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Advances in Applied Science Research, 2011, 2 (3):455-460

Pelagia Research Library

ISSN: 0976-8610
CODEN (USA): AASRFC

Isolation and characterization of micro-organism from oil contaminated sites

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ABSTRACT

A study was conducted in order to decipher the microorganisms from oil contaminated sites for oil degradation abilities. Four used oil degrading Bacterial samples were isolated from oil contaminated sites in Lucknow. One isolate MJH1101 showed maximum oil degradation abilities, only 1 ml oil was recovered out of 25 ml after seven days incubation period and there was a decrease in width of oil layer from 6mm to 1mm after seven days incubation. The isolate was characterized for staining and biochemical activities based on Bergey's Manual.

Key words: Carcinogenicity, *Bacillus*, Degradation, Bioremediation.

INTRODUCTION

Oil spills have been a major issue across decades. One of the famous oil spill which is also ongoing is in Taylor Energy Well in Gulf of mexico ,U.S.A caused due to Hurricane (sept 16, 2004 till present date) and almost 0.03- 0.05 tonnes oil/perday is estimated to leak. Another recent oil spill is in Mumbai (India) and caused due to the leakage in Mumbai-Uran pipeline dated january 21 2011 and about 55 tonnes of oil was leaked in Arabian sea.

Various such accident occurs throughout the years and it causes damage to our surrounding ecosystem.

Used engine oil can be considered as one of the source responsible for polluting the soil with hydrocarbons. Used engine oil consists of Petroleum ether or Benzine ,Gasoline, Naptha, Mineral spirits, Kerosene, Fuel oil, Lubricating oil, Paraffin wax, Asphalt or Tar. Used motor oil typically has much higher concentrations of PAHs (polycylic aromatic hydrocarbons) than new

motor oil . Chronic effects of naphthalene, a constituent in usedmotor oil, include changes in the liver and harmfuleffects on the kidneys, heart, lungs, and nervous system. Due to their relative persistence and potential for various chronic effects (like carcinogenicity), PAHs (and particularly the alkyl PAHs) can contribute to longterm (chronic) hazards of jet fuels in contaminated soils, sediments, and groundwaters [Irwin, *et al.*,1997].

One of the most significant impact associated with workshop seepage of used engine oil includes lose of soil fertility, water holding capacity, permeabilty and binding capacity. [Moorthi, *et al.*, 2008]

Its a very costly approach to treat oil contaminated site by conventional methods such as use of chemicals or peat moss (a plant which absorbs hydrocarbons). These conventional methods can be replaced by modern methods such as micro-organism or engineered micro-organism which can detoxify the contaminants in to lesser toxic compounds.

Bioremediation method is considered to be more economical and safe method for the treatment of oil contaminated site. It has been observed that micro-organism that grows on oil contaminated soil are much capable of of degrading oil than those microrganisms which are found on non-contaminated site of oil.this can be a very good example of Adaptation. The natural process of biodegradation can be speed up if we add some nutrient to it which help in the growth of microrganisms or we can isolate the mo's from contamination site inoculate in nutrient broth and mixed it in contaminated region.

Looking at the previous studies on bioremediation [Moorthi, *et al.*, 2008]; [Emtiazi, *et al.*,2005]; [Bragg, *et al*.,1994]; [Singh and Lin 2008]; [Udeani, *et al*., 2009]; [Barathi and Vasudevan., 2001];[Head and Swannell., 1999]; [Ortega *et al.*, 2003]., the present study is also based on the biodegradation of used engine oil in order to decipher the cultures found in oil contaminated soil for there oil degradation abilities.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from two different oil contaminated sites: (A) Popular Service Garage,city station, Lucknow, (B) Rajesh Garage, Jama masjid, Lucknow. Soil was collected randomly 5-10 cm beneath the surface using spatula and were packed in sterile polybags and transferred to the laboratories. [Okoh, 2003;Ojo, 2006].

Used Engine Oil: Collected from Rajesh Garage, Jama Masjid, Lucknow. pH= 11, Colour: Blackish Brown.

Isolation of bacteria from soil sample:

Bacterial species were isolated from the collected soil samples by serial dilution and agar plating method wherein the soil sample was diluted from 10^{-1} to 10^{-5} dilutions, and the diluted soil samples were spread on sterile Nutrient agar paltes. The inoculated plates were incubated at 37^{o}C for 24 hours.

Mixed cultures obtained after incubation were named as MJH1101, MJH1102, MJH1103, MJH1104 tentatively and were purified by quadrant streaking on sterile NA plates. The purity of cultures was cross checked by gram staining procedure.

Staining and biochemical activities of purified cultures:

In order to identify the purified cultures tentatively on the basis of Bergey's manual [Aneja, K.R., 2003] various staining and biochemical tests were performed namely Gram staining, Endospore staining, Catalase test, Mannitol fermentation, Glucose fermentation, fructose fermentation, and Lactose fermentation.

Screening of purified cultures for degradation of used oil:

Oil degradation studies of purified cultures were performed against used oil obtained from two sites in lucknow, wherein the components for preparing 100ml Nutrient broth were dissolved in 75ml distilled water and 25ml of used engine oil was added, pH was maintained to 7. Media with oil was autoclaved at 15 psi for 20 minutes. Cooled media was innoculated with 1ml of 24 hour old grown culture of the respective pure cultures. The innoculated flasks were incubated at 120rpm in a shaking incubator at 37 ^{o}C for 7 days. Width of oil and media layer in the flask was recorded on zero day and 7 th day. And also the oil degradation was quantified by studying the oil recovery after 7th day of incubation. In this way the oil degradation study was carried out for all the purified cultures.

RESULTS

Isolation of bacteria from soil sample:

Bacterial species were isoalted from soil samples and mixed cultures were obtained, **figure 1** below shows the plates showing mixed culture.

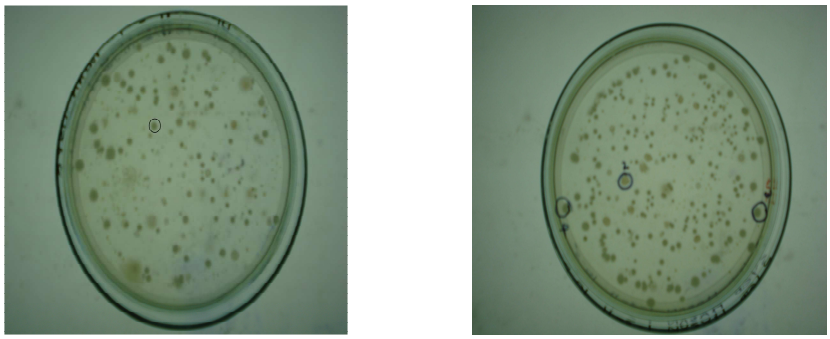

Figure 1: Mixed Culture

Figure 2 shows the pure cultures of the four cultures MJH1101, MJH1102, MJH1103, MJH1104, purified by quadrant streaking.

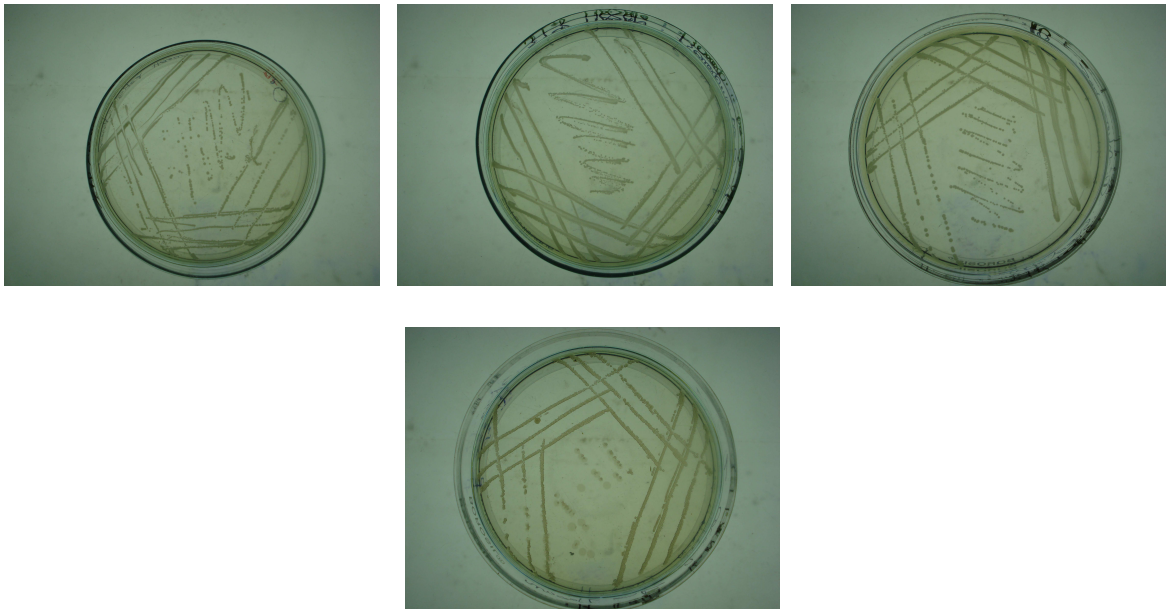

Figure 2: Pure Cultures

Staining and other biochemical tests of the obtained pure culture:

Table 1 below shows the results of variuos staining and biochemical activities of all the four cultures purified for oil degradation studies.

Table 1: Staining and Biochemical activities of purified cultures.

TEST	MJH1101	MJH1102	MJH1103	MJH1104
GRAM STAINING	+ ve, Bacillus	+ve, Coccus	+ve, Coccus	+ve, Bacillus
ENDOSPORE STAINING	+ ve	+ ve	+ ve	+ ve
CATALASE TEST	+ ve	+ ve	+ ve	+ ve
MANNITOL FERMENTATION	-ve	-ve	-ve	-ve
GLUCOSE FERMENTAION TEST	+ ve	-ve	+ ve	+ ve
SUCROSE FERMENTAION	-ve	-ve	+ ve	+ ve
LACTOSE FERMENTATION TEST	+ ve	-ve	+ ve	+ ve

Screening of purified cultures for degradation of used oil:

Table 2 & 3 below show the quantification of oil degardation by two methods used in this study. From the Table 2 it can be depicted that the isolate MJH1101 was showing maximum oil degradation in both the parameters.

Table 2: Oil degardation studies (width).

CULTURE	WIDTH OF OIL ON ZERO DAY(mm)	WIDTH OF OIL ON 7TH DAY(mm)
MJH1101	6	1.0
MJH1102	6	3
MJH1103	6	2.5
MJH1104	6	3.5

Table 3: Oil ddegradation studies (oil recovery)

CULTURE	VOLUME OF OIL ON ZERO DAY(ml)	VOLUME OF OIL ON 7th DAY (RECOVERY) (ml)
MJH1101	**25**	**1.5**
MJH1102	**25**	**7.5**
MJH1103	**25**	**2.5**
MJH1104	**25**	**9.0**

DISCUSSION

Soil sample was collected from two oil contaminated sites as done earlier by [Ojo, 2006; Okoh, 2003]; [Emtiazi, *et al.,* 2005]. Further microorganism was isolated by serial dilution method and agar plating method as done previously by [Udeani *et al*., 2009]. In motor mechanic shop there is constant change in microorganism of oil contaminated soil as the colour and texture changes. Cultures were purified by streaking techniques and the purity was cross checked by Grams staining procedure.

Purified cultures were characterized for the various staining and biochemical activities and was compared with bergey's manual as done earlier by [Udeani *et al*., 2009]. The isolate showing maximum oil degradation abilities was gram positive, *Bacillus spp.* and catalase positive. Few studies [Annweiller *et al*., 2000]; [Ijah and Antai, 2003]; [Sorkhoh *et al*., 1993]; [Korda *et al.*, 1997]; [Rahman *et al.*, 2002;Sepahi *et al.*, 2008] have been reported on the roles of *Bacillus spp.* in hydrocarbon bioremediation; although there are several reports on bioremediation of pollutants by the action of *Bacillus spp*. occurring in extreme environments. [Ijah and Antai, 2003] reported *Bacillus spp*. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples (30 and 40% crude oil).

It has been postulated that *Bacillus spp*. are more tolerant to high levels of hydrocarbons in soil due to their resistant endospores. There is growing evidence that isolates belonging to the *Bacillus spp.* could be effective in clearing oil spills [Ghazali *et al*., 2004].

Prelimimary screening of purified culure was also done by recovering oil from the flask and estimating the amount of oil left after degredation. This is one of the few reports on this method of quantifying oil degradation abilities.

Acknowledgement:
We are thankful to the Management of MRD LifeSciences (P) Limited, Lucknow, Mr. R. P Mishra, MRDLS, Lucknow, Mr. Amit Pandey, MRDLS, Lucknow, for there kind support throughout the research work, we are also thankful to the Almighty without whose consent nothing is possible.

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