Aplinkos tyrimai, inžinerija ir vadyba, 2014. Nr. 2(68), P. 41-52
Environmental Research, Engineering and Management, 2014. No. 2(68), P. 41-52
http://erem.ktu.lt

ISSN 1392-1649 (print)
ISSN 2029-2139 (online)

Large Scale Bioremediation of Petroleum Hydrocarbon Contaminated Waste at Various Installations of ONGC. India: Case Studies

Ajoy Kumar Mandal[1], Priyangshu Manab Sarma[1], C Paul Jeyaseelan[1], Veeranna A Channashettar[1], Bina Singh[1], Anil Agnihotri[2], Banwari Lal[1] and Jayati Datta[3]

[1]*The Energy and Resources Institute (TERI), New Delhi, India*
[2]*Corporate HSE, Oil and Natural Gas Corporation Limited (ONGC), Lakshmi Nagar, New Delhi, India*
[3]*Bengal Engineering and Science University, Shibpur, India*

crossref http://dx.doi.org/10.5755/j01.erem.68.2.5632

(Received in April, 2014; accepted in June, 2014)

In situ and *ex situ* bioremediation of oil contaminated effluent pits, sludge pits, oil spilled land and tank bottom, and effluent treatment plant (ETP) oily sludge was carried out at Ankleshwar, Mehsana, Assam and Cauvery Asset of Oil and Natural Gas Corporation Limited (ONGC), India. The types of contaminant were heavy paraffinic, asphaltic and light crude oil and emulsified oily sludge /contaminated soil. An indigenous microbial consortium was developed by assembling 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 on a large scale field applied to the above oil installations and it successfully bioremediated 30,706 tonnes of different types of oily waste. In 65 case studies of different batch size of *in situ* and *ex situ* bioremediation processes, the initial TPH content varying from 69.20 to 662.70 g/kg of oily waste has been biodegraded to 5.30 – 16.90 g/kg of oily waste in a range of 2 to 33 months. Biodegradation rate varied in the range of 0.22 – 1.10 Kg TPH /day/m^2 area due to the climatic condition of the treatment zone and the type of waste treated. The bioremediated soil was non-toxic and natural vegetation was found to be grown on the same ground. Successful eco-restoration of one large effluent pit of 26,000 m^2 area was carried out by cultivation of local fish species after completion of bioremediation. Bioremediation technology has helped ONGC with the management of their hazardous oily wastes in an environment friendly manner.

Keywords: *bioremediation, biodegradation, oily waste, microbial consortium, total petroleum hydrocarbon*

1. Introduction

Oil and Natural Gas Corporation Limited (ONGC), the largest oil exploration company owned by the Government of India, has been carrying out exploration and production activity at various oil installations located all over India since 1960. ONGC has installed thousands of oil wells in farmers' fields, at various states of India, especially Gujarat and Assam, with a large pipeline network that transports crude oil from the oil well to group gathering stations (GGS). During daily exploration and production, ONGC developed a number of effluent pits and sludge pits containing oil-contaminated effluent water as well as oily sludge. Every year during the rainy season these effluent and sludge pits overflow and generate huge quantities of oil-contaminated land. During transportation of crude oil through the surface as well as underground pipeline, there are frequent accidents leading to the leakage due to which farmer's land gets contaminated. Also, these effluent and sludge pits are located near the village creating the potential danger for grazing cattle and other animals to fall into the pits and die. The waste generated from these effluent and oily sludge pits and oil contaminated soil in the farmer's land, termed as "oily waste", has been designated as hazardous waste by India, US EPA (United States Environmental Protection Agency) and OECD (Organization for Economic Co-operation and Development) countries (Zhu et al., 2001; Ministry of Environment and Forest, Government of India, 2000). Due to stringent regulatory norms, the disposal of these oily wastes in an environment friendly manner is a prime concern for ONGC authorities.

Total petroleum hydrocarbons (TPH) and their components like alkane; aromatic; nitrogen, sulfur, and oxygen containing compounds (NSO); and asphaltene fractions and water, and sediments are the constituents of hazardous oily waste (Bhattacharya et al., 2003). Oil contamination has severe impacts on the plant and animal ecosystem including human health. The majority of the chemical ingredients of crude oil are carcinogenic, mutagenic, teratogenic and health hazards. Oil contaminated soil loses its fertility and has an impact on seed germination. (Mandal et al., 2007, 2009b, 2011, 2012a). 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, thermal desorption, soil washing, etc. have been applied for remediation of oily waste since early times (Vidali, 2001 and Mandal et al., 2007, 2012a). It is observed that none of the conventional methods are the environment friendly solution as they are not the permanent solution for the environmental pollution, and sometimes they are not cost effective (Sood et al., 2009, Ouyang et al., 2005)

During the recent two decades it has been established that virtually all types of hydrocarbons are susceptible to microbial degradation, and hence the relevance of 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 options for treatment of oil contamination in terms of affordability, ecologically approachable and efficient in treating the contamination of hydrocarbon polluted soils (Bragg et al., 1994 and Prince et al., 1994, Chikere et al., 2009). Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to nontoxic compounds (Lal 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 a small quantity of oily sludge, which may provide the most convincing demonstrations of this technology, have been carried out in India and abroad. (Mearns et al., 1997, Raghavan et al., 1999; Mishra et al., 2001; B K Gogoi et al., 2003; Ouyang et al., 2005; Chikere et al., 2009, Liu et al., 2009).

In the present investigation, in situ and ex situ bioremediation of waste oily sludge and oil contaminated soil / land was carried out on a large scale in the fields at various ONGC installations located at different climatic zones in India. *In situ* bioremediation was carried out for oil contaminated land, effluent pits and oily sludge pits; whereas *ex situ* bioremediation was carried out in case of the waste oily sludge generated from tank cleaning and ETP operation and oil contaminated soil generated due to accidental oil spill. Some of the large effluent pits were restored for ecological development after bioremediation. For bioremediation studies at all ONGC installations an indigenously developed microbial consortium was applied (Mandal et al, 2007, 2007a, 2009, 2009a, 2011, 2012). Successful laboratory feasibility study was carried out for bioremediation of oily waste (from ONGC) using the said microbial consortium prior the field application.

2. Methods

2.1. Selection of Microbial Consortium

In course of the study, conducted by the authors' institute during the past few years, indigenous microbial strains had been isolated from fifteen different oil contaminated sites located at different geo-climatic regions in India. The efficacies of the strains were evaluated for biodegradation of TPH component of the oily waste and based on the functional diversity of the isolated strains, the best degraders for the major components of the TPH fractions were selected to form a consortium whose details have been reported in earlier studies of the institute (Bhattacharya et al., 2003; Sarma et al., 2004, 2004a, 2010, Lal & Khanna, 1996, 1996a; Mishra et al., 2001, 2001a, 2004, Prasad et al., 2005; Sood et al. 2009, 2009a, Krishnan et al. 2006, 2001, Lavania et al. 2012). This consortium has been previously reported for application of biodegradation studies carried out either in the laboratory or on the field scale (Mandal et al, 2007, 2007a, 2009, 2009a, 2011, 2012, 2012a, Sarma et al. 2006).

2.2. Selection and Preparation of Bioremediation Sites

The current study summarizes the *in situ* and *ex situ* field scale bioremediation of a total quantity of 30,706 tonnes of waste oily sludge and oil contaminated soil in 65 different batches at various ONGC installations located at different geo-climatic regions in India. The contaminant was lying in different sizes of effluent and sludge pits located randomly within or outside ONGC installation premises. The details of geo-climatic locations, types and quantity oily waste bio remediated under this study are listed in Table 1.

The batch size was decided based on the availability of the oily waste to be undertaken for bioremediation at one time in each installation. Hence the number of batches and the quantity of waste in each batch varied from installation to installation. For example, at ONGC Mehsana Asset, the largest and oldest ONGC asset in India, the waste oily sludge pits of different sizes were lying scattered at random places within the Asset area, hence maximum number (42) of bioremediation batches were carried out there. Whereas, Cauvery Asset, being very small Asset, there was only 3 batches of bioremediation job carried out (Table 1). In few batches the site was inside the installation premises, where *ex situ* bioremediation was carried out. For *ex situ* bioremediation studies, secured HDPE (high density poly ethylene) lined bioremediation sites were prepared near the sludge storage pit inside the ONGC 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. In few studies where the waste was liquid in nature, the required quantity of dry garden soil was mixed with the oily waste so as to make the transportation convenient. The bioremediation study under different climatic conditions was challenging one. In some installations, the size of the effluent pits (area of one pit 27,000 m^2 with depth of 3.5 m at Mehsana asset) has made the study more challenging. Wild shrubs / bushes on the boundary of the effluent pits had made the access very difficult. Most of the effluent and sludge pits were containing redundant pipes, metallic and rubber scraps and other non-biodegradables, which were removed by the ONGC authority before initiation of bioremediation.

The contaminants bio-remediated at the ONGC oil installations were crude petroleum hydrocarbon contaminated sludge and soil and multiple types of contaminated area covering a large waste effluent pit, a number of sludge pits, oil spilled land, the oil contaminated tank farm area and pipeline leakage points. The type of contaminated oil was varying in nature i.e. in few locations it was heavy paraffinic oil of API < 10, and in the other area it was light crude oil of API in the range of 28 – 35. At Cauvery asset the contaminant was emulsified oily sludge generated from its phase separation process. Data collected from ONGC indicated that the type of Mehsana crude oil varied in the range from paraffinic to highly viscous asphaltenic, density @ 15^0C was 0.8064 – 0.9768 g/cm^3, pour point 0–45^0C, API gravity <10 to >45, asphaltene content 0.2 % - 10 %, resin content 1.6 % - 25 % and wax content 1% - 27%. This indicated the variation in the chemical composition of oily waste undertaken for bioremediation at different oil installations of ONGC.

Table 1. Details of field case studies on the bioremediation jobs carried out at various oil installations of ONGC in India

Particulars of oil installation	**Geo-climatic conditions of the oil installation (location/ elevation*/ climate / temperature/ average annual rainfall)**	**Type of oily waste under-taken for bioremediation**	**Quantity (Tonne) of oily waste bioremediated / (Quantity in each batch)**	**No. of batches**
ONGC Ankleshwar Asset (Gujarat state)	$21^0$42’N & $72^0$58’E/ 15m / extreme & tropical savanna climate/ ~ 23 ^{0}C – 40 ^{0}C /~800-1200 mm (*Western India*)	Light crude oily sludge from exploration, tank cleaning and ETP	3,063 / (min. 193 t – max. 625 t)	7
ONGC Cauvery Asset (Tamilnadu state)	$10^0$56’N & $79^0$50’E/ 1m / coastal region with tropical maritime climate/ ~16 ^{0}C – 35 ^{0}C / ~1,260 mm (*Southern India*)	Light crude oily sludge from exploration, tank cleaning and ETP. Emulsified oily sludge	966 / (min. 120 t – max. 450 t)	3
ONGC Mehsana Asset (Gujarat state)	23° 35' N & 72° 23' E/ 81m / semi-arid & extreme dry or semi dry / ~ 15^0C – 50 ^{0}C max. / ~ 625 to 875 mm (*Western India*)	Heavy viscous asphaltic oil contaminated land, soil & water; effluent & sludge pits	16,938 / (min. 11 t – max. 2196 t)	42
ONGC Assam Asset (Assam state)	$26^0$55’N & $94^0$44’E/ 132m / humid subtropical monsoon / ~ 10 ^{0}C – 40 ^{0}C max./ ~ 2485 mm (*North Eastern India*)	Light crude oily sludge from exploration, tank cleaning and ETP & oil contaminated site	9,739 / (min. 83 t – max. 1261 t)	13
Total	**-**	**-**	**30,706 / (11-2,196 t)**	**65**

*Comments: *average elevation above mean sea level, min. – minimum, max. – maximum, t – tonne*

2.3. Application of microbial consortium to oily waste

The microbial consortium was manufactured in the 1500 litre bioreactor (Bioengineering AG, Switzerland) at TERI, New Delhi, India and transported to the respective sites for its application to oily waste by manual spreading at regular intervals of 30 days. Specially designed nutrient formulation was dissolved in water and spread uniformly to the bioremediation site with the help of water sprinkler for enhancing the population of the microbial consortium. Mixing of oily waste and microbes was done by mechanical tilling of bioremediation sites. In the control site, microbial consortium was not added, however the rest of the other activities like tilling, watering etc. were carried out in the same manner as at the experimental bioremediation site.

2.4. Tilling and watering

Tilling of the bioremediation sites was done at a regular interval once in a week to maintain aeration for the microbial consortium at the bioremediation sites. This was done with the help of a tractor with an attached 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.

2.5. Sampling

Oily waste samples were collected from the bioremediation sites at zero day (before bioremediation i.e. before application of microbes but after mixing of soil, if any, to the oily waste) and at regular intervals after application of the microbial consortium till the completion of the study. The bore well water samples were collected, in sterile plastic bottles from each bore wells installed at the nearby area of the bioremediation site, for monitoring the environmental impact (Mandal et al., 2012a).

2.6. Monitoring of bioremediation process

For monitoring the performance of the bioremediation process, the samples of oily waste and bore well water were analysed for the selected parameters as described below.

Characterszation of oily waste. TPH was extracted from a known quantity of oily waste by a solvent extraction method with the Soxhlet extractor using various solvents like hexane, methylene chloride and chloroform consecutively and calculated gravimetrically. The extracted TPH was further fractionated for various fractions like alkane, aromatic, NSO and asphaltene fractions. Alkane and aromatic fractions were concentrated by evaporation of solvents and then analysed 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 the authentic standards (Mishra et al., 2001).

Determination of microbial count. Total Bacterial Count (TBC) to monitor the bacterial population was determined by a standard spread plate method using the standard Luria Bertini agar plate (Himedia catalog no. M 557) as described earlier by Mishra et al., 2004.

Determination of pH, moisture content and selected heavy metals. The pH of the oily waste sample was measured using the standard pH meter (Orion Expandable Ion Analyzer model no. EA – 940) by taking 20% (w/w) solution, in distilled water, of the sample for measurement. 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 the Atomic Absorption Spectrophotometer AAS- TJA (SOLAAR M Series, Unicam, USA) and also by the Stripping Voltammetric method using the Voltammetry-Amperometry (VA) trace analyzer (757 VA Computrace) by Metrohm 663 VA Stand (Swiss made) combined with AUTOLAB 30 Potentiostat–Galvanostat. Oil and grease in the ground water samples were determined as per standard method IS 3025 (P 39): 1991.Selected heavy metals in residual oily waste and ground water quality monitoring was done to check the environmental impact of the bioremediation process and also impact of toxic heavy metals on the survival of microbes, if any.

Biodegradation of TPH in the oily waste. The decrease in the TPH content and its fractions with time and the percent biodegradation were calculated from the samples collected periodically from the sites and analysed gravimetrically as described earlier. Simultaneously, biodegradation of alkane and aromatic fractions was also assessed by quantitative measurement of the peaks from the GC chromatogram with the help of the 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 the IS standard methods) as well as soil toxicity like fish toxicity (by method no IS: 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 as per the method described by Mandal et al., 2012a.

3. Results and discussions

3.1. Development of microbial consortium

As described above in Section 2.1, an indigenously developed microbial consortium was selected for large scale field bioremediation studies at various ONGC installations in India. The consortium contained four different most efficient strains to biodegrade different fractions of TPH of the oily waste of ONGC installations. Successful laboratory feasibility study was carried out for bioremediation of oily waste (from ONGC) using the said microbial consortium before field application. (Mishra et al. 2001, 2001a, 2004; Lal & Khanna, 1996 and Mandal et al., 2007, 2007a, 2009, 2009a, 2011).The quantity of microbes for field application was decided so as to maintain the microbial count in the range of 10^7 to 10^9colony forming a unit (CFU) per g of oily waste in the bioremediation site. Average quantity of nutrient formulation applied to the bioremediation study was in the range of 0.06 – 0.10 kg per tonne of oily waste.

3.2. Composition of oily waste

While characterising the composition of oily waste under this study, it was observed that in all the samples the steam extractable TPH was nil. The initial zero day composition indicated that one kg of oily waste contained in the range of 69.20-662.70 g solvent extractable TPH, 182.50-552.20 g moisture and 121.40-748.30 g residue containing sediments and other organic and inorganic compounds. After fractionation it was observed that one kg of solvent extracted TPH contained in the range of 440-680 g alkane, 210-370 g aromatic and 100-220 g NSO and asphaltene fractions. Table 2 describes detailed composition ranges of oily waste bioremediated at different ONGC installations under this study. After statistical analysis of all the 65 batches of bioremediation studies, it was thus observed that in majority of the cases the initial TPH was in the range of > 200 to ≤ 500 g/kg waste (Table 3).

Table 2. Characteristics of oily waste undertaken for bioremediation at various oil installations of ONGC in India

Particulars of ONGC installation /Assets	Composition range in the oily waste					
	TPH (solvent extractable), g/kg waste	Moisture, g/kg waste	Residue, g/kg waste	Alkane, g/kg TPH	Aromatic, g/kg TPH	NSO + Asphaltene, g/kg TPH
Ankleshwar	424.80 - 662.70	215.90 - 274.20	121.40 - 306.30	610 - 680	200 - 260	110 - 140
Cauvery	161.00 - 515.00	227.90 - 503.00	196.00 - 336.00	600 - 620	210 - 230	160 - 180
Mehsana	69.20 - 475.40	182.50 - 350.00	184.60 - 748.30	450 - 660	230 - 370	100 - 220
Assam	166.50 - 641.90	191.20 - 552.20	166.90 - 381.50	440 - 583	250 - 360	101 - 200
Range	**69.20 – 662.70**	**182.50 – 552.20**	**121.40 – 748.30**	**440 - 680**	**210 - 370**	**100 - 220**

Table 3. Statistical analysis of TPH before and after bioremediation at oil installations of ONGC in India

TPH before Bioremediation (i.e. Initial TPH value)			TPH after Bioremediation (i.e. Final TPH value)		
TPH range, g/kg waste	No. of batch	Percentage of total batches, %	TPH range, g/kg soil	No. of batch	Percentage of total batches, %
0 to ≤ 100	3	5	0 to ≤ 10	49	75
> 100 to ≤ 200	7	11	> 10 to ≤ 20	16	25
> 200 to ≤ 300	12	18	> 20 to ≤ 30	0	0
> 300 to ≤ 400	17	26	> 30 to ≤ 40	0	0
> 400 to ≤ 500	18	28	> 40 to ≤ 50	0	0
> 500 to ≤ 600	6	9	-	-	-
> 600 to ≤ 700	2	3	-	-	-
Total	**65**	**100**	**Total**	**65**	**100**

3.3. Biodegradation

The initial TPH content in the oily waste, undertaken for bioremediation at different ONGC installations, was in the range of 69.20 - 662.70 g/kg waste. After bioremediation, the TPH content came down to the range of 5.30 – 16.90 g/kg. The time required for bioremediation in each batch was also varied, as it ranged from 2 to 33 months depending upon the type of oil contamination. Highly viscous asphaltenic crude at Mehsana took up to 33 months and the emulsified oily sludge at Cauvery asset was over in 21.5 months. The factor of the initial oil content (e.g. CTF Ankleshwar & Assam) and the climatic condition of the site (e.g. ONGC Assam -) also played a role in the bioremediation rate. The rate of biodegradation of TPH varied in the range of 0.22–1.10 Kg TPH/day/m^2 area of the bioremediation site with biodegradation percent of 90.98 % - 98.91 % in the above period (Table 4). Whereas the degradation of oily waste in the control sites, where no microbes and nutrients were added, were hardly up to18% in the same time period. After statistical analysis of all the studies carried out at various oil installation of ONGC, it was observed that TPH content after bioremediation was in the range of 0 to ≤ 10 g/kg in 75% samples i.e. in major cases and in the range of > 10 to ≤ 20 g/kg in 25% samples (Table 3).

Figure 1 describes the trend of biodegradation in one of the case studies carried out in three different batches in three different sites nearby CTF Geleky, ONGC, Assam Asset, India, where the initial TPH of 575.80 – 641.90 g/kg waste had been biodegraded to less than 8.80 – 9.30g/kg of within 15 - 19 months indicating average biodegradation of 98.5%. Whereas in the control site of the TPH content in oily waste was found to be decreased from 581.30 to 476.67g/kg oily waste in 19 months period indicating biodegradation of 18% only. The above results indicate that the bioremediation process by using the microbial consortium is an efficient process for treatment of oil contamination. Figure 2 presents the photographs of the large effluent pit (of 27,000 m^2 area) at Santhal – 1, ONGC, Mehsana Asset, before and after the bioremediation study. While analysing the GC chromatogram of the TPH fractions, it was observed that most of the alkane and aromatic fractions of the oily waste were efficiently biograded within the remediation period.

3.4. pH and microbial count of residual oily waste samples at the bioremediation site

Throughout the bioremediation process, pH of the oily waste samples was within 6.5 to 8.5 in all the batches. The microbial counts were maintained in the range of 10^7 to 10^9 CFU/g soil 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/g soil. Table 5 describes the pH and microbial count in one case study at CPF Gandhar, ONGC Ankleshwar asset.

3.5. Ground water quality

There was no oil and grease content found in the ground water samples before and after bioremediation (Table 6). Also the pH of the ground water before and after bioremediation was in the range of 6.5-8.5. The selected heavy metals in the ground water before and after bioremediation was within the permissible limits of WHO (World Health organisation)/ BIS (Bureau of Indian Standard). This indicates that there was no leaching of oily waste to the ground water during the bioremediation process confirming the environment friendliness of the technology.

Table 4. Biodegradation of TPH of oily waste at various oil installations of ONGC in India

Particulars of oil installation	**TPH (g/kg oily waste) at site**		**% Biodegradation (w / w)**	**Time for bio-remediation (months)**	**Biodegradation rate (Kg TPH /day/m^2 area)**
	Initial (Before bioremediation)	**Final (After bioremediation)**			
Ankleshwar Asset	424.80 - 662.70	6.70 - 12.80	97.75 - 98.60	5 - 15	0.50 ± 0.21
Cauvery Asset	161.00 - 515.00	5.30 - 6.80	96.71 - 98.91	14 - 21.5	0.34 ± 0.31
Mehsana Asset	69.20 - 475.40	5.80 - 15.00	90.98 - 97.78	4.5 - 33	0.22 ± 0.15
Assam Asset	109.60 - 641.90	2.10 - 16.90	91.09 - 98.49	2 - 19	1.10 ± 0.84
Total / Range	**69.20 - 662.70**	**5.30 - 16.90**	**90.98 - 98.91**	**2 - 33**	**0.22 - 1.10**

3.6. Heavy metal analysis

There was no considerable change in the concentration of selected heavy metals in oily waste before and after bioremediation (Table 7). This indicates that the selected heavy metals are not biodegraded by the applied microbial consortium. Similarly, the selected heavy metals do not have any impact on the performance of the microbes on biodegradation of oily waste. It was also observed that the concentrations of all the selected heavy metals were within the regulatory limit as per Hazardous Waste (Management and Handling) Rules, amendment 2008, of the Government of India,(HWMS 2008, GoI) indicating no environmental impact of the bioremediation process.

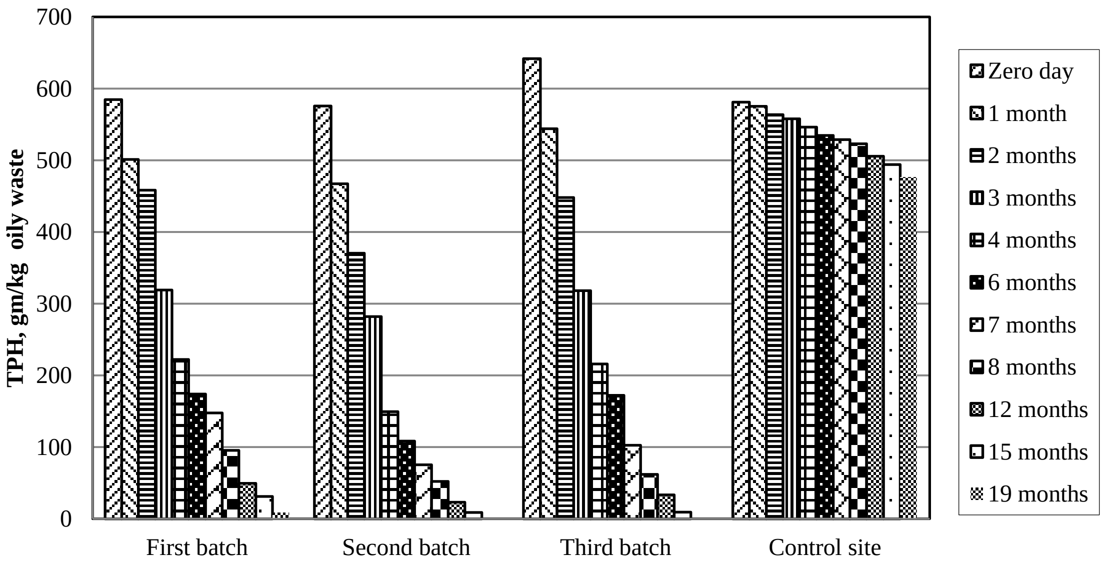

Fig. 1. Biodegradation of TPH of the oily waste at CTF Geleky, ONGC, Assam Asset (1st batch – 1260 t oily sludge in sludge pit, 2nd batch – 128 t oil spilled soil on land inside CTF premises and 3rd batch – 120 t oil spilled soil on farmland outside CTF boundary, Control site - 25 t oil spilled soil on land inside CTF premises)

Fig. 2. Effluent pit at South Santhal, ONGC, Mehsana, Asset: before (left) and after (right) bio-remediation

Table 5. pH and microbial count at the bioremediation site at CPF Gandhar, Ankleshwar

Time of treatment	Study Batch # 1		Study Batch # 2		Study Batch # 3		Control site	
	pH	TBC *	pH	TBC *	pH	TBC *	pH	TBC *
Zero day	7.4	4.6×10^9	7.4	3.1×10^9	7.4	2.9×10^9	7.4	2.1×10^5
1 month	7.5	3.8×10^9	7.5	3.2×10^9	7.6	4.9×10^9	7.5	1.3×10^5
2 months	7.3	1.2×10^9	7.4	4.8×10^9	7.5	3.7×10^9	7.7	1.2×10^5
3 months	7.5	4.7×10^8	7.6	8.1×10^8	7.6	7.6×10^8	7.8	6.1×10^4
4 months	7.5	2.6×10^8	7.6	5.4×10^8	7.7	4.4×10^8	7.8	4.2×10^4
5 months	7.7	4.3×10^7	7.9	6.8×10^7	7.9	7.8×10^7	7.9	2.5×10^4
6 months	-	-	-	-	8.1	2.3×10^7	8.1	1.8×10^4

*Comments: * TBC – Total bacterial count in the oily waste in bioremediation site (CFU/g waste)*

3.7. Soil toxicity

The soil after bioremediation was found to be nontoxic by fish toxicity test and after bioremediation the seed germination index of the soil improved significantly. Figure 3 describes the seed germination study carried out for the oil contaminated soil before and after bioremediation at CTF, ONGC, Ankleshwar, where the germination index has been considerably increased in the soil after bioremediation. Before bioremediation, the bioremediation site was ecologically non-functional due to the toxic moieties of the TPH. However, after bioremediation and decrease in the TPH content, the natural vegetation was restored at the site (Figure 2). Eco-restoration of one large effluent pit was successfully carried out at Mehsana asset by cultivating local fish species in the pit after bioremediation (Mandal et al., 2011). The grown fish samples were analysed for a histopathology test and no bioaccumulation of petroleum hydrocarbon component was observed in the fish tissues. The above indicates that using microbes bioremediation can help in eco-restoration of the hydrocarbon contaminated sites.

Table 6. Ground water characteristics near the bioremediation sites of ONGC Installations, India

Particulars	Permissible limits		Ground water quality near bioremediation site before and after bioremediation							
	BSI / WHO*	EPA (LIE)**	ONGC, Ankleshwar		ONGC, Cauvery		ONGC, Mehsana		ONGC, Assam	
			Before	After	Before	After	Before	After	Before	After
Selected Heavy Metals			(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Zinc (Zn)	5 ppm	1 ppm	0.081	0.076	2.312	2.131	7.368	7.286	2.044	1.790
Manganese (Mn)	0.1 ppm	1.5 ppm	0.033	0.030	0.076	0.069	0.336	0.302	0.651	0.579
Copper (Cu)	1 ppm	1 ppm	0.030	0.026	0.008	0.006	< 0.001	< 0.001	0.047	< 0.001
Nickel (Ni)	5 ppb	1 ppm	< 0.001	< 0.001	< 0.001	< 0.001	0.031	0.026	0.197	0.186
Lead (Pb)	5 ppb	0.5 ppm	0.002	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Cobalt (Co)	5 ppb	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.044	< 0.001
Arsenic (As)	5 ppb	0.5 ppm	0.002	0.001	< 0.001	< 0.001	0.025	0.021	0.071	0.068
Cadmium (Cd)	1 ppb	0.01 ppm	0.008	0.006	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Chromium (Cr) (Total)	5 ppb	1 ppm	0.007	0.005	< 0.001	< 0.001	< 0.001	< 0.001	0.05	< 0.001
Selenium (Se)	0.5 ppb	0.5 ppm	< 0.001	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	< 0.001	< 0.001
Physico-chemical properties:										
pH	-	6 - 10	7.48	7.35	7.26	7.15	7.62	7.35	7.39	7.61
EC (mS/cm)	-	-	1.89	1.65	45.3	43.7	2.12	1.89	2.29	2.16
Oil / Grease	-	10 ppm	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

*Comments: *BSI – Bureau of Indian Standards, WHO – World Health Organization, **EPA – Environment Protection Agency, LIE – Liquid Industrial Effluent*

Table 7. Selected heavy metals in residual oily waste at the bioremediation sites of ONGC Installations, India

Heavy metals	Permissible Limit, mg/kg waste*	Concentration of heavy metal (mg/kg waste) in oily waste before and after bioremediation study (typical) at							
		ONGC, Ankleshwar		ONGC, Cauvery		ONGC, Mehsana		ONGC, Assam	
		Before	After	Before	After	Before	After	Before	After
Zinc (Zn)	20000	6.82	5.96	4.23	3.67	2.60	2.40	1.20	1.16
Manganese (Mn)	5000	< 0.001	< 0.001	0.76	0.71	2.70	2.40	27.40	26.89
Copper (Cu)	5000	1.13	0.96	3.22	2.87	2.15	1.40	1.20	1.18
Nickel (Ni)	5000	0.85	0.74	0.49	0.08	1.10	0.75	2.83	2.78
Lead (Pb)	5000	1.46	1.17	0.06	0.05	0.04	0.06	5.63	5.39
Cobalt (Co)	5000	1.23	1.08	< 0.001	< 0.001	0.20	0.18	0.11	0.09
Arsenic (As)	50	1.07	0.89	4.09	4.38	0.10	0.20	1.82	1.77
Cadmium (Cd)	50	< 0.001	< 0.001	< 0.001	< 0.001	0.03	0.02	0.05	0.04
Total Chromium (Cr)	5000	1.43	1.21	1.51	< 0.001	1.30	1.20	6.85	5.99
Selenium (Se)	50	1.26	0.97	< 0.001	< 0.001	< 0.001	< 0.001	0.06	0.05

*Comments: *As per Schedule – II of Hazardous Waste Management, Handling and Transboundary Movement Act (Amendment 2008), by Government of India*

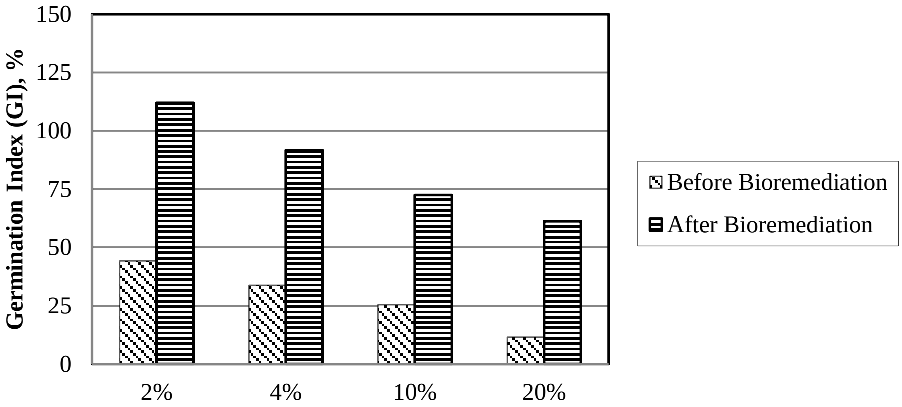

Fig. 3. Comparison of germination index (GI) in the seed germination study with soil before and after bioremediation at CTF, ONGC Ankleshwar Asset, India

4. Conclusions

- In situ and ex situ bioremediation of oil contaminated effluent pits, sludge pits, oil spilled land and tank bottom and ETP oily sludge at Ankleshwar, Mehsana, Assam and Cauvery Asset of ONGC, India and remediation of large effluent pits of even 27,000 m^2area were carried out. The type of contamination was heavy paraffinic and asphaltic crude oil (API <10), light crude oil (API: 28-35) and emulsified oily sludge /contaminated soil.
- A total of 30,706 tonnes of oily waste was treated from a pre-treatment TPH concentration of 69.20-662.70 g/kg waste to a post-treatment TPH concentration of 5.30-16.90 g/kg waste in 2-33 months. Biodegradation rate was in the range of 0.22-1.10 Kg TPH /day/m2 area depending upon the type of contamination, initial TPH concentration and climatic conditions of the site.
- After bioremediation soil was nontoxic and had no adverse effect on seed germination. Natural vegetation was found to grow on the site after bioremediation. Successful eco-restoration of a large effluent pit was carried out by cultivation of local fish species after completion of bioremediation. There was no accumulation of petroleum hydrocarbon on the grown fish tissues.
- Selected heavy metals concentration in residual oily waste was within the permissible limit of HWMS 2008, and was not having any impact on the performance of the bioremediation process. There was no leaching of oil contamination to the underground water during the bioremediation process. Hence the bioremediation process is considered to be environment friendly.
- Bioremediation technology has helped ONGC in disposal of their oily waste and restoration of oil contaminated farmer's land in an environment friendly manner.

Acknowledgements

The authors are thankful to Dr. R. K. Pachauri, Director-General of TERI, for providing the infrastructure to carry out the present study. The authors also thank the management of ONGC in India for providing the opportunities for field study at their installation. Authors acknowledge the co-operation and support of the Department of Chemistry, Bengal Engineering and Science University, for providing the infrastructure facility in carrying out part of the physico-chemical analysis related to the present study.

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PhD Ajoy Kumar Mandal – Fellow & Area Convenor, Bioremediation Technology Area.
Main research area: Environmental and Industrial Biotechnology – Bioenergy, Bioremediation, Fermentation.
Address: The Energy and Resources Institute (TERI), Habitat Place, Lodhi Road, New Delhi - 110003, India.
Tel.: +91-11-2468 2100,
E-mail: *akmandal@teri.res.in*, *ajoy_mandal@hotmail.com*

PhD Priyangshu Manab Sarma – Fellow & Area Convenor, MicrobialBiotechnology Area.
Main research area: Environmental and Industrial Biotechnology – Bioenergy, Bioremediation, Fermentation.
Address: The Energy and Resources Institute (TERI), Habitat Place, Lodhi Road, New Delhi - 110003, India.
Tel.: +91-11-2468 2100,
E-mail: *priyanms@teri.res.in*

MSc C Paul Jeyaseelan – Fellow, Bioremediation Technology Area.
Main research area: Environmental and Industrial Biotechnology –Fermentation technology.
Address: The Energy and Resources Institute (TERI), Habitat Place, Lodhi Road, New Delhi - 110003, India.
Tel.: +91-11-2468 2100,
E-mail: *jeyaseelan@teri.res.in*

MSc Veeranna A Channashettar – Fellow, Bioremediation Technology Area.
Main research area: Environmental and Industrial Biotechnology –Fermentation technology.
Address: The Energy and Resources Institute (TERI), Habitat Place, Lodhi Road, New Delhi - 110003, India.
Tel.: +91-11-2468 2100,
E-mail: *veeranna@teri.res.in*

PhD Bina Singh – Associate Fellow, Microbial Biotechnology Area.
Main research area: Environmental and Industrial Biotechnology –Analytical Chemistry.
Address: The Energy and Resources Institute (TERI), Habitat Place, Lodhi Road, New Delhi - 110003, India.
Tel.: +91-11-2468 2100,
E-mail: *bina@teri.res.in*

PhD Banwari Lal – Director of the Environmental and Industrial Biotechnology Division.
Main research area: Environmental and Industrial Biotechnology – Bioenergy, Bioremediation, Fermentation.
Address: The Energy and Resources Institute (TERI), Habitat Place, Lodhi Road, New Delhi - 110003, India.
Tel.: +91-11-2468 2100,
E-mail: *banwari@teri.res.in*

PhD Anil Agnihotri – Dy. General Manager, Corporate HSE.
Main research area: Environmental Science-Bioremediation & Bioenergy.
Address: Corporate HSE, Oil and Natural Gas Corporation Limited (ONGC), Lakshmi Nagar, New Delhi, India.
E-mail: *agnihotri@ongc.co.in*

PhD Jayati Datta – Professor at the Department of Chemistry.
Main research area: Electro Analytical Chemistry – Fuel Cell.
Address: Bengal Engineering and Science University, Shibpur, PO: Botanic Garden, Dist: Howrah, West Bengal – 711103, India.
E-mail: *Jayati_datta@rediffmail.com*

Stambaus masto naftos angliavandeniliais užterštų atliekų biologinis valymas įvairiose NGDK gamyklose. Indijos atvejų tyrimai

Ajoy K. Mandal[1], Priyangshu M. Sarma[1], C Paul Jeyaseelan[1], Veeranna A. Channashettar[1], Bina Singh[1], Anil Agnihotri[2], Banwari Lal[1], Jayati Datta[3]

[1]*Energijos ir išteklių institutas (TERI), Naujasis Delis, Indija*

[2]*HSE Korporacija, Naftos ir gamtinių dujų akcinė bendrovė (NGDAB), Naujasis Delis, Indija*

[3]*Bengalijos inžinerijos ir mokslų universitetas, Vakarų Bengalija, Indija*

(gauta 2014 m. kovo mėn.; atiduota spaudai 2014 m. birželio mėn.)

Buvo atlikta *in situ* ir *ex situ* biologinio naftos nuotekų duobių, dumblo duobių, nafta užterštos žemės bei rezervuaro dugno valymo ir naftos produktais užterštų nuotekų valymo įrenginių (NVĮ) analizė *Ankleshwar*, *Mehsana*, *Asamo* ir *Cauvery Asset* Naftos ir gamtinių dujų korporacijos įmonėse (NGDK), Indijoje. Teršalus sudarė sunkioji parafininė, asfaltinė ir žaliavinė naftos rūšys ir dumblas su emulsine nafta (užterštu dirvožemiu). Vietinių mikrobų bendrija buvo išplėtota sujungus keturias bakterijų rūšis, išskirtas iš skirtingų naftos produktais užterštų vietų Indijoje. Atrinktos bakterijos gali biologiškai skaidyti įvairias naftos angliavandenilių (BNA) frakcijas aliejingose atliekose į aplinkai palankius galutinius produktus. Mikrobų bendrija buvo paskleista dideliame plote, minėtose naftos produktais užterštose srityse, kuriose sėkmingai biologiškai suskaidė 30 706 tonų įvairių rūšių angliavandeniliais turtingų atliekų. Analizuojant 65 atvejų, atliekant įvairaus masto *in situ* ir *ex situ* bioremediacijos procesus, pradinė BNA sudėtis, įvairavusi nuo 69,20 iki 662,70 g/kg aliejingų atliekų, buvo biologiškai suskaidyta iki 5,30–16,90 g/kg atliekų masės intervale nuo 2 iki 33 mėnesių. Biodegradacijos rodiklis tiriamosiose srityse svyravo nuo 0,22 iki 1,10 kg BNA/d./m^2 dėl klimato kaitos valomojoje zonoje ir apdorojamų atliekų tipo. Nustatyta, kad biologiškai išvalytas dirvožemis buvo netoksiškas, o toje pačioje žemėje galima ir natūrali vegetacija. Sėkmingai atkūrus ekologinį būvį po bioremediacijos procesų didelėje nuotekų surinkimo duobėje (26 000 m^2) sėkmingai buvo įveistos vietinių žuvų rūšys. Biologinio valymo technologija padėjo NGDK įmonėms, generuojančioms pavojingas naftos atliekas, tvarkyti jas tausojant aplinką