

Bioremediation of Lubricant Oil Pollution in Water by *Bacillus megaterium*

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ABSTRACT: Lubricant oil from Pump motors, automobile engines and engines of boats and ships constitute an important pollutant of water and soil ecosystems, leading to devastating damage to the aquatic ecosystem at times of accidents. There are wide varieties of microorganisms in water and soil known to utilize petroleum hydrocarbons as an energy source and degrade them. Hydrocarbon utilizers (HCUs) found to be useful in the remediation of oil pollution in soil and water, includes certain species of *Pseudomonas*, *Rhodococcus*, *Bacillus*. This work is primarily based on degradation study of engine oil and evaluation of the effectiveness of indigenous microorganisms in the process. Microbes isolated from polluted sites have been screened for their ability to grow under in vitro culture, where used engine oil was the sole carbon source. Evaluation of residual oil content and percentage degradation of oil in culture medium was measured at regular intervals of 7 days for a period of 28 days. Residual oil was estimated by the toluene cold extraction method, percentage degradation of hydrocarbons was evaluated using Gas Chromatography (GC). And heavy metals in the sample before and after remediation were also analyzed by Energy diffraction spectroscopy (EDS) and characterised using Scanning Electron microscope (SEM). Results of the study have indicated that microbes are potential agents studied under in vitro conditions for restoring oil contamination and thereby biodegrading the harmful hydrocarbon pollution

KEYWORDS: Hydrocarbon utilizing Bacteria, biodegradation, bioremediation, Hydrocarbons.

I. INTRODUCTION

Oil derived from crude oil or mixture containing synthetic oil including engine oil, gear oil, hydraulic oil, spent oil, tank bottom sludge and slop oil generated from petroleum refineries are said to be source of spent oil which is a major environmental problem in most developing countries like India. There are 36,165 Hazardous waste recycling industries, generating 62, 32,507 metric tons of HW every year. Mechanical methods such as electrolysis, re-refining are too expensive labour intensive and no technological upgradation P K Selvi et al [1]. When the used oil is accidentally or deliberately released into the environment can cause serious problems to both biotic and a biotic ecosystem such as carcinogenicity and mutagenicity was stated by Anthony [2]. In [3] authors said release of untreated or spent oil into estuaries, lakes and ponds causes immediate and obvious problems to animals and plants. There by causing long term effects on ecosystem which related to prolong period depending on the concentration of toxicants in their top of food chain.

In most oil contaminated sites some highly toxic derivatives like benzene, xylene, alkyl benzenes, 1, 3-butadiene are present. And these components are well known carcinogens for both animals and humans [4]. Illegal dumping of spent oil into environment is a global ramification, prolong exposure of high level polycyclic aromatic hydrocarbons can develop liver or kidney disease and high level risk of cancer [5].

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II. RELATED WORK

In 1946 Claude U. Sable as recognised that the microorganisms has the potential to utilize the hydrocarbon as the sole source of carbon and energy, and found these organisms are widely distributed in nature [2]. The cheap, effective and safe method for reducing hydrocarbon pollution in environment could perfectly do by microbial degradation [6]. The widespread application of using microorganisms to remediate polluted sites in natural environment is called bioremediation. A wide range of Hydrocarbon utilizers (HCUs) found to be useful in the soil include the following species, *Flavobacter*, *Mycobacterium*, *Streptococcus*, *Acinetobacter*, *Providencia*, *Pseudomonas*, *Carynebacterium*, *Bacillus*, *Rhodococcus*. Other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent, but they take longer periods of time to grow when compared to their bacterial counterparts [7].

The objectives of this study are to isolate petroleum hydrocarbon utilizing bacteria and heavy metal degrading which grow on used engine oil as sole source of carbon and energy. The study also focused on efficiency of the isolated organisms in degrading oil and mainly in break down or degrading long chain hydrocarbons.

III. MATERIALS AND METHODS

- A. *Source of Engine Oil*: Contaminated oil and soil samples were collected from mechanical workshop. Samples were analyzed for microbiological and some physical- chemical parameters [8] such as temperature, pH and moisture content. The used engine oil collected from the mechanical workshop branded as 15W40 was used as fresh engine oil sample.
- B. *Isolation of petroleum Hydrocarbon degrading microorganism*: The collected samples are enriched in Nutrient broth for 7 days. Sample was serially diluted and spread plated in nutrient agar plate for bacterial culture and Rose Bengal chloronphenicol agar for fungal culture. Bacterial culture was incubated at 37°C for 24hours, while fungal cultures are incubated at room temperature for 7 days [9]. After incubation pure strains are isolated by quadrant streak plating method based on their ability to degrade used oil. The strains are identified by Gram's staining technique and Biochemical test based on Bergey's manual.
- C. *Efficiency of bacteria in consuming used oil*: [1] In order to test the efficiency of bacterial degradation the experiment as follows, isolated strains are sub cultured in 10 ml of nutrient broth and shakes at 200RPM, in 37°C for 24 hours. The pre-cultured samples are transferred for 100ml conical flask containing 5 ml of used oil and 50 ml of distilled water and then incubated room temperature (25 ±2°C). The experiment is conducted in duplicates at regular intervals of 7 days for the period of 28 days. The oil is extracted by gravity separation method. The sample is centrifuged at 10000 RPM for 20 minutes and kept at vertical position overnight, in order to measure the efficiency of microbes in reducing the oil volume. Then the sample are analysed in GC for quantifying the percentage of degradation of hydrocarbons present in the oil sample compared with the control used oil sample.
- D. *Growth medium and culture condition*: Minimal salt medium (MSM) and oil dispensed distilled water medium was used as growth medium for the organisms containing used engine oil has the sole carbon source for their growth and proliferation. The pH of the medium was adjusted to 7.2 for bacterial growth. The medium was autoclaved for 121°C at 15 psi before inoculation. Experiment was conducted in both bacterial optimised incubator temperature (37°C) and Room temperature (25 ±2°C). Bacterial growth was monitored by withdrawing a sample from culture medium every 7 days interval. The sample is analysed in UV visible spectrophotometer at 600 nm.
- E. *Toluene Cold Extraction method*: Residual oil was extracted by gravity separation method. Then the residual oil was extracted by liquid- liquid extraction by adding 2 ml of Toluene to the broth culture in flask and shake for 30 mints. After removing the aqueous phase with separating funnel, the residual oil concentration was determined by Gas Chromatography [10]. Similarly, other sample and control flasks were also extracted.

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F. Analytical measurement: GC analysis was done by HP GC equipped with flame ionization detector (FID) with capillary column MS 100% dimethyl polysiloxane. Helium was used as gas carrier and speed was maintained at 155ml/min. 1 µl of sample was injected into the injection port at 280°C; oven column temperature at 50°C. Pressure at 90.7 mmHg, column temperature maintained at 50°C for 5 min, the increased to 260°C for 15 min. The samples were analysed in split less mode ranging from 30 to 500 molecular mass for 5 cycles. The consecutive peaks for the presences of hydrocarbon can be found compared with the control and the samples.

G. Identification of heavy metals: Heavy metals in the samples are identified by plating the sample in a silica plate and analysed using Electron dispersion spectroscopy (EDS) and been characterised using Scanning electron microscope (SEM)

IV. EXPERIMENTAL RESULTS

A. Isolation and screening of petroleum hydrocarbon utilizing bacteria

Bacteria were isolated based on the ability to grow on used oil as single carbon source. 2 pure strains of hydrocarbon utilizing bacteria are been isolated from the enriched contaminated oil and soil samples. Microscopic observation showed Gram negative rods. Biochemical analysis based on Bergey’s manual of determinant microbiology as showed similarity to bacillus sp. Micrococcus, and pseudomonas sp. The source and corresponding representations are tabulated below

Table 1: Information about the Isolates from different polluted sites

S. No	Source of soil sample	Representative isolates
1	Railway workshop	A1, A2, A3, A4, A5
2	Bike workshop	B1, B2, B3
3	Car workshop	C1, C2, C3, C4

B. Growth Analysis of isolates in MSM and Oil dispensed distilled water medium at room temperature

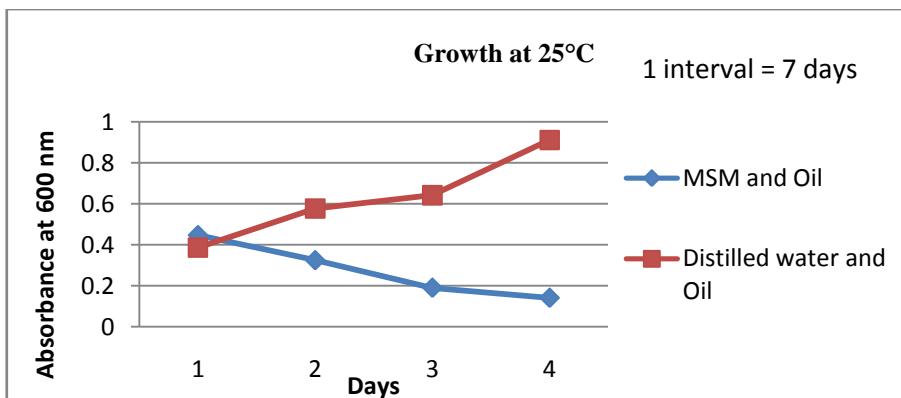


Fig : Growth of A2 at 25°C for 7 days interval for period of 28 days

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C. Growth Analysis of isolates in MSM and Oil dispensed distilled water medium at Incubated temperature

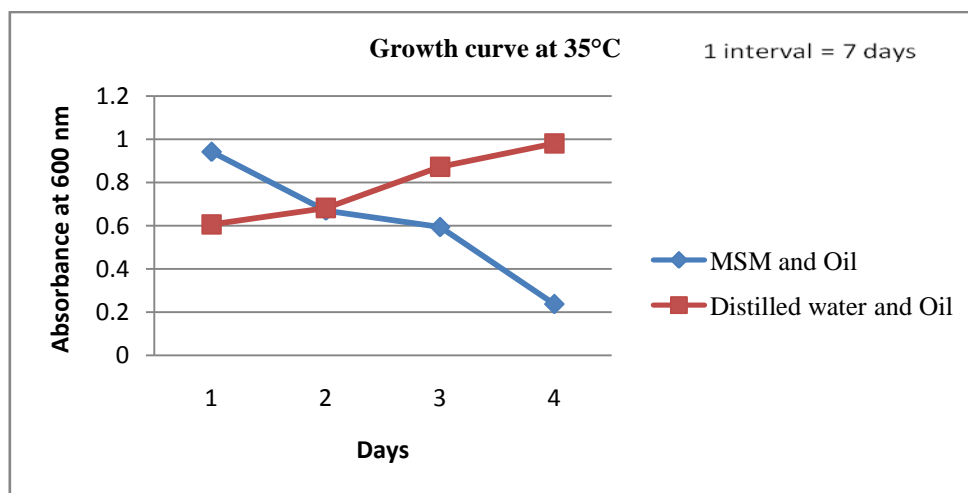


Fig 2: Growth of A2 at 35°C for 7 days interval for period of 28 days

D. Biochemical Identification:

Biochemical analysis of the isolates which showed maximum growth in oil dispensed medium was done and the results are tabulated in table2.

Table 2: Biochemical and physiological identification of isolated strains

Characteristic	A1	A2
Gram Stain	-	-
Morphology	Rods	Rods
Indol	+	-
Citrate	+	+
Methyl red	-	+
Vp	+	-
Fermentation	-	-
Catalyse	-	-
6.5% NaCl	+	-
pigmentation	White & smooth	White powdery
Relative identification of Species	<i>Bacillus Subtilis</i>	<i>Bacillus Megaterium</i>

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E. Efficacy of Microbes in utilizing used oil

The volume reduction was analysed at 4 intervals of 7 days each and percentage reduction was analysed by the above mentioned formula.

Table 3: Volume reduction

Sample Name	Initial volume	Final volume				Percentage reduction
		7 th day	14 th day	21 st day	28 th day	
A1	5	4.5	4.2	3.9	3.6	28%
A2	5	4.1	3.4	3.3	3.0	40%

F. Gas Chromatography Analysis

Percentage reduction of hydrocarbon content in the sample was compared with the control used oil sample and the sample after bioremediation.

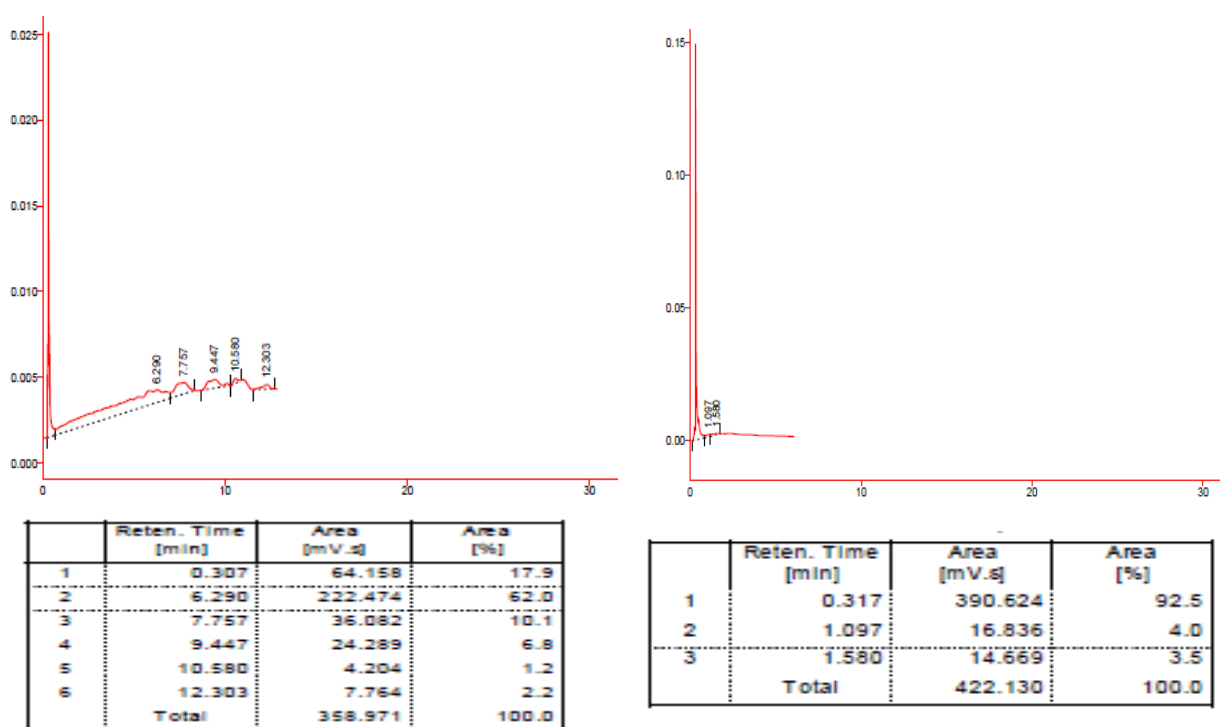


Fig 3: GC FID spectra for control used oil (before degradation) and A2 after 28 days fermentation

G. Heavy metal analysis

Presence of trace amount of heavy metals are been detected by EDS showing the peaks of lead (Pb) present in the sample and images of SEM showing the metallic clumps in the control sample and whereas heavy metals are been dispersed in the A2 sample after 28 days fermentation without any degradation.

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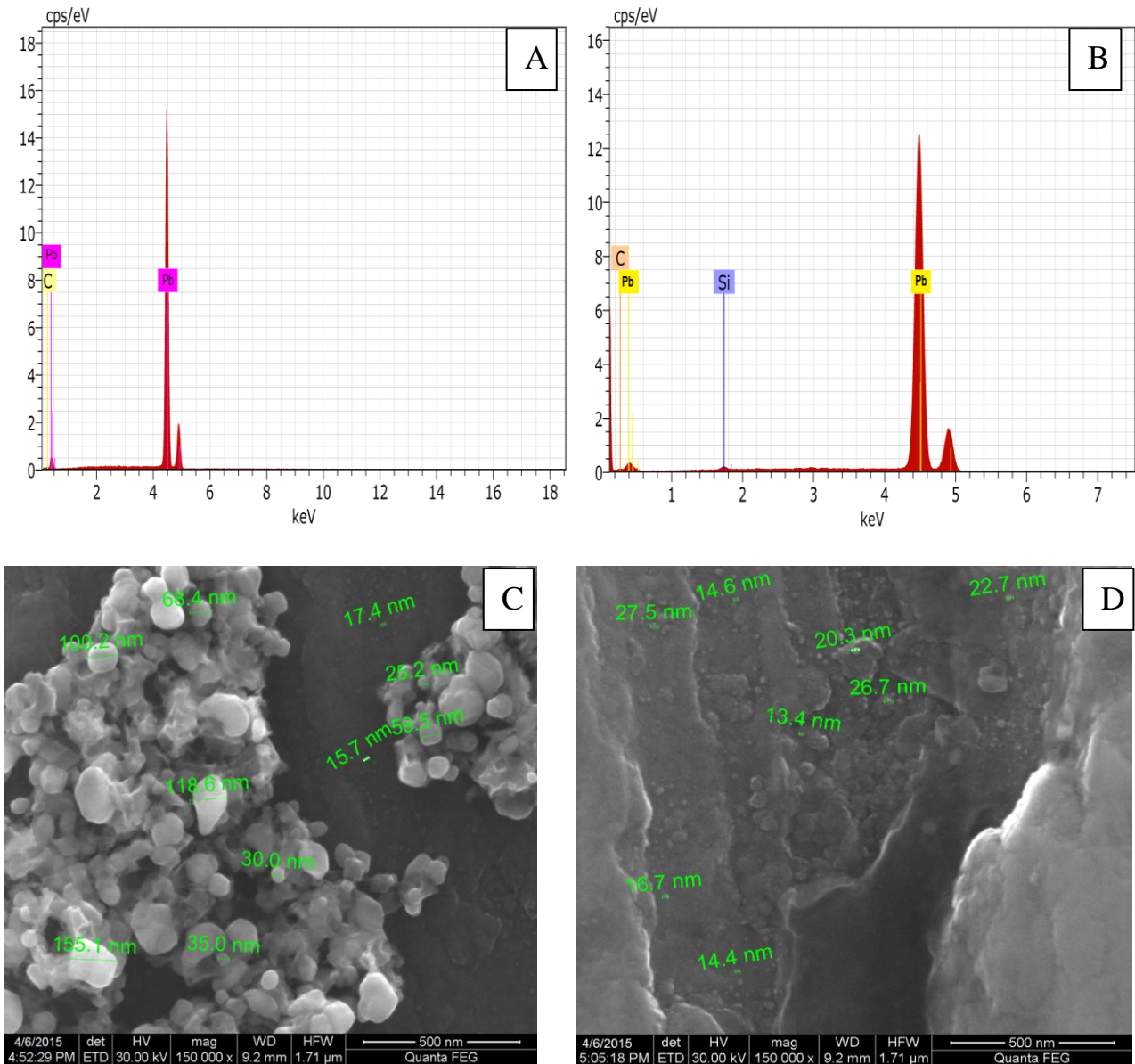


Fig 4: [a & b] chromatogram of heavy metals analysed in EDS and SEM images [c] of Control used oil containing clumps of heavy metals, Heavy metals are dispersed after fermentation of 28 days by A2 [d].

V. DISCUSSION

Environmental pollution is a major problem in the modern world and developing countries like India. Petrochemical waste is a major pollutant in ponds, lakes, rivers, etc and is more resistant to normal water treatments, hence contaminating water and soil. Over the past few decades, there has been tremendous work has been done on emphasizing bioremediation using a variety of microorganisms alone or mixed [13].

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The Initiation of this study is to isolate and identify efficient microorganism involved in remediating petrochemical waste or pollution and also determining the best condition for degradation process. Organisms obtained in this study were isolated from the contaminated sites near mechanical workshops. Various strains of bacteria's including *Bacillus Sp.*, *pseudomonas Sp.*, *Rhodo Sp.* and *E. Coli Sp.* has been isolated and selected for biodegradation under in-vitro conditions. Optimization of Physio-chemical parameters for the degradation of used LO in stimulated conditions for each organism has been studied. Comparative evaluation of the time course and extent of biodegradation of used oil in 2 different mediums was conducted with the selected organisms. In which *Bacillus Sp.* A2 showed maximum reduction in oil volume about 40% reduction within the period of 28 days under stimulated conditions. And the pattern of degradation was observed by GC analysis for *Bacillus Sp.* showing 92.5% degradation of hydrocarbon content in used oil after fermentation for 28 days. The results was comparable with [11] where the same *Bacillus Sp.* showed 83.7% degradation in minimal media, whereas in the present work oil dispensed in distilled water containing used oil showed maximum result of about 92.5% degradation of hydrocarbons.

Energy dispersion Spectroscopy (EDS) and Scanning Electron Microscope (SEM) analysis showed trace elements lead (Pb) in control used oil. But there was no degradation of heavy metals happened during this fermentation process which was seen in the EDS chromatogram. Only metallic clumps have been dispersed over oil content, which was seen in the SEM images.

VI. FUTURE SCOPE

The isolated strains that are screened for the degradation of hydrocarbons and heavy metals can be enhanced by growing in various crude substrates and thereby increasing the efficiency in cleaning up the environmental pollution

VII. CONCLUSION

The present study indicates that microorganisms are the potential agents for remediating oil polluted sites, particularly in ponds, lakes etc. The microorganisms can able to bio remediate polluted sites without any effects on the environment and more cost efficient also.

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