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BIOREMEDIATION : AN ENVIRONMENT FRIENDLY SUSTAINABLE BIOTECHNOLOGICAL SOLUTION FOR REMEDICATION OF PETROLEUM HYDROCARBON CONTAMINATED WASTE

Ajoy Kumar Mandal ^{1*}, Priyangshu Manab Sarma ¹, Bina Singh ¹, C Paul Jeyaseelan ¹, Veeranna A Channashettar ¹, Banwari Lal ¹ and Jayati Datta ²

¹The Energy and Resources Institute (TERI), Lodhi Road, New Delhi, India. e-mail:

²Bengal Engineering and Science University, Sibpur, West Bengal, India.

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 hydrocarbon 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 and successfully bioremediated >200,000 tonnes of different types of oily waste in India. In >200 field case studies of different batch size on in situ and ex situ bioremediation process, the initial TPH content varying from 5% to 52% has been biodegraded to <1% in major cases in 2 – 12 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.

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

INTRODUCTION

Petroleum industries unavoidably generate enormous quantity of waste oily sludge and oil contaminated soil which constitutes a major challenge for hazardous waste management as well as environment management. Apart from this, oil transportation is one of the major causes of environmental pollution where the land and water 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 (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). Due to war enormous quantity of land and sea water gets contaminated by oil spill (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 TPH constitutes a complex mixture of alkane; aromatic;

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nitrogen, sulfur, and oxygen containing compounds (NSO); and asphaltene fractions (Bhattacharya et al., 2003). Oil contamination has severe impacts in the plant as well as animal ecosystem including human health (Mandal et al., 2007; EPA, undated). Some of the environmental impacts due to oil contamination include: physical and chemical alteration of natural habitats, physical smothering effect on the marine life, lethal or sub-lethal toxic effects on the marine life, changes in the marine ecosystem, etc. (Szaro et al., 1978; Agnes et al., 2003; Moacir et al., 2008; Samiullah, 1985 and Peterson, 2001). Polycyclic aromatic hydrocarbons (PAH) may lead to skin erythema (reddening), skin cancer, sinonasal cancer, gastrointestinal cancer, and bladder cancer, Inhalation leads to headache, nausea, dizziness, respiratory irritation, BTEX (Benzene, Toluene, Ethyl benzene & Xylene) present in the oil contamination, cause mutations, cancers, birth defects, endocrine disruptions, still births, nervous disorders, and liver disease, carcinogen, effect on central nervous system, depression, irregular heartbeats (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 loses its fertility and effects on seed germination. (Yoshida et al., 2006 and Gong et al., 2001). Hence disposal of the same in an improper manner may cause a serious environmental problem (Yustle et al., 2000).

Various conventional methods like land filling, incineration, air sparging, 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 an 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 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 with small quantity of oily sludge, which may provide the most convincing demonstrations of this technology, have been carried out. (Raghavan et al., 1999; Mishra et al., 2001; Ouyang et al., 2005 and Liu et al., 2009).

The present paper describes our experience on field case studies on bioremediation of oily waste at various oil installations in India and abroad using indigenously developed microbial consortium.

METHODS AND PROCEDURES

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 10⁸ 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 TM (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.

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 10⁸ 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.

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. 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.

Selection and Preparation of Bioremediation Sites:

More than 200 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

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Table- 1 (Mandal et al., 2007, 2010, 2009, 2007a, 2009a; Lal et al., 2007 and Sarma et al., 2007, 2006). The bioremediation jobs were 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.

Application of Microbial Consortium on Petroleum Hydrocarbon Contaminated 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 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 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 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.

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. Samples were collected from random points in the bioremediation site using a statistical sampling method. 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. The samples were collected in sterile plastic containers. The ground water samples were collected in sterile plastic bottles from each bore wells installed near the bioremediation site where required. The bore wells were flushed thoroughly before collecting the samples.

Table – 1: Location of bioremediation sites in the field case studies by TERI, India.

Name of the oil Installation / type of oily waste	Quantity of oily waste (cubic meter)	Number of batches	TPH Content (%) in oily waste	
			Before bioremediation	After Bioremediation
Abu Dhabi National Oil Company (ADNOC), Abu Dhabi / Oil contaminated drill cuttings	200	1	17.26	0.98
BG Exploration and Production India Limited (BGEPI), India / Oil based mud (OBM)	2,428	3	5.75 – 6.23	0.26 - 0.57
Bharat Petroleum Corporation Limited (BPCL), India / Oily sludge	5,000	1	19.3 – 26.5	0.9 - 1.2

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Cairn Energy Pty. India Limited, India / Oil contaminated drill cuttings	567	2	14.93 – 18.81	0.82 – 1.09
Chennai Petroleum Corporation Limited (CPCL), India / Oily sludge	4,444	2	26.12	0.89
Hindustan Petroleum Corporation Limited (HPCL), India / Oily sludge	5,010	3	16.7 – 52.81	0.9 – 1.60
Indian Oil Corporation Limited (IOCL) Refineries in India / Oily sludge (acidic + non acidic)	75,412	48	9.6 – 38.4	0.37 – 0.95
Kuwait Oil Company (KOC), Kuwait / Oil contaminated soil	778	1	4.6 – 12.75	0.09 – 0.10
Mangalore Refinery and Petrochemicals Limited (MRPL), India. / Oily sludge	2,222	2	8.35 – 19.86	0.84 – 0.97
Oil and Natural Gas Corporation Limited (ONGC) installations in India / Oily sludge & oil contaminated soil	95,499	145	12.0 – 51.5	0.5 – 1.2
Oil India Limited (OIL), Assam / Oily sludge & oil contaminated soil	15,921	14	21.6 – 37.7	0.49 – 0.53
Reliance Energy Limited (RIL), India / Oily sludge	611	2	19.15	0.5
Total	208,092	224		

Monitoring of Bioremediation Process

Samples of oily waste ground water 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:

Characterization of oily sludge

Total petroleum hydrocarbon (TPH) was extracted from a known quantity of oily sludge by solvent extraction method (USEPA 9071B) 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).

Determination of microbial count

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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

pH of 20% (w/w) solution of the sludge 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 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 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).

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, polycyclic aromatic hydrocarbon(PAH), Polychlorinated biphenyls (PCBs) etc. (by USEPA methods).

RESULTS

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°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 sludge.

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)		Temperature range ($^{\circ}\text{C}$)	Total number of culturable bacterial strains isolated	Total number of species among the bacterial strains
	Latitude	Longitude			
IOCL, Mathura refinery (Northern)	27 $^{\circ}$ 26' N	77 $^{\circ}$ 43' E	10 – 40	30	14
IOCL, Barauni refinery (Eastern)	25 $^{\circ}$ 28' N	85 $^{\circ}$ 59' E	19 – 35	16	8
IOCL, Haldia refinery (Eastern)	22 $^{\circ}$ 00' N	88 $^{\circ}$ 05' E	15 – 35	26	6

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IOCL, Gujarat refinery (Western)	22°16' N	73°14' E	20 – 38	30	9
IOCL, Panipat refinery (Northern)	29°23' N	76°58' E	15 – 40	20	5
ONGC oil well, Jorhat (North Eastern)	26°40' N	95°35' E	10 – 35	11	4
Oil well of Oil India Ltd., Duliajan, (North Eastern)	27°15' N	95°15' E	10 – 35	26	12
IOCL, Digboi refinery (North Eastern)	27°15' N	95°15' E	12 – 35	33	11
IOCL, Guwahati refinery (North Eastern)	26°09' N	91°46' E	15 – 35	22	10
BPCL, Mumbai refinery, (Western)	18°56' N	72°51' E	24 – 35	18	7
HPCL, Visakhapatnam (South Eastern)	17°41' N	83°17' E	21 – 42	19	6
CRL, Cochin refinery (Southern)	9°55' N	76°14' E	19 – 37	12	6
BRPL, Bongaigoan refinery (North Eastern)	22°16' N	73°14' E	18 – 32	13	6
Vadinar refinery (Western)	23°44' N	72°39' E	15 – 45	16	3
Reliance refinery, Jamnagar (Western)	22°26' N	70°26' E	15 – 45	32	3
Total				324	110

Composition of Oily Sludge

The 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(nitrogen, sulfur & oxygen fraction) asphaltene, resins etc. (Table – 1).

Biodegradation

After complete application of the microbial consortium to the bioremediation sites, it was observed that within 2 – 12 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 below describes the trend of biodegradation 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.

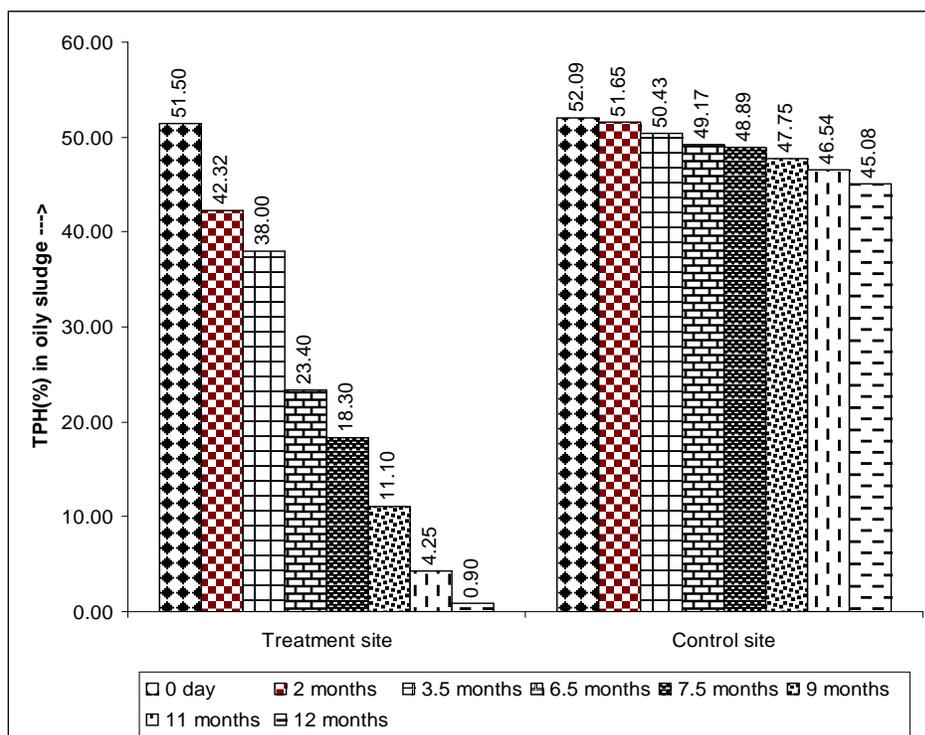


Figure – 1: Biodegradation of oil contaminated soil at ONGC Cauvery Asset, India.

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

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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.

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 – 2). 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. Hence bioremediation by microbes helps in eco-restoration of the hydrocarbon contaminated sites.



Figure – 2 : Site near GC-2, Burhan, at Kuwait Oil Company (KOC), Kuwait, before and after bioremediation.

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 more than 200,000 metric tonnes of oil contamination at various oil installations in India and abroad. Bioremediated soil has been found containing TPH content to the extent of <1% which is not toxic. Bioremediation technology has helped the oil industries in eco-restoration of the hydrocarbon contaminated sites.

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REFERENCES

- Agnès Gelin, Vincent Gravez, Graham J. Edgar (2003). Assessment of *Jessica* oil spill impacts on intertidal invertebrate communities. *Marine Pollution Bulletin*, 46(11),1377-1384.
- Atlas R. M. (1991). Microbial hydrocarbon degradation – biodegradation of oil spills. *Chem. Tech. Biotechnol.*, 52, 149 – 156.
- Bartha, R., Bossert, I., (1984). The fate of petroleum in the soil ecosystems. In: Atlas, R.M. (Ed.), *Petroleum Microbiology*. Macmillan, New York, 435–473.
- Bhattacharya D, Sarma P M, Krishnan S, Mishra S, Lal B (2003). Evaluation of genetic diversity among *Pseudomonas citronellolis* strains isolated from oily sludge contaminated sites. *Appl Environ Microbiol*,69, 1431–1441.
- Bragg, J. R., R. C. Prince, J. B. Wilkinson, and R. M. Atlas. (1994). Effectiveness of bioremediation for the *Exxon Valdez* oil spill, *Nature*, 368, 413–418.
- Carpenter C. P., D. L. Geary Jr., R. C. Myers, D. J. Nachreiner, L. J. Sullivan, J. M. King, (1977), Petroleum hydrocarbon toxicity studies : XIV. Animal and human response to vapors of “High Aromatic Solvent”, *Toxicology and Applied Pharmacology*, 41(2), 235-249.
- Chen Colin S., You C. Hseu, Shih. H. Liang, Jar-Yi Kuo, Ssu. C. Chen, (2008), Assessment of genotoxicity of methyl-*tert*-butyl ether, benzene, toluene, ethylbenzene, and xylene to human lymphocytes using comet assay, *Journal of Hazardous Materials*, 153(1-2), 351-356.
- Chikere C. B., G. C. Okpokwasili and B. O. Chikere. (2009). Bacterial diversity in a tropical crude oil polluted soil undergoing bioremediation, *African Journal of Biotechnology*, 8(11), 2535-2540.
- Dabbs C.W (1999). Oil Production and Environmental Damage. <http://www1.american.edu/ted/projects/tedcross/soilpr15.htm>
- Dibble J T and Bartha R (1979). Effect of environmental parameters on the biodegradation of oil sludge. *Appl Environ Microbiol*, 55, 729–739.
- Enzler M.S (2006). Environmental Disasters. Lenntech. <http://www.lenntech.com/environmental-disasters.htm>
- EPA (Undated).Wildlife and Oil Spills. EPA Office of Emergency and Remedial Response. http://www.epa.gov/oem/docs/oil/edu/oilspill_book/chap5.pdf
- Gomer Charles J. & David M. Smith. (1980). Acute skin phototoxicity in hairless mice following exposure to crude shale oil or natural petroleum oil, *Toxicology*, 18(1), 75-85.
- Gong P, B. M. Wilke, E. Strozzi, S. Fleischmann. (2001). Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils. *Chemosphere*, 44(3), 491-500.
- Head I. M. (1998), Bioremediation: towards a credible technology. *Microbiology*, 144(3), 599.
- Knafla A., K.A. Phillipps, R.W. Brecher, S. Petrovic, M. Richardson, (2006). Development of a dermal cancer slope factor for benzo[*a*]pyrene, *Regulatory Toxicology and Pharmacology*, 45(2), 159-168.
- Lal B. and S. Khanna, (1996), Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorans*, *Journal of Applied Bacteriology*, 81, 355-362.
- Lal Banwari, P M Sarma, Manish Dwivedi, Ajoy Kumar Mandal, Anil Agnihtori, V K Jain. (2007). Bioremediation of oil spill and oily sludge contaminated sites. Proceedings of 48th Annual Conference Association of Microbiologists of India, Chennai, India, Paper no. IL-EN2, 42.

<http://www.ejournalofscience.org>

- Lee Ada S., Michael R. Bye, Robert B. Mellins, (2006), Lung Injury from Hydrocarbon Aspiration and Smoke Inhalation, *Kendig's Disorders of the Respiratory Tract in Children*, (Seventh Edition), 653-660.
- Lewis Ceri, Chris Pook, Tamara Galloway, (2008), Reproductive toxicity of the water accommodated fraction (WAF) of crude oil in the polychaetes *Arenicola marina* (L.) and *Nereis virens* (Sars), *Aquatic Toxicology*, 90(1), 73-81.
- Liu Wuxing, Yongming Luo, Ying Teng, Zhengao Li, Peter Christie, 2009. Prepared bed bioremediation of oily sludge in an oil field in northern China. *Journal of Hazardous Materials*, 161, 479-484.
- Mandal A. K, Manish Dwivedi, Abu Swaleh, P. M Sarma, B. Lal, A. Agnihotri, U. S. Hazra, S A Fazal, A K Rout & U S Prasad. (2007a). REMEDIATION OF OILY SLUDGE at various installations of ONGC: A BIOTECHNOLOGICAL APPROACH, Proceedings of Petrotech 2007: 7th International Oil & Gas Conference and Exhibition, New Delhi, India, Paper no. 753.
- Mandal Ajoy Kumar, Priyangshu Manab Sarma, Manish Dwivedi, Abu Swaleh, Bina Singh, Banwari Lal, S Ganesan, B. R. Jatob, V. K. Jain and Anil Agnihotri (2009). Bioremediation of tank bottom waste oily sludge at CPF Gandhar, India : a case study. Proceedings of Petrotech 2009: 8th International Oil & Gas Conference and Exhibition, New Delhi, India, Paper no. 657.
- Mandal Ajoy Kumar, Priyangshu Manab Sarma, Manish Dwivedi, Abu Swaleh, Banwari Lal, A. Agnihotri, U.S. Hazra and K. S. Dwivedi (2009a). Bioremediation of oil contaminated soil at CTF Kalol, ONGC, Ahmedabad Asset, India. Proceedings of National Conference on Energy Resources of North East India, Guwahati, Paper no. S09.HSE.0004.
- Mandal Ajoy Kumar, Priyangshu Manab Sarma, Manish Dwivedi, Abu Swaleh, Banwari Lal, A. Agnihotri, U. S. Hazra, A. Doraiah and S. S. Patidar (2007). Bioremediation Of Oil Contaminated Soil At South Santhal CTF, ONGC, Mehsana Asset, India. Proceedings of 2007 Asia Pacific Oil and Gas Conference and Exhibition, Jakarta, Indonesia, Society of Petroleum Engineers (SPE), Paper no. 109571.
- Mandal Ajoy Kumar, Shuvendu Acharya, Priyangshu Manab Sarma, Bina Singh and Banwari Lal. (2010). Bioremediation Of Oil Contaminated Drill Muds At Bhavnagar Shorebase, India : A Field Case Study. Proceedings of Petrotech 2010: 9th International Oil & Gas Conference and Exhibition, New Delhi, India, Paper no. 20100515.
- Mearns, A. J., Venosa, A.D., Lee, K., Salazar, M. (1997). Field-testing bioremediation treating agents: lessons from an experimental shoreline oil spill. Proceedings of 1997 International Oil Spill Conference. American Petroleum Institute, Washington DC, 707-712.
- Ministry of Environment and Forest (MoEF), Government of India, *Hazardous Wastes (Management and Handling) Rules Amendment 2000*.
- Mishra S., Jyot J. & al. (2001a), *In situ* bioremediation potential of an oily sludge degrading bacterial consortium, *Current Microbiology*, 43, 328.
- Mishra Sanjeet, Jeevan Jyot, Ramesh C. Kuhad, Banwari Lal, (2001). Evaluation of Inoculum Addition To Stimulate In Situ Bioremediation of Oily-Sludge-Contaminated Soil, *Applied and Environmental Microbiology*, 67(4), 1675-1681.
- Mishra Sanjeet, Priyangshu M. Sarma, Banwari Lal, (2004). Crude oil degradation efficiency of a recombinant *Acinetobacter baumannii* strain and its survival in crude oil-contaminated soil microcosm, *FEMS Microbiology Letters*, 235, 323-331.

<http://www.ejournalofscience.org>

- Moacir A. Torres, Marcelo P. Barros, Sara C.G. Campos, Ernani Pinto, Satish Rajamani, Richard T. Sayre, Pio Colepicolo (2008). Biochemical biomarkers in algae and marine pollution: A review. *Ecotoxicology and Environmental Safety*, 71(1),1-15.
- Ouyang Wei, Hong Liu, V. Murygina, Yongyong Yu, Zengde Xiu, S. Kalyuzhnyi. 2005. Comparison of bio-augmentation and composting for remediation of oily sludge : A field scale study in China, *Process Biochemistry*, 40, 3763 – 3768.
- Peterson Charles H., (2001). The “Exxon Valdez” oil spill in Alaska: Acute, indirect and chronic effects on the ecosystem, *Advances in Marine Biology*, 39, 1-103.
- Prince, C.R., 1993. Petroleum spill bioremediation in marine environments. *Critical Review of Microbiology* 19, 217-242.
- Prince, R. C., Elmendorf, D. L., Lute, J. R., Hsu, C. S., Haith, C. E., Senius, J. D., Dechert, G.J., Douglas, G. S, and Butler, E. L. (1994). 17 α (H), 21 β (H)-Hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environmental Science and Technology*, 28, 142-145.
- Raghavan P U M & M. Vivekanandan (1999). Bioremediation of oil-spilled sites through seeding of naturally adapted *Pseudomonas putida*. *International Biodeterioration & Biodegradation*, 44, 29 – 32.
- Rice Stanley D., Jeffrey W. Short, Mark G. Carls, Adam Moles, Robert B. Spies, (2007), The *Exxon Valdez* Oil Spill, *Long-term Ecological Change in the Northern Gulf of Alaska*, 419-520.
- Samiullah Yusaf, (1985). Biological effects of marine oil pollution. *Oil and Petrochemical Pollution*, 2(4), 235-264.
- Sarma Priyangshu Manab, Ajoy Kumar Mandal, Teeka Singh, Anju Singh and Banwari Lal (2007). Remediation of drill cuttings and drilling muds, the potential environmental hazard of petroleum exploration: A biotechnological intervention. Proceedings of the Second International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld 2007), Seville, Spain.
- Sarma Priyangshu Manab, Dhruva Bhattacharya, S. Krishnan, and Banwari Lal (2004), Assessment of intra-species diversity among strains of *Acinetobacter baumannii* isolated from sites contaminated with petroleum hydrocarbons, *Can. J. Microbiol.*, 50, 405 – 414.
- Sarma Priyangshu Manab, Ajoy Kumar Mandal, Abu Swaleh, Manish Dwivedi and Banwari Lal (2006). Remediation of petroleum wastes and reclamation of waste lands : A biotechnological approach, Proceedings of Brownfield Asia 2006 : International conference on remediation and management of contaminated land : Focus on Asia, Kualalumpur, Malaysia, 185 – 198.
- Sood Nitu & Banwari Lal (2009). Isolation of a novel yeast strain *Candida digboiensis* TERI ASN6 capable of degrading petroleum hydrocarbons in acidic conditions, *Journal of Environmental Management*, 90, 1728–1736
- Swannell, R.P.J., Lee, K., and Mcdonagh, M. (1996) Field evaluations of marine oil spill bioremediation. *Microbiological Reviews*, 60(2), 342-365.
- Szaro Robert C., Michael P. Dieter, Gary H. Heinz, John F. Ferrell (1978). Effects of chronic ingestion of south Louisiana crude oil on mallard ducklings. *Environmental Research*, 17(3), 426-436.
- Vidali M., (2011), Bioremediation An Overview, *Pure Applied Chemistry*, 73(7), 1163 – 1172, ©2001, IUPAC.
- Walker, J D. & al., (1975) Microbial petroleum degradation: application of computerized mass spectroscopy, *Can. J. Microbiol*, 21, 1760.
- Wikipedia (2011). Oil Spills. The Free Encyclopedia. http://en.wikipedia.org/wiki/Oil_spill

<http://www.ejournalofscience.org>

- Yoshida M, H. Michel, L.A. Sazanov, S. Yoshikawa, J. Barber, J.K. Lanyi, S.P. Balashov, V.A. Shuvalov, A.G. Yakovlev, T.A. Shkuropatova, L.G. Vasilieva, A.Y. Shkuropatov, P. Gast, S.D. Dunn, P.A. Del Rizzo, Y. Bi, K.S. Wood, D.J. Cipriano, P. Turina, A. Rebecchi, et al. (2006). *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1757(5-6)(1), 1-551.
- Yuste L, Corbella M E, Turiegano M J, Karlson U, Puyet A, Rojo F (2000). Characterization of bacterial strains able to grow on high molecular mass residues from crude oil processing. *FEMS Microbiol Ecol*, 32, 69–75
- Zhang Litai, Kristin Sannes, Alan J. Shusterman, Corwin Hansch, (1992), The structure-activity relationship of skin carcinogenicity of aromatic hydrocarbons and heterocycles, *Chemico-Biological Interactions*, 81(1-2), 149-180.
- Zhu Xueqing, Albert D. Venosa, Makram T. Suidan, and Kenneth Lee, U.S. Environmental Protection Agency, USA, (2001), *Guidelines for the bioremediation of marine shorelines and freshwater wetlands*.