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Biodegradation of Oil Spill by Petroleum Refineries Using Consortia of Novel Bacterial Strains

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Abstract Feasibility study carried out at the site prior to the full scale study showed that the introduced bacterial consortium effectively adapted to the local environment of the soil at bioremediation site. The soil samples were collected from the contaminated fields after treatment with bacterial consortium at different time intervals and analyzed by gas chromatography after extraction with hexane and toluene. At time zero (just before initiation of bioremediation), the concentration of total petroleum hydrocarbons in the soil (25-cm horizon) of plot A, B, C and D was 30.90 %, 18.80 %, 25.90 % and 29.90 % respectively, after 360 days of treatment with microbial consortia was reduced to 0.97 %, 1.0 %, 1.0 %, and 1.1 % respectively. Whereas, only 5 % degradation was observed in the control plot after 365 days (microbial consortium not applied).

Keywords Novel bacterial strain · Aromatic · Aliphatic · Corncob

During the production and transportation of crude oil, unsuitable operation and leakage may result in contamination of soil with petroleum hydrocarbons. Petroleum contamination causes significant environmental impacts and presents substantial hazards to human health (US EPA. 2010). The BP spill spewed 4.1 million barrels of oil into the Gulf of Mexico over 87 days, making it the biggest unintentional off shore oil spill in the history of the petroleum industry.

In recent years crude oil spills incidents are increasing at an alarming rates. The oil refineries are also generating enormous quantity of hazardous hydrocarbon waste as oily sludge. Many of the constituents of oily wastes are toxic and potential carcinogens. Disposal of hydrocarbon contaminated soil is usually carried out with reference to government legislations which vary in different countries. Sludge contaminated soil can be revived using a variety of technologies such as excavation and containment of contaminated soils in secured landfills, stabilization and solidification, thermal desorption and incineration (Varanasi et al. 2007). These treatments are not environmental friendly and have substantial cost implications with remediation cost per cubic meter of polluted soil ranging from $880 for landfill disposal, $700 for incineration and $260 for thermal desorption (Cookson 1995).

Removing of oil waste by air purging or by use of surfactants is costly and hazardous to health and can pollute the environment. One in situ technique for the remediation of oil contaminated soil is bioremediation, which is safe and cost effective.

The Energy and Resources Institute (Teri), India, has developed a cock tail of five microbes (microbial consortium) (Banwari and Khanna 1996a, 1996b) isolated from oil contaminated soil for cleanup of oil spills and treatment of oily sludge (Das and Mukherjee 2007; Unterbrunner et al 2007).

The aim of the paper is to carry out the field study in Ahmedabad area (four different sites), India, to bio-remediate land contaminated with an oily sludge, the land belonging to an oil refinery by using a carrier based

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hydrocarbon-degrading bacterial consortium and nutrient mixture.

Materials and Methods

All the chemicals used were of analytical grade (AR) and were obtained from commercial supplier.

The contaminated soil was collected from a crude oil spill site located in Ahmadabad, Gujarat (India). The soil samples were collected from three depths 0–25 cm, 26–50 cm, and 51–75 cm, air dried and passed through a 2 mm mesh sieve, homogenized by hand and stored at 4 °C in the dark until use. Physiochemical properties of soil were analyzed by standard methods and total petroleum hydrocarbon (TPH) content of the soil was determined gravimetrically.

Corn cob powder was used as a carrier material to immobilize bacteria owing to its properties of being highly granular, absorbent, biodegradable and inexpensive. Corn cob was harvested at maturity, air-dried and mechanically ground to obtain a particle size of approximately 0.5–1.5 mm. Before use, corn cob powder was sterilized by autoclaving at 121 °C for 20 min.

A bacterial consortium that could degrade oily sludge was developed in minimal salt media, with oily sludge as the sole source of carbon and energy, from a sample of soil contaminated with crude oil. The sample was collected from an oil well located in one of the site at Ahmedabad in Gujarat, India.

Prior to the field study, a feasibility study on bioremediation of soil contaminated with oily sludge was carried out at bioremediation site. The sludge lying on the contaminated land was characterized by estimating total petroleum hydrocarbon (TPH), alkane, aromatic, NSO, asphaltene, and heavy metals. The total area (4,000 m^2) of the feasibility study was grouped as gathering station GGS-1 Kalol (A), group gathering station GGS-1 Limbodera (B), group gathering station GGS-II Limbodera (C), and group gathering station GGS-II Gamij (D) and one site was marked as (E, Control).

Four sites were selected for bioremediation; one of the blocks was maintained as untreated control. Depending on the sludge quantity and quality, the bacterial consortium was applied; One kilogram of the carrier-based bacterial consortium and 50 L of a nutrient mixture (67.5 g KNO_3, 8.75 g K_2HPO_4, 2.5 g $MnSO_4{\cdot}2H_2O$, 0.005 g $FeSO_4$, 0.05 g, $CaCl_2{\cdot}2H_2O$, 0.005 g, $ZnSO_4{\cdot}7\ H_2O$, 0.005 g, $CuSO_4{\cdot}5H_2O$, 0.05 g, $COCl_2$, 0.005 g, Al K $(SO_4)_2$, 0.005 g H_3BO_4, and 0.005 g Na_2MoO_4) for every 10 m^2 were added to all the blocks except the control. The land was thoroughly tilled at 15-day intervals with tractor fitted with a harrow.

Physical and chemical properties of the soil samples withdrawn from 0 to 25 cm horizon at the onset and at the end of the treatment were characterized. Air dried and pulverized soil samples were analyzed for organic carbon, nitrogen, available phosphorous, potassium, moisture level, and pH using standard method. Various heavy metals were analyzed from the mixed soil samples collected from Plot A, Plot B, Plot C, Plot D, and Plot E at time zero (before initiation of the experiments) and at the end of the study. Heavy metals in oily sludge were also analyzed using atomic absorption spectroscopy.

Oil was extracted from the oily sludge samples by Soxhlet extraction using EPA method (EPA 9071 C) (Makadia et al. 2011; Eikania et al. 2011). Each treated block was sampled at 28 points. Samples were collected at time zero (just before initiating the bioremediation), after 3, 6 months and at the end of the study (12–18 months). The efficiency of the method was checked by the recovery experiment. The dried soil (10 g) was spiked with crude oil (1 gm) and mixed properly. The spiked soil sample was extracted with hexane (200 ml) by Soxhlet extraction and analyzed by gravimetric method (Eikania et al. 2011). Recovery of oil by this method was 0.99 g (99 %). Ten-gram soil from each experimental site was extracted similarly and quantity of oil was calculated gravimetrically.

Total Petroleum Hydrocarbon (TPH) extracted from oil-contaminated soil was fractionated into aliphatic hydrocarbons and poly-aromatic hydrocarbons by silica gel column, eluting with hexane followed by toluene (Luque de Castro and Priego-Capote 2010). Both hexane and toluene fractions were dried completely on a rotary evaporator, and by simple gravimetric method, the weight of the eluted alkane and aromatic fractions were calculated. Fractions eluted from the column with hexane and toluene were analyzed by GC (Hewlett Packard 5890 Series II) fitted with FID (flame ionization detector) and DB-2887 column (30 m × 0.22 mm id and 2.64 mμ film thickness). The operating conditions used for hexane fraction are as follows: injector and detector temperature 300 °C respectively; oven temperature was programmed as follows: 80 °C for 1 min @ 15 °C min^{-1} to 220 °C. Helium was used as a carrier gas with a flow rate of 2.5 ml min^{-1} (total run time 63.0 min). The aromatic hydrocarbons were analyzed on a DB-23 column (30 m × 0.20 mm × 2.64 mμ film thickness) with operating parameter: injector and detector temperature 300 °C respectively; oven temperature 60 °C for 1.0 min @ 8 °C/min to 280 °C for 20.0 min. Helium was used as a carrier gas at a flow rate 1.1 ml/min. The percent decrease in the peak areas of the different hydrocarbons relative to the controls was adopted as a measure of hydrocarbon degradation (Mishra et al. 2001).

Results and Discussion

Of the four treatments employed for the feasibility study, the addition of the bacterial consortium and nutrients resulted in the maximum bioremediation response. The selected bacterial strains were able to degrade more than 95 % of the aliphatic fractions, 75 % of aromatic fractions in 15 days under laboratory conditions and 95 % of NSO fractions of oily sludge (total petroleum hydrocarbon) in 24 h into carbon-dioxide and water (Bajpai et al 1998) as the end products. It was observed that the cocktail of five microbial strains could biodegrade crude oil and oily sludge at a very fast rate under laboratory conditions.

Based on the above results, the treatment consisting of the selected bacterial consortium and nutrients was chosen for the field scale bioremediation study.

The soil at bioremediation site was black due to heavy contamination of oil. The physiochemical properties of soil at bioremediation site during contamination and after bioremediation are as follows: the bulk density of the soil remained unchanged (1.40–1.45 g/cm^3) water holding capacity increased significantly from 60.00 % to 71.00 %, organic carbon decreased from 4.20 % to 2.60 % and not much variation in pH of the soil, total nitrogen increased from 0.043 % to 1.15 %, potassium from 129 % to 115 % and available phosphorus from 12.00 % to 27.00 %.

The chemical composition of oily sludge showed the highest content of organic matter followed by sediments and ash. The solvent-extractable TPH (total petroleum hydrocarbons) content in oily sludge was found to be 31 %, water (11 %,), organic matter (27 %,), sediment and ash (30 %.). The TPH contained aliphatic hydrocarbons (46 %), aromatic hydrocarbons (37 %), NSO (7 %) and asphaltene (10 %).

A total of 13 heavy metals were detected in oily sludge and mixed soil samples collected from plot A, B, C, D, and E (Table 1). Nine heavy metals were detected in the oily sludge with Al (aluminum), Mn (manganese) and Fe (iron) in high concentrations. The soil samples collected from 25-cm horizon showed the presence of seven heavy metals at time zero and after 1 year of bioremediation and soil collected from 50 cm horizon showed seven heavy metals at time zero and eight at the end of study. However, the concentration of these heavy metals in the sludge and soil was much lower than the prescribed safe limits and therefore were safe to soil bacteria.

The concentration of total petroleum hydrocarbons (TPH), alkane, aromatic hydrocarbons in oil contaminated soil at time zero and after treatment with microbial consortium on 365 days from plot A, B, C, D and E is reported in (Table 2). At time zero (just before initiation of bioremediation), the concentration of oil (TPH) in the soil (25-cm horizon) of plot A was 39.2 %. After 365 days it was reduced to 0.97 % indicating the removal of 97.03 % of TPH. Limbodara I Plot (B) at time zero, the concentration of oil (TPH) in the soil (25-cm horizon) was 18.9 %, after 365 days, it was reduced to 1.1 % which means the removal of 99.9 % of TPH, similarly at Limbodara II (C) and Gamij plot (D) the initial TPH was 25.80 29.90 % reduced to 1, and 1.1 %, whereas in control plot (E) it was reduced from 40 % to 35 % (without application of consortia) (Table 2).

The waste oily sludge and oil soaked soil lying in four pits situated at oil well near petroleum refinery at Ahmedabad Asset (Oil and Natural Gas Corporation) was used for bioremediation. Microbial consortia were applied in these pits to degrade the oily sludge. All the pits at Ahmedabad Asset were used for bio-remediation.

At the area A (GGS-I Kalol) 278.9 tons of oily sludge was used for bioremediation by applying 1,515.00 kg of microbial consortium. Before bioremediation the oil sludge contained 39.2 %, of TPH, which was reduced to 0.97 % in 365 days after application of microbial consortia.

At GGS-I Limbodera (B) l 222.8 tons of oily sludge was used for bioremediation by application of 1,880.0 kg of microbial consortia. The initial concentration of TPH in the oily sludge was found from 13.3 % to 18.9 %, which were reduced to <1 % after bioremediation (after 1 year).

At GGS-II Limbodera (C), 248.8 tons of oily sludge was treated with1900.0 kg of microbial consortium. The concentration of TPH (Total Petroleum Hydrocarbon) at zero time was found to 25.85 %, which was reduced to 1 % after bioremediation (after 365 days).

AT GGS-Gamij (D) which is around 60 km from the Ahmedabad 324.4 tons of oily sludge was treated with l 1,725.0 kg of bacterial consortia. The initial concentration of TPH was 25.80–29.90 % reduced to 1–1.1 % after 365 days of treatment with microbial consortia where as in control (E) it was reduced from 40 % to 35 % without application of consortium.

Initial concentration of TPH contamination in the soil of plot A, plot B, plot C, plot D, Plot E at different time periods during the course of the study are shown in (Fig. 1).

The aliphatic and aromatic hydrocarbons present in the oil contaminated soil were separated before remediation and after 365 days of treatment with microbial consortium by column chromatography eluting with hexane followed by toluene. These fractions were analyzed by GC. The GC chromatograph of hexane fraction of 0 day sample showed the presence of aliphatic hydrocarbons from C11 to C29 (Fig. 2). The same sample when analyzed after 365 days of treatment with microbial consortium showed reduced concentration of aliphatic hydrocarbons.(Fig. 3).

Similarly analysis of zero day treated sample of the toluene fraction showed the presence of aromatic hydrocarbons namely phenanthrene, anthracene, flouranthrene,

Table 1 Concentration of heavy metals in oily sludge and soil before and after bioremediation

Heavy metals	Oily sludge	Conc (mg/kg)[a]			
		Soil at time zero		Soil at 360 days	
		25 cm	50 cm	25 cm	50 cm
Al	77.6 ± 5.00	5.89 ± 0.08	52.1 ± 1.20	13.9 ± 1.0	1.25 ± 0.01
Si	ND	ND	ND	ND	0.69 ± 0.01
Zn	0.59 ± 0.04	ND	ND	ND	ND
Mn	2.52 ± 0.05	0.34 ± 0.02	1.34 ± 0.02	0.51 ± 0.01	0.03 ± 0.00
Cu	0.03 ± 0.00	ND	ND	ND	ND
Co	0.0005 ± 0.00	0.02 ± 0.00	0.006 ± 0.00	0.003 ± 0.00	002 ± 0.00
Fe	38.6 ± 1.20	10.6 ± 0.92	33.80 ± 0.75	17.8 ± 1.10	1.14 ± 0.01
Cd	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Pb	ND	ND	ND	ND	ND
Cr	0.03 ± 0.00	0.001 ± 0.00	0.007 ± 0.00	0.003 ± 0.00	0.02 ± 0.00
Ni	ND	ND	ND	ND	ND
As	0.003 ± 0.00	0.001 ± 0.00	0.006 ± 0.00	0.002 ± 0.00	0.01 ± 0.0
Se	ND	ND	ND	ND	ND

[a] Values are average ± standard deviation for 28 samples

Table 2 Presence of TPH, alkane, aromatic hydrocarbons NSO and asphaltene in soil at before and after treatment with microbial consortium

Block	Concentration (%) at 0 day				Concentration (%) after 365 days (%)			
	TPH	Alkane	Aromatic	NSO + Asph.	TPHAlkane	Aromatic		NSO + Asph
GGS-1Kalol (A)	39.2[a]	20.2	14.5	4.1	0.97[a]*	0.70	00.0	0.0
GGS-1Limbodara(B)	18.9[b]	7.8	5.6	3.6	1.1[b]*	0.09	00.0	0.0
GGS-I1Limbodara(C)	25.8[c]	11.5	8.2	3.2	1.0[c]*	0.05	00.	0.0
GGS-IIGamij(D)	29.9[d]	17.2	9.1	3.2	1.1[d]*	0.87	00.0	0.0
Control(E)	40.1[e]	18.8	12.4	5.8	35.2[e]*	18.6	9.8	6.1

TPH could not be detected at 26–50 cm, and 51–75 cm. Value is means and Standard deviation for ± 6 for 28 samples

[a] Means of 28 samples in plot A and a* value obtained after 365 days

[b] Means of 28 samples in plots and b* value obtained after 365 days

[c] Means of 28 samples in plot c and c* value obtained after 370 days

[d] Means of 28 samples in plot d and d* value obtained after 365 days

[e] Control

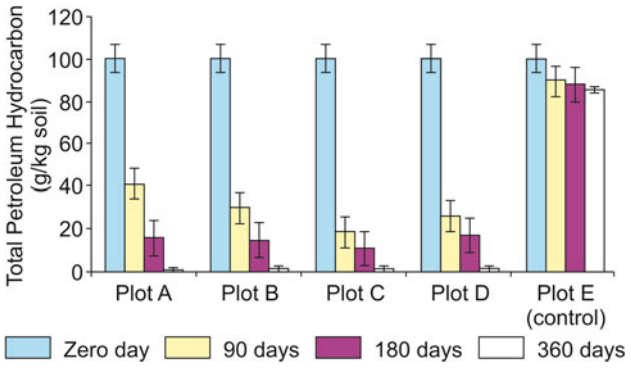

Fig. 1 Concentration of Total petroleum hydrocarbon (TPH) in the oil contaminated soil of plot A, B, C, D and E. before (0 day) and 365 days after treatment with microbial consortium

pyrene, chrysene, benzo(a)anthracene, bnzo(k) fluoranthene, benzo(b) fluoranthrene, benzo(a) pyrene, indeno (1,2,3,-c, d) pyrene, dibenzo (a, h) anthracene, benzo (g, h, i) perylene (Fig. 3), whereas the treated sample analyzed after 1 year showed complete degradation of aromatic hydrocarbons by microbial consortium (Fig. 3).

The microbial consortia developed by TERI were able to bio-remediate about 20 % contaminations by oil (200 grams per kilogram of oil) in 2 months. The bioremediation has been found to cheap technology to clean up hazardous waste than the conventional physico-chemical treatments.

The bioremediation work at Ahmedabad Asset, India was undertaken to clean up the waste oily sludge pits at various well head sites. Before bioremediation Ahmedabad

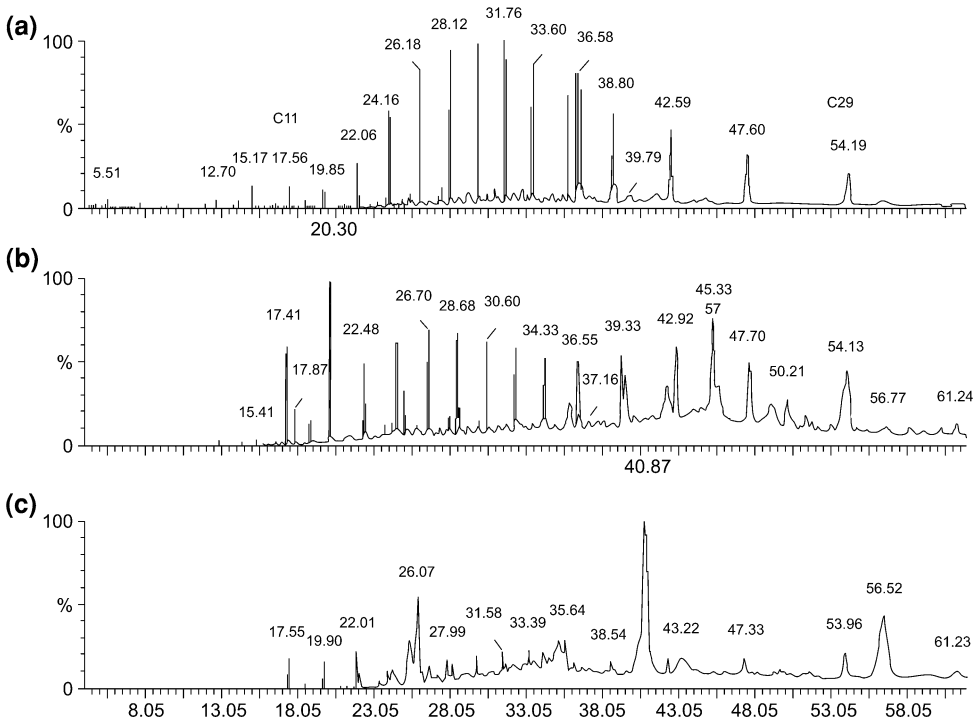

Fig. 2 GC chromatograph showing the presence of aliphatic hydrocarbons **a** 0 day **b** 6 months after treatment **c** 1 year after treatment

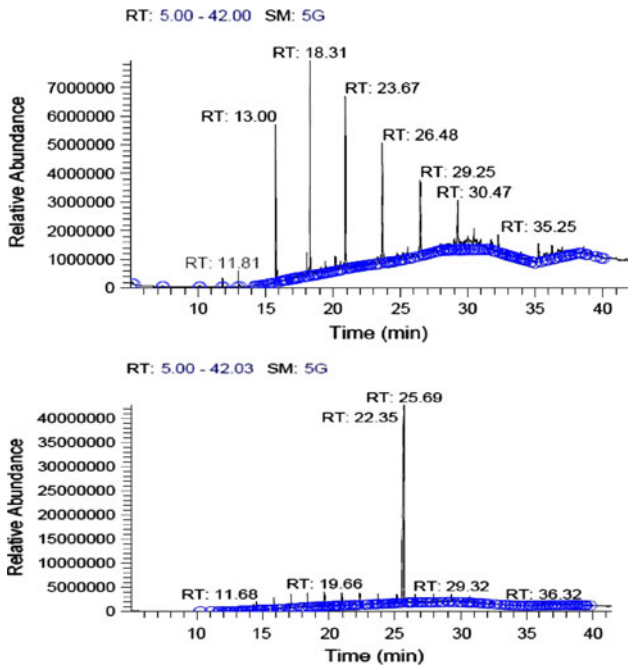

Fig. 3 GC chromatograph showing the presence of aromatic hydrocarbons in the untreated and treated soil after 365 days

Asset used to pay huge amount as compensation to the farmers due to oil spill in their fields. However, after bioremediation the cases of compensation reduced significantly and Ahmedabad Asset saved considerable amount of money which was earlier given as compensation. Bioremediation process is the most eco friendly and viable approach for cleanup of oil spill, oil contaminated sites and waste oily sludge pits.

Conclusions

A feasibility study at the site prior to the full scale study showed that the introduced bacterial consortium effectively adapted to the local environment of the soil. The carrier material used for the purpose is an agricultural residue, corn cob powder, which is very good soil conditioner. The carrier material may also augmented the degradation rate by providing air pocket in the soil, thereby making it porous and facilitating aeration for growth and survival of the introduced bacterial consortium. The main advantage of in situ treatment is that it allows soil to be treated without being excavated and transported, resulting in potentially significant cost savings.

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