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A Study on oil degradation potential of bacteria isolated from two different oil contaminated sites in Allahabad

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Abstract
The most common problems today are caused due to petroleum products, their use and their degradation. If exposed to the environment they can be hazardous to the surroundings as well as life forms. Thus remediation of these is of utmost importance. Bioremediation is a non-invasive and cost effective technique for the clean-up of these petroleum hydrocarbons. The present study was investigated to evaluate the ability of microorganisms present in the soil itself to degrade these hydrocarbons so that contaminated soils can be treated using microbial consortium. Soil from areas near garages is used to isolate the microorganism which is further used to create an inoculum, whose ability to degrade heavy as well as light crude oil is assessed quantitatively. The bacterial species C3 and D2 showing maximum degradation of petroleum (37.55% and 45%) were identified based on Bergey's manual and was tentatively identified as *Bacillus subtilis* and *Bacillus megaterium* for the samples from Civil lines and Dandi respectively.

Keywords: MSM media, Bioremediation, biodegradation, oil spills *Bacillus subtilis*, *Bacillus megaterium*

Introduction
Environmental Pollution with petroleum and petroleum products like (engine oil, petrol, diesel etc), has been recognized as one of the most serious problems (Udeani *et al*., 2009) [6]. Engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents (Butler and Mason, 1997) [1]. That is used to lubricate the parts of an automobiles engine, in order to keep everything running smoothly (Hagwell *et al*., 1992) [2]. Contamination of soil and water bodies with engine oil is a serious ecological problem, primarily in the mechanic workshops, garages. Oil production activities releases a large amount of hydrocarbon in terrestrial & aquatic environment. The level of soil pollution by petroleum products and oil sludge has reached millions of cubic meters (Zukauskaite and Viktorija, 2008; Sarma and Sarma, 2010) [7, 5]. Prolonged exposure and high oil concentration may cause the development of liver or kidney disesase, possible damage to bone marrow and an increased risk of cancer (Mishra *et al*., 2001; Mandri and Lin, 2007) [4, 3]. The present study was designed to study the oil degradation potential of microbes inhabiting oil contaminated sites for oil degradation potential.

Materials and Methods
Collection of soil and oil sample
The soil samples were collected from two garages situated in Dandi (near Naini) and Civil lines area of Allahabad respectively. The soil sample was dug from six inches below the soil surface and stored in the sterilized polythene. The soil texture in comparatively black than normal soil, which shows the spill of the oil in garage during vehicle repairs. The unused oil samples were also collected from the same garages in sterilized bottle.

Isolation and enumeration of bacteria
Oil degrading bacteria were isolated by serial dilution agar plating method or viable plate count method by diluting the soil up to 10^{-5} dilutions. The mixed colonies obtained were differentiated on the basis of colony morphology and were purified by quadrant streaking. Purity of the cultures was cross checked by Gram's staining.

Screening of purified cultures for oil degradation
Oil degradation studies of the purified cultures isolated from oil contaminated sites were carried out in minim al salt media supplemented with 5% used engine oil, incubating the same

at 37° C for seven days in shaking incubator at 120 rpm and tracking the growth of culture in the flask throughout seven days incubation. Also the oil degradation was quantified by recovering the left out oil after seven days by dispensing the culture broth from both the control and test flask in centrifuge tube and spinning the same at 10,000rpm for 10 minutes, recovering and weighing the left out engine oil.
The obtained values were put in the formula given below to obtain the percentage oil degradation. A control flask was also maintained along with the test flask having no inoculation, and percentage oil degradation was calculated.

$$\% \text{ oil degradation} = \frac{\text{weight of oil on 0 day-weight of oil on 7 day}}{\text{Weight of oil on 0 day}}$$

Identification of the culture showing maximum oil degradation potential
The cultures showing maximum oil degradation potential was identified based on the key of Bergey's manual by performing various staining and biochemical tests.

Results and Discussion
Isolation of bacteria from soil sample
Bacterial species were isolated from soil samples and mixed cultures were named C1, C2, C3, C4 for sample from civil lines and D1, D2, D3, D4 for Naini, Allahabad sample and they were further purified by quadrant streaking.

Evaluation of Oil Degradation Potential of Isolated Cultures
The ability of isolated cultures to utilize oil as a source of carbon and energy was confirmed by studying their growth profile. Figure 1shows the growth profile of all the four cultures. It can be seen from the results that the culture C3 and D2 shows maximum growth throughout the incubation period as indicated by OD at 600nm and also the oil degradation potential of the purified cultures was studied based on their ability to grow in MSM supplemented with 5% oil for a period of seven days giving an indication of the capacity of culture to utilize oil as a source of carbon and energy.

Oil degradation % of purified cultures from civil lines sample
Oil degradation was also quantified by calculating the percentage oil degradation by using the formula described in materials and methods. The percentage oil degradation by the samples after 7 days incubation were shown in Figure 1 and Table1.

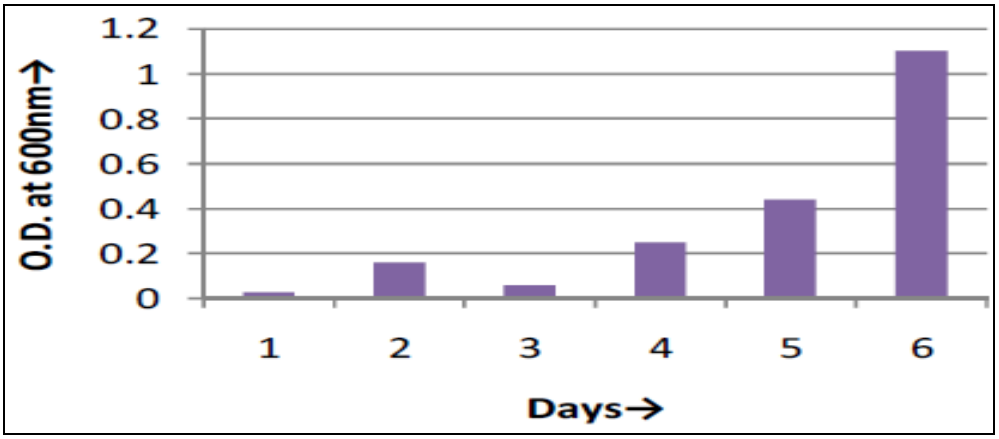

Fig 1: Graph based on OD reading for C3 sample

Table 1: Oil degradation % of purified cultures from civil lines sample

S. No.	Culture No.	Weight of oil on 0 day	Weight of oil on 7th day	% of oil degradation
1.	Control flask (without inoculation)	20	20	0%
2.	C1	20	18.5	7.5%
3.	C2	20	15.70	21.5%
4.	C3	20	12.49	37.55%
5.	C4	20	14.30	28.5%

The results of the enumeration of the cultures carried out in MSM supplemented with 5% oil.

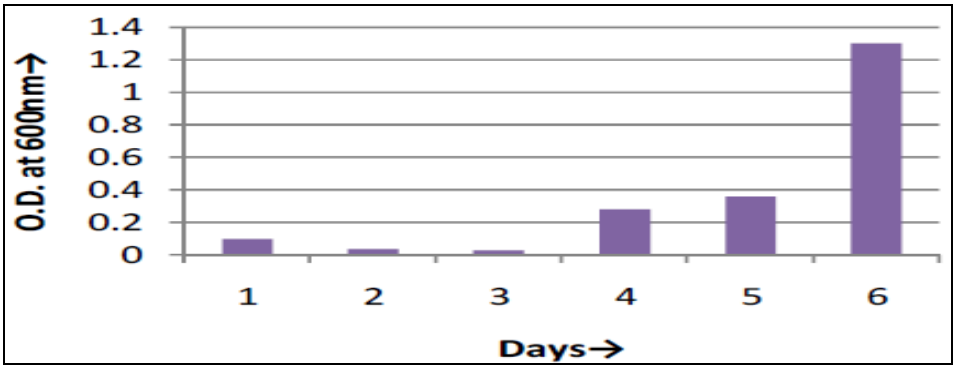

Fig 2: Graph based on OD reading for D2 sample

Oil degradation was also quantified by calculating the percentage oil degradation by using the formula described in materials and methods. The percentage oil degradation by the samples after 7 days incubation as shown in Figure 2 and Table 2.

Table 2: Oil degradation % of purified cultures from Naini sample

S. No.	Culture No.	Weight of oil on 0 day	Weight of oil on 7th day	% of oil degradation
1.	Control flask (without inoculation)	20	20	0%
2.	D1	20	17.5	12.5%
3.	D2	20	11.00	45%
4.	D3	20	14.49	27.55%
5.	D4	20	16.30	18.5%

Identification of the culture showing maximum oil degradation (C3, D2) during screening

Table 3 shows the results of Gram staining, catalase test, endospore staining and Voges Proskeurs test of the culture C3 and D2 showing the maximum oil degradation potential. Based on Bergey's manual the isolated strain was identified as ***Bacillus magaterium shown*** as in Table 3.

Table 3: Staining and Biochemical activities of samples C_3 and D_2

Sample C3		Sample D2	
Gram staining	Positive	Gram staining	Positive
Cellular morphology	*Bacillus*	Cellular morphology	Bacillus
Endospore staining	Positive	Cellular arrangement	*Streptobacillus*
Catalase test	Positive	Endospore staining	Positive
VP test	Negative	Catalase test	Positive
		VP test	Positive

References

1. Butler CS, Mason. Structure-function analysis of the bacterial aromatic ring-hydroxylating dioxygenases. Advanced microbial physiology, 1997; 38:47-84.
2. Hagwell IS, Delfino LM, Rao JJ. Partitioning of polycyclic aromatic hydrocarbons from oil into water. Environ. Sci. Technol. 1992; 26:2104-10.
3. Mandri T, Lin J. Isolation and Characterization of engine oil degrading indigenous microorganism in Kwazulu-natal, south africa. African J. of Biotechnol. 2007; 12:67-71.
4. Mishra S, Jyot J, Lal B. Evaluation of inoculum addition to stimulate in situ bioremediation of oily sludge contaminated soil. Appl. Environ.microbiol. 2001; 16:75-81.
5. Sarma A, Sarma H. Enhanced biodegradation of oil products by some microbial isolate supplemented with heavy metals. 2010; 7:116-120.
6. Udeani TKC, Obroh AA, Azubike N. Isolation of bacteria from mechanic workshops soil environment contaminated with used engine oil. African J. Biotechnol; 2009; 8(22):6301-03.
7. Zukauskaite A, Viktorija J. Impact of heavy metals on the oil products biodegradation process. Waste Manage. Res, 2008; 26: 500-07.