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Remediation of Hydrocarbon Contaminated Soil through Microbial Degradation- FTIR based prediction

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ABSTRACT

Samples of polluted soil contaminated with used motor oil spillage were collected to study whether such soils may be remediated, i.e. whether the remediation of hydrocarbon contaminated soil is achievable using microbial strain sampled from the same site where from the contaminated soil has been collected for isolating bacterial strains. Sampled soil has been divided in to two sets and the one set of the two was inoculated with the isolated microbial strain (treated sample) and the other set left as such without any inoculation (untreated). The inoculation of microbial strain was performed after assessing the water holding capacity of the sampled soils. FTIR spectra were obtained of the untreated samples and have been referred in the study as zero day. Spectra of treated samples were obtained on 12th day of incubation and on 25th day of incubation. A comparison of FTIR spectra of treated (12th day and 25th day) and untreated soil revealed the presence of new bands pertaining to aliphatic and polycyclic aromatic hydrocarbons including various alcohols, aldehydes and ketones. The isolated bacterial strain seems to have substantial potential to remediate the hydrocarbon contamination of the soil and indicate signs of bioremediation of soils contaminated with used motor oil.

Key words: - Fourier-Transform Infrared Spectrometer (FTIR), Hydrocarbons, Used motor oil.

INTRODUCTION

Along with other developing economies, India is also suffering with ever increasing consequential environmental threats. Rapid industrialization can only move hand in hand with the efficient and optimum feasibility of transport, which results in to increased use of

automobiles. With the increase in vehicle use, India has also witnessed a mushroom growth of automobiles and also the service stations where significant amount of used motor oil is erroneously discharged in to the environment. The used motor oil during manual oil changing operation is not recycled but spilled and dumped at station sites, thereby polluting both soil and water.

Used motor oils are likely to contain greater percentage of aromatic and aliphatic hydrocarbons (C_{15}-C_{50}), nitrogen and sulphur compounds, and metals (Mg, Ca, Zn, Pb *etc*.) than fresh oils [4]. In addition to the aforesaid compounds, presence of naphthalene, benzo[a] pyrene and anthracene can not be ruled out [2]. An erroneous and accidental release of such compounds over a long period of time may accumulate potential hazard to environmental and human health [8]. It is likely that petroleum based new wastes could be generated at all times which can be linked to newer technologies for oil manufacture, which along with new additives can be linked to different engine types and operation practices [3].

Although micro-organisms are known to degrade the various ingredients present in hydrocarbons, there is a need to isolate novel microbes that can not only degrade the major components of oil but can selectively deplete other compounds as well, such as naphthalane, benzo[a] pyrene and anthracene [1]. Most of the petroleum based hydrocarbons are degradable subsequent to their disposal [5], however, very little information is available towards the chemical transformation of such compounds under natural environmental conditions. The objective of the present study was isolation of a hydrocarbon degrading microbe and the evaluation of its petroleum hydrocarbon degradation potential in soil, using FTIR spectroscopy.

MATERIALS AND METHODS

Study site

Lucknow (26^{o}5/N latitude, 80^{o}56/E longitude, 128 m above the sea level), the Capital of Uttar Pradesh, spread over an area of 310 sq. km in the central plain of the Indian subcontinent, supporting a population of 36.48 lakhs (2001 Census) has a number of two-wheeler service stations scattering at different sites. For the study, a service station namely Speed Automobiles and Repairing Center located at Telibagh, Lucknow, was selected as the sampling site. The top soil sampled from around the vicinity of the service station so as to know the most contaminated site of the sampled sites.

Isolation and inoculation of microbial culture

Soil sample contaminated with mobil oil were collected in sterile plastic bags and immediately brought to the laboratory, preserved at 4 ^{0}C till further analysis. Bacterial strains were isolated from the same contaminated soil by serial dilution agar plate method. Soils samples collected from the study site were divided in to two sets. One set (50 g) not inoculated with isolated bacterial strains was named as untreated or zero day (control) and was analyzed using FTIR and the other set as treated in which 50 g of soil was inoculated with the isolated bacterial strains. Mixing was done manually every third day. Moisture was kept at 50 %; temperature range was 20-25 ^{0}C. Water holding capacity was checked before inoculating the soil samples so as to inoculate them with the adequate amount of microbial culture. The treated set was analyzed after

12th day and after 25th day of incubation using FTIR. The isolated bacterial strain was analyzed morphologically on the basis of Gram staining, colony morphology, shape, etc. The isolated strain was characterized as Gram negative, rod. Treated and untreated samples were analyzed for residual hydrocarbons using FTIR (Fourier-Transform Infrared Spectrometer), Nicolet model-6700, which works in mid IR range of 4000-400 $cm^{-1.}$ For the analysis, the samples were grounded with KBr in the ratio of 100:1 (100 mg KBr and 1 mg sample) in an agate motor to make the pellets by using a Hydraullic press (CAP.- 15T) at a pressure of ten tons. Spectra were obtained with a total of 32 scans against a KBr background.

RESULTS AND DISCUSSION

The microorganism was isolated from used motor oil contaminated soil by a selective enrichment culture technique. Following biochemical tests it was tentatively identified as *Pseudomonas* species. The used motor oil adsorbed soil was inoculated with the isolated bacterial strain and the FTIR spectrum was recorded for both the soils, i.e., one treated with the bacterial strain and the untreated control. The FTIR spectrum of the treated soil was recorded after the 12th and 25th day of microbial inoculation. The FTIR analysis of used motor oil contaminated soil on different days of microbial inoculation is given in Table-1. The FTIR spectrum (Fig. 1) of untreated used motor oil contaminated soil revealed bands at 3648.9 cm^{-1} indicating an O-H stretch corresponding to low concentration of phenols ; at 2361.5 cm^{-1} corresponding to C-H stretch of alkenes and at 1557.8 due to the presence of carboxylates [6].

However, the FTIR spectrum (Fig. 2) of the used motor oil contaminated soil, recorded on 12th day of microbial incubation, indicated new bands at 2925.1 cm^{-1}, 1027.2 cm^{-1} and 777.4 cm^{-1}, corresponding to C-H stretching in aliphatic compounds, C-O stretch for primary alcohols and alkyl halides, respectively [9]. Interestingly on 25th day of microbial incubation, all the bands observed on zero day (control) disappeared and a new band appeared (Fig. 3) in the range 3100-3000 cm^{-1} at 3168.6 cm^{-1} indicating C-H stretching in aromatic compounds [7, 3]. Altogether the results suggest that the isolated strain prefer both C-H aliphatic and aromatic stretches for degradation of long chain alkanes present in used motor oils.

Table 1: FTIR analysis of used motor oil contaminated soil on different days of microbial incubation.

S. No.	Range of Wave numbers (cm^{-1})	Band position in cm^{-1}			Band assignment
		Zero Day	12th Day	25th Day	
1.	3670-3610	3648.9	-	-	-OH stretch
2.	3100-3000	-	-	3168.6	=C-H stretching in aromatic compounds
3.	3000-2850	-	2925.1	2925.3	-C-H stretching in aliphatic compounds
4.	2455-2265	2361.5	2361.5	-	C-C, C≡N (Nitriles)
5.	1650-1550	1557.8	-	-	C=O stretching
6.	1400-1000	1027.2	1027.2	1017.3	-C-O stretching for primary alcohols
7.	850-550	-	777.4	-	Alkyl halides (CCl)

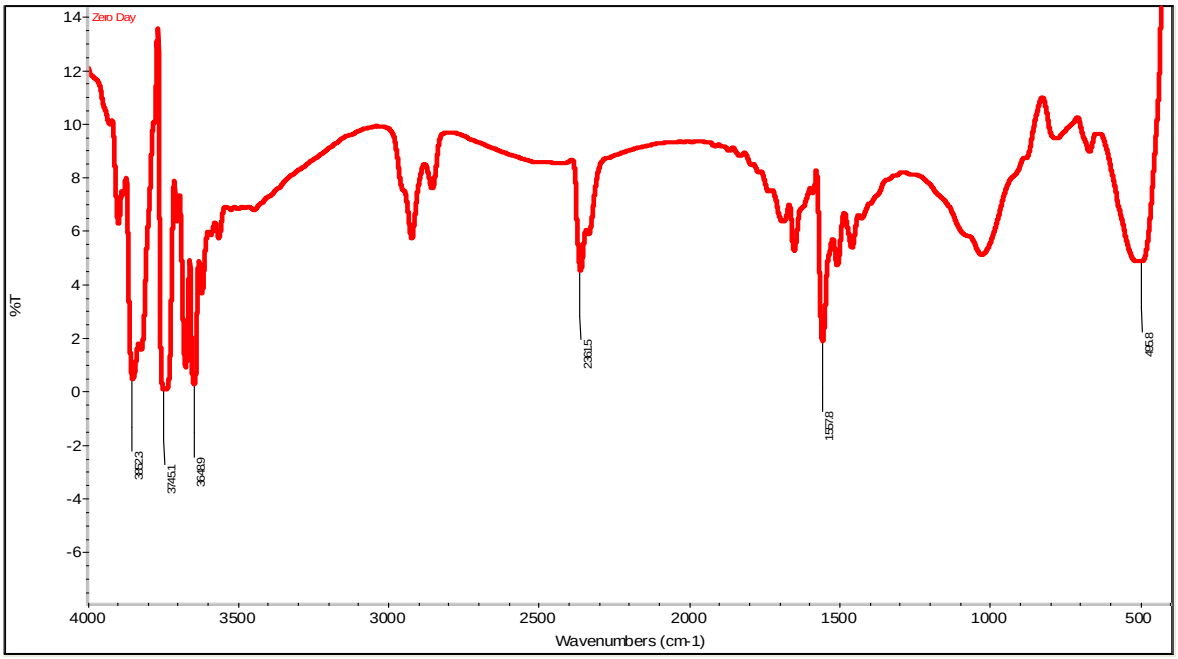

Fig. 1: FTIR spectrum of used motor oil contaminated soil on the first day (zero hour) of microbial inoculation.

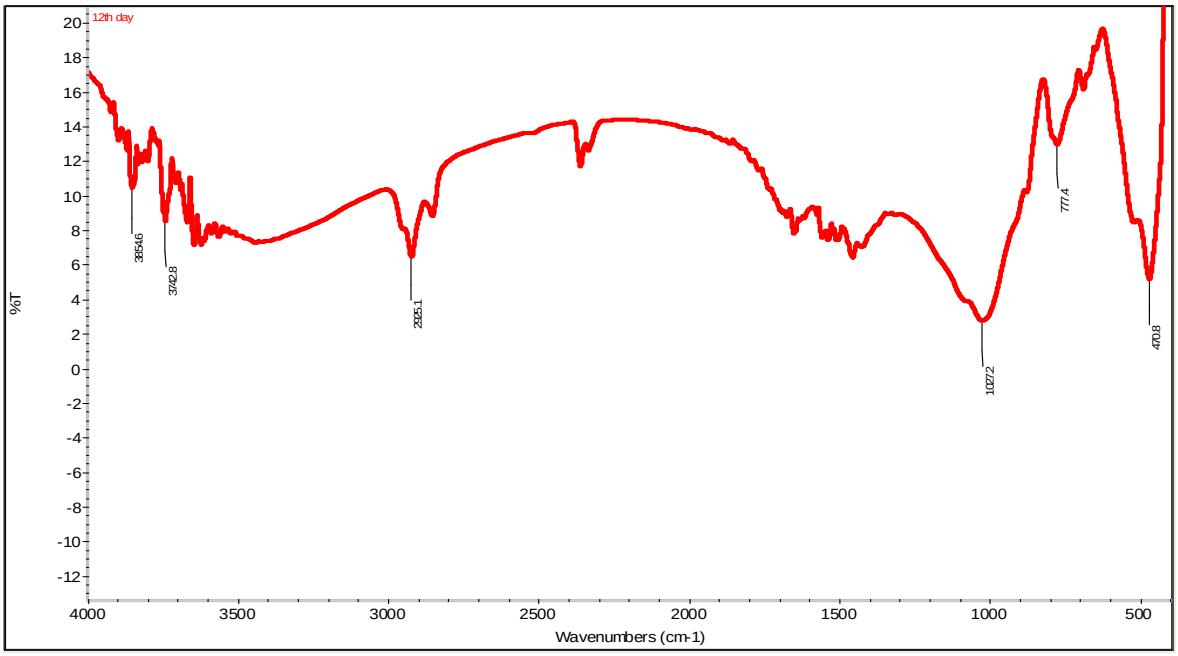

Fig. 2: FTIR spectrum of used motor oil contaminated soil on 12th day of microbial incubation.

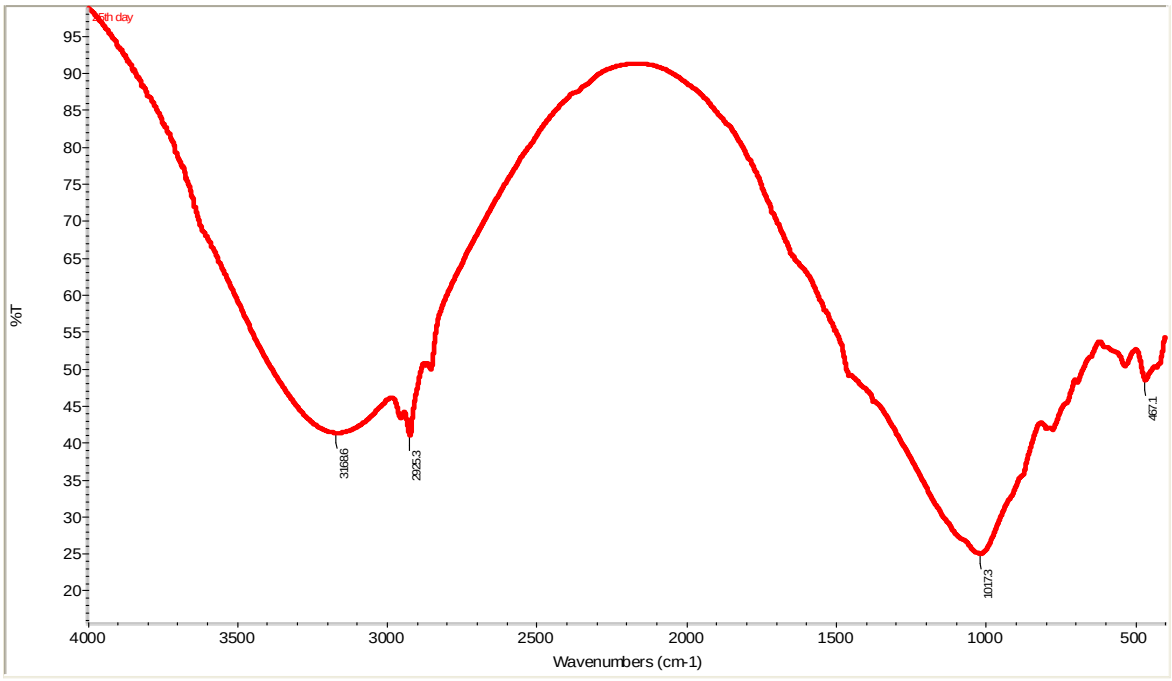

Fig. 3: FTIR spectrum of used motor oil contaminated soil on 25th day of microbial incubation.

Another important finding was an increase in transmittance at 2361.5 cm^{-1} which was merely 4.25 % at zero day but was found to be 11.5 % at the 12^{th} day of incubation. However, the transmittance peak at 2361.5 cm^{-1} completely disappeared on the final day of experiment (25^{th} day). Since the wave number corresponds to the possible nitrile stretch in the sample a possible degradation of the same can not be ruled out. The total viable counts of bacteria in the used motor oil contaminated soil increased with the increase in incubation period from the day of inoculation (1.5± 0.15 x 10^5 CFU / ml) to the end of experimentation (10^9 CFU/ml). The excellent growth of bacteria in the used motor oil contaminated soil indicated the ability of the bacterial isolate to utilize petroleum hydrocarbons as an organic nutrient source and its potential in bioremediation perspective.

CONCLUSION

The FTIR spectra recorded at different time intervals validates positive indication of hydrocarbon degradation in soil by the isolated bacterial strain. The results suggest that the isolated bacterial strain has affinity for C-H aliphatic and aromatic stretches and hence degrades long chain alkanes present in used motor oil. On the basis of the results obtained, it is safe to record that the soil fertility which has been lost due to oil spills has been restorated back by the use of the isolated bacterial strain as the FTIR spectra indicates. It is therefore concluded that by using the isolated bacterial strain, the soil which has lost its fertility because of the oil may be remediated and be turned back as fertile soil. Moreover, FTIR spectroscopy can be a very useful tool in performing preliminary tests in order to predict remediation performance so as to select an appropriate approach for clean- up technologies. Our next step shall be to design experiments

to establish optimum conditions to characterize the remediation behavior at the most overlooked sensitive sites of hydrocarbon pollutants such as motor service stations.

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