, sediments, other organics, etc. (397.70 – 612.40 gm/kg). TPH extracted from the oily waste contained alkane fraction in the range of 313.40 – 660.00 gm/kg TPH, aromatic fraction in the range of 221.40 – 514.10 gm/kg TPH and heavy fractions like NSO (nitrogen, sulfur & oxygen fraction), asphaltene, resins etc. in the range of 95.70 – 172.50 gm/kg TPH (Table – 3). In IOCL Digboi refinery, the oily sludge undertaken for bioremediation was highly acidic in nature (pH< 3). In other refineries the pH of the oily sludge was in the neutral range.

Table – 2: ***TPH degrading bacterial strains isolated from sampling sites situated in different geoclimatic regions in India***

Isolation sites (Regional location in India)	Geographical location (Latitude & Longitude)		Tempe-rature range (^{0}C)	Total number of culturable bacterial strains isolated	Total number of species among the bacterial strains
	Latitude	Longitude			
IOCL, Mathura refinery (Northern)	$27^0 26' N$	$77^0 43' E$	10 – 40	30	14
IOCL, Barauni refinery (Eastern)	$25^0 28' N$	$85^0 59' E$	19 – 35	16	8
IOCL, Haldia refinery (Eastern)	$22^0 00' N$	$88^0 05' E$	15 – 35	26	6
IOCL, Gujarat refinery (Western)	$22^0 16' N$	$73^0 14' E$	20 – 38	30	9
IOCL, Panipat refinery (Northern)	$29^0 23' N$	$76^0 58' E$	15 – 40	20	5
ONGC oil well, Jorhat (North Eastern)	$26^0 40' N$	$95^0 35' E$	10 – 35	11	4
Oil well of Oil India Ltd., Duliajan, (North Eastern)	$27^0 15' N$	$95^0 15' E$	10 – 35	26	12
IOCL, Digboi refinery (North Eastern)	$27^0 15' N$	$95^0 15' E$	12 – 35	33	11
IOCL, Guwahati refinery (North Eastern)	$26^0 09' N$	$91^0 46' E$	15 – 35	22	10
BPCL, Mumbai refinery, (Western)	$18^0 56' N$	$72^0 51' E$	24 – 35	18	7
HPCL, Visakhapatnam (South Eastern)	$17^0 41' N$	$83^0 17' E$	21 – 42	19	6
CRL, Cochin refinery (Southern)	$9^0 55' N$	$76^0 14' E$	19 – 37	12	6
BRPL, Bongaigoan refinery (North Eastern)	$22^0 16' N$	$73^0 14'E$	18 – 32	13	6
Vadinar refinery (Western)	$23^0 44' N$	$72^0 39' E$	15 – 45	16	3
Reliance refinery, Jamnagar (Western)	$22^0 26' N$	$70^0 26' E$	15 – 45	32	3
Total				**324**	**110**

4.4 Biodegradation

The initial TPH content in the oily waste, undertaken for bioremediation at different oil refineries, was in the range of 83.50 – 531.30 gm/kg oily waste. After complete application of the microbial consortium to the bioremediation sites, it was observed that, in major cases, within 2 – 12 months period the TPH content of the oily waste has been biodegraded to less than 10 gm/kg of oily waste indicating more than 95% biodegradation (Table - 4). Whereas the degradation of oily waste in the control sites, where no microbes and nutrients were added, were hardly 5 – 15% in the same time period. The rate of biodegradation of TPH varied in the range of 0.07 – 0.84 Kg TPH/day/m^2 area of bioremediation site (Table – 4).

Figure – 1 below describes the trend of biodegradation in one of the case studies carried out at IOCL Panipat refinery, India, where the initial TPH of 206.50 – 231.00 gm/kg oily waste has been biodegraded to less than 10 gm/kg of oily waste within 3 months in 1st and 2nd batch, 4 months time in 3rd batch and 7 months time period in 4th batch of bioremediation job in the same bioremediation site indicating an average biodegradation rate of 0.40 kg TPH/day/m^2 area of bioremediation site. Whereas in the control site of IOCL Panipat refinery the TPH content in oily waste was found to be decreased from 225.60 to 193.50 gm/kg oily waste in 17 months time period. To note that in the case study at Mangalore refinery, the time for bioremediation was more than 20 months and the rate of biodegradation of TPH was 0.07 Kg TPH/day/m^2 area of bioremediation site (Table – 4). This was due to continuous heavy rain during the bioremediation process which has affected the mobilization of equipment like excavator etc. It was quite interesting to note that in some places the climate was arid (Panipat refinery) or semi arid zones (Gujarat refinery), however in those sites also the bioremediation job was successfully completed within 2 – 12 months time with a biodegradation rate of 0.38 and 0.41 Kg TPH/day/m^2 area of bioremediation site respectively. The biodegradation rate was maximum at IOCL Digboi refinery, 0.84 Kg TPH/day/m^2 area of bioremediation site, where the oily sludge was highly acidic in nature and specific acidic sludge degrading microbes were applied. The above results indicate that the bioremediation process by using the microbial consortium is an efficient process for treatment of oil contamination. Figures 2 & 3 below describes the GC chromatogram indicating biodegradation of Alkane and Aromatic fractions of the oily sludge at IOCL Panipat refinery. It can be observed that most of the alkane and aromatic fractions of the oily waste has biodegraded efficiently within the remediation time.

Table – 3: ***Characteristics (average composition) of oily waste undertaken for bioremediation at various refineries in India.***

Particulars of oil refinery	Composition (average) in the oily waste					
	TPH (solvent extractable) (gm/kg oily waste)	Moisture (gm/kg oily waste)	Residue (gm/kg oily waste)	Alkane (gm/kg TPH)	Aromatic (gm/kg TPH)	NSO + Asphaltene (gm/kg TPH)
CPCL, Chennai	279.40 ± 128.00	297.10 ± 101.40	424.70 ± 90.40	582.50 ± 17.10	302.50 ± 12.60	115.00 ± 12.90
IOCL, Barauni	185.40 ± 16.70	328.60 ± 11.90	486.00 ± 15.00	538.10 ± 36.10	344.60 ± 25.30	117.30 ± 15.60
IOCL,Digboi	372.50 ± 131.20	228.40 ± 51.50	399.10 ± 86.20	313.40 ± 47.20	514.10 ± 33.20	172.50 ± 50.60
IOCL, Gujarat	196.20 ± 51.10	323.90 ± 70.50	479.80 ± 64.20	660.00 ± 14.10	244.30 ± 12.70	95.70 ± 5.30
IOCL, Haldia	231.00 ± 25.60	371.30 ± 16.30	397.70 ± 32.40	512.00 ± 44.40	342.00 ± 25.70	146.00 ± 21.70
IOCL, Mathura	195.60 ± 37.80	191.90 ± 15.60	612.40 ± 51.90	596.70 ± 15.30	280.00 ± 20.00	123.30 ± 32.10
IOCL, Panipat	224.00 ± 10.80	255.90 ± 119.30	520.10 ± 121.90	621.40 ± 18.60	221.40 ± 9.00	157.10 ± 26.90
MRPL, Mangalore	160.30 ± 54.20	290.20 ± 51.20	549.50 ± 105.40	625.00 ± 7.10	250.00 ± 14.10	125.00 ± 7.10
Total / Range	**160.30 - 372.50**	**191.90 - 371.30**	**397.70 - 612.40**	**313.40 - 660.00**	**221.40 – 514.10**	**95.70 - 172.50**

4.5 pH and Microbial Count of the Oily Waste Samples at the Bioremediation Site

Throughout the bioremediation treatment process, pH of the samples was within 6.5 to 8.5 in all the cases, except in the case of acidic oily sludge. In case of Digboi refinery the initial pH of oily sludge was less than 3 which has increased slightly up to 5.5 after biodegradation. This slight increase in pH might be due to the addition of required quantity of uncontaminated soil at the bioremediation site, which was mixed with the acidic oily waste of higher TPH content at the initial stage so as to decrease the initial contamination load for the microbes. The microbial counts were maintained in the range of 10^7 to 10^9 CFU per gram of sample in the experimental bioremediation sites. However, in the control site the microbial count was found to be in the range of 10^3 to 10^5 CFU per gram of sample.

Table – 4: ***Biodegradation of TPH of oily waste undertaken for bioremediation at various oil refineries in India.***

Particulars of oil refinery	TPH (gm/kg oily waste) at bioremediation site		% Biodegradation (w / w)	Time taken for bioremediation (months)	Biodegradation rate (Kg TPH /day/m^2 area)
	Initial (Before bioremediation)	Final (After bioremediation)			
CPCL, Chennai	129.50 - 437.10	8.80 - 14.30	93.20 - 97.80	3 - 13	0.21 ± 0.07
IOCL, Barauni	162.00 - 212.20	3.70 - 50.70	70.18 - 98.14	5 - 5.5	0.43 ± 0.17
IOCL,Digboi	170.40 - 531.30	8.70 – 48.70	86.93 - 97.74	2.5 - 15	0.84 ± 0.64
IOCL, Gujarat	132.00 - 270.00	3.90 - 34.50	82.54 - 98.13	2 - 12	0.41 ± 0.36
IOCL, Haldia	193.00 - 269.00	5.60 - 12.50	94.47 - 97.44	6 - 10	0.19 ± 0.05
IOCL, Mathura	152.50 - 223.10	3.50 - 8.50	96.19 - 97.70	4 - 12	0.37 ± 0.20
IOCL, Panipat	206.50 - 238.00	2.60 - 8.00	96.51 - 98.86	3 - 10	0.38 ± 0.09
MRPL, Mangalore	83.50 - 198.60	8.40 - 9.10	89.94 - 95.12	21 - 24	0.07 ± 0.03
Total / Range	**83.50 - 531.30**	**2.60 – 50.70**	**70.18 - 98.86**	**2 - 24**	**0.07 - 0.84**

4.6 Ground Water Quality

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. The pH in all the ground water samples were within 6.5 to 8.5. To note that the pH of ground water near IOCL Digboi refinery, where highly acidic oily sludge was bioremediated, was also in the range of 6.5 – 8.5. This clearly indicated that there was no leaching of acidic oily sludge contamination to the underground water during bioremediation process. There was no change in the concentration of selected heavy metals in the ground water samples collected before and after the bioremediation job. This further confirms that there is no leaching of the contaminant to the underground water during the bioremediation process.

4.7 Heavy Metal Analysis

All the selected heavy metals concentration in the residual oily waste collected from the bioremediation site before and after bioremediation were within the permissible limit as per Hazardous Waste (Management and Handling) Rules, amendment 2008, of the Government of India. However, there was no sign of biodegradation of heavy metals in any of the case studies.

4.8 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. The bioremediated soil had no adverse effect on the seed germination. Also natural vegetation was found to be grown on the sites after bioremediation. Figure – 4 describes the results of the seed germination study carried out for the oil contaminated soil before and after bioremediation at IOCL Panipat Refinery. It has been observed that the Germination Index has been increased considerably in the soil after bioremediation. Hence bioremediation using microbes can help in eco-restoration of the hydrocarbon contaminated sites.

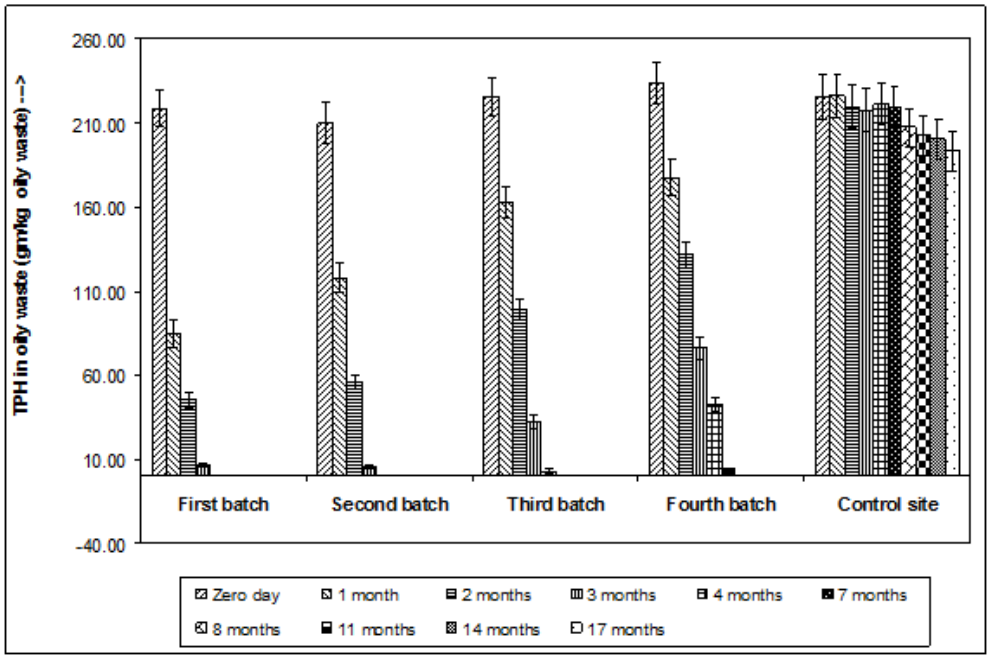

Figure 1 : ***Biodegradation of TPH of the oily waste at IOCL Panipat Refinery, India.***

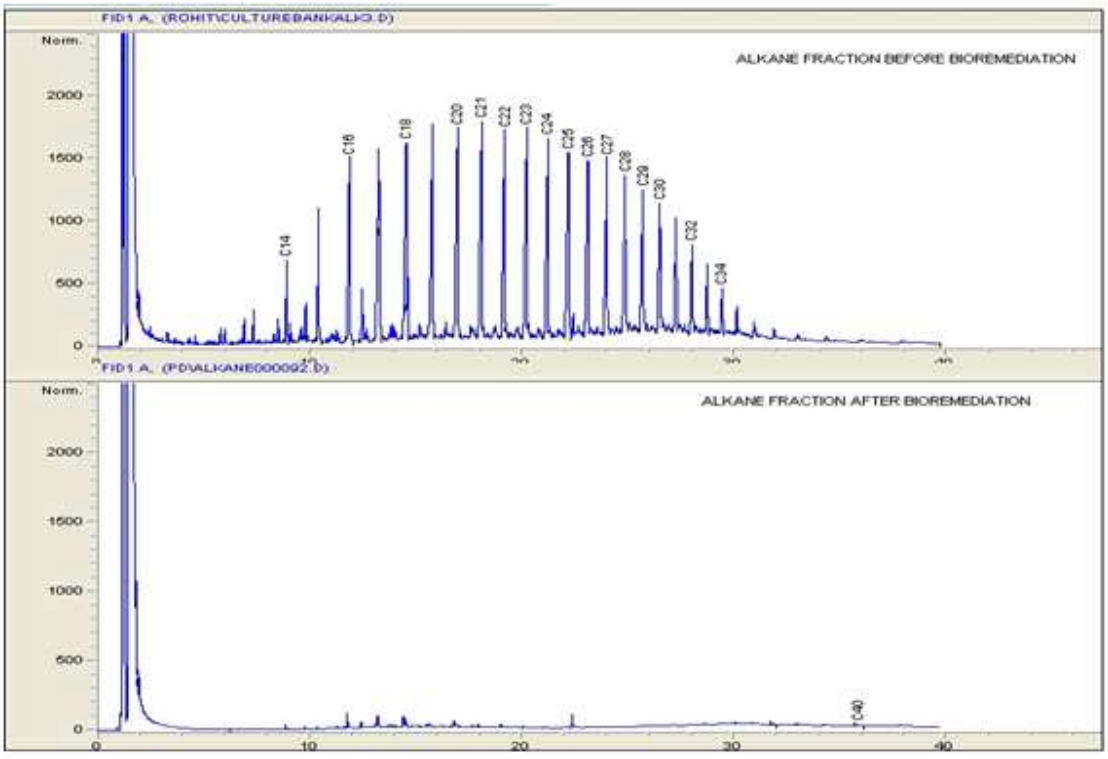

Figure 2 : ***Biodegradation of Alkane fraction of TPH of the oily waste at IOCL Panipat Refinery, India.***

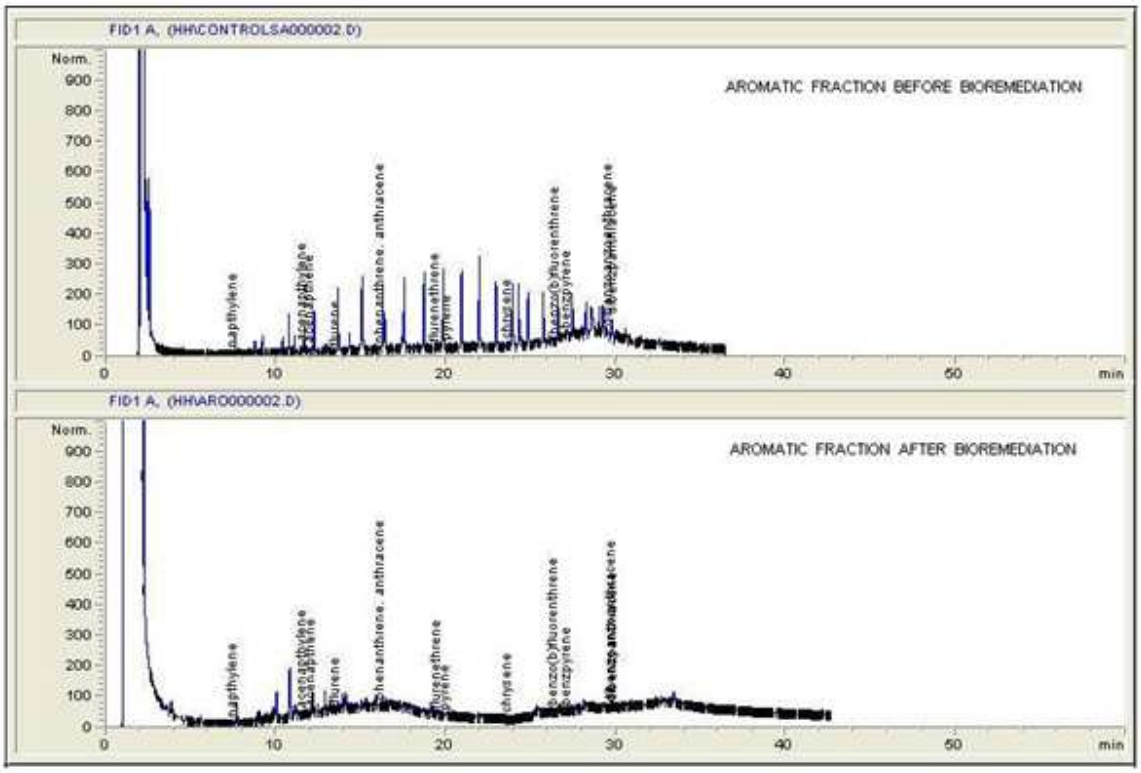

Figure 3 : ***Biodegradation of Aromatic fraction of TPH of the oily waste at IOCL Panipat Refinery, India.***

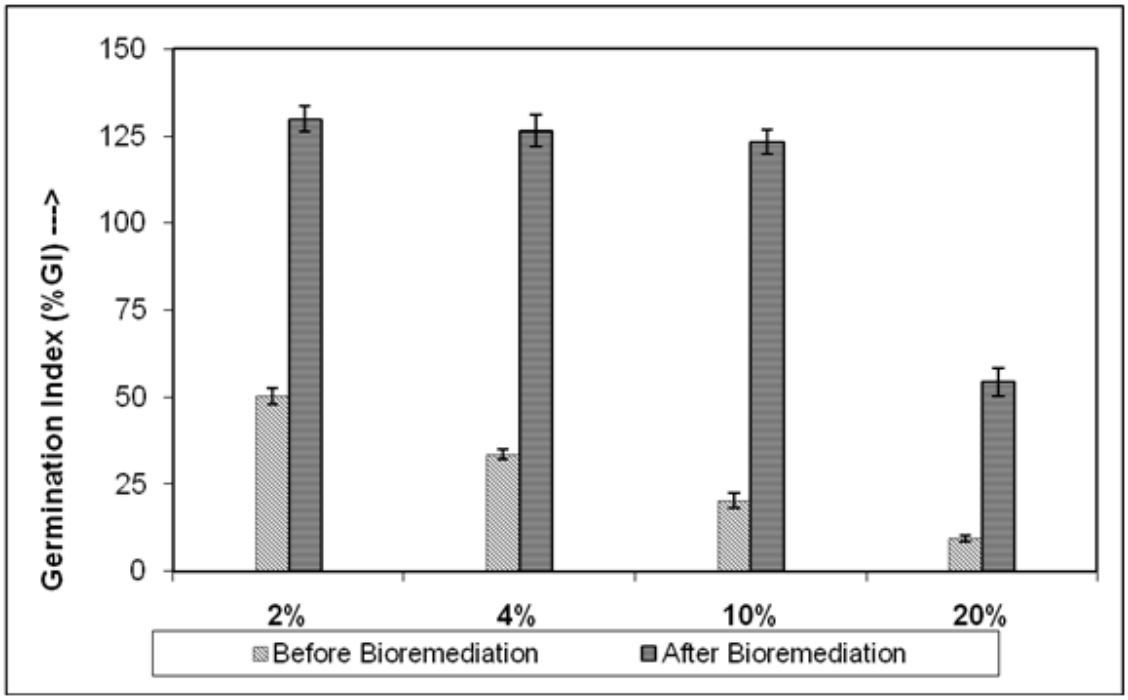

Figure 4 : ***Comparison of Germination Index (GI) in the seed germination study with soil before and after bioremediation at IOCL Panipat Refinery, India.***

CONCLUSION

Oil and gas industries contributes 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 48,914 tons of waste oily sludge in batches at various oil refineries in India. Bioremediated soil has been found containing TPH content to the extent of < 10 gm/kg of oily waste and was found to be not toxic to the environment. The time for bioremediation was within 2 – 12 months from the date of first application of microbes on the oily waste. In one refinery more than 20 months time was required for bioremediation which was due to continuous heavy rain during the bioremediation process which has hampered the execution activities. Bioremediated soil is found to be non toxic and has no adverse effect on seed germination. Bioremediation technology has helped the Indian oil refineries in disposal of their waste oily sludge in an environment friendly manner.

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