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IN VITRO EVALUATION OF OIL DEGRADATION POTENTIAL OF *Bacillus subtilis*

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ABSTRACT: Continuous release of oil in the area of mechanic workshop, not only causes soil pollution but also degrade fertility and water holding capacity of soil. In this study, four Bacterial cultures were isolated from oil contaminated sites in Lucknow. Percentage oil degradation by the isolates namely MJAN1101, MJAN1102, MJAN1103 & MJAN1104 was determined and was found out to be 63.375%, 54.79%, 40.35% and 34.49% respectively. Further the isolate MJAN1101 showing maximum oil degradation was identified based on Bergey's manual and was tentatively identified as *Bacillus subtilis*.

Key Words: MSM media, Bioremediation, Oil degradation, Oil spills, *Bacillus subtilis*.

INTRODUCTION

Used motor oil is the largest single source of oil pollution in lakes, streams, and rivers. Oil doesn't dissolve in water. Oil and petroleum products are toxic to people, wildlife, and plants. One quart of motor oil can pollute 250,000 gallons of water, and one gallon of gasoline can pollute 750,000 gallons of water. Oil that leaks from our cars onto roads and driveways is washed into storm drains, and then usually flows directly into a lake or stream. Americans spill 180 million gallons of used oil each year into the nation's waters. This is 16 times the amount spilled by the Exxon Valdez in Alaska.

Petroleum is a complex mixture of many thousands of compounds. Contamination of the soil by oil causes it to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity. Contamination of groundwater is also a potential problem. The other significant impact is on surface water, mostly the nearby streams, which receive a lot of untreated effluent from service stations containing oil and grease as well as non-biodegradable detergents **[1].**

It's 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 **[2].**
Bioremediation is a natural process, useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. **[3].**

Keeping in mind the harmful effects of oil pollution and the previous research work being carried out in this field viz [4-7;1-2] etc. the present study was designed to explore the oil contaminated sites for the inhabitance of oil degrading Bacteria and further exploration of the inhabiting Bacteria for their oil degradation potential.

MATERIALS & METHODS

Sample Collection

Soil samples for study were collected in sterile polybags from a mechanic workshop situated near Ram Manohar Lohia Hospital, Gomti nagar, Lucknow , the soil was collected after digging the earth upto 15cm. Soil was Blackish in colour.

Isolation of Oil Degrading Bacteria

Oil degrading bacteria were isolated by serial dilution agar plating technique by diluting the soil upto 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 Degradation of Used Oil

Oil degradation studies were carried out in MSM (Minimal Salt Media) media supplemented with emulsified used engine oil. 50ml MSM quantity in g^{-1} being (KH_2PO_4 3; Na_2HPO_4 6; NaCl 5; NH_4Cl 2.0; $MgSO_4$ 0.1; Glucose 8.0; pH 7), supplemented with 5% emulsified (gum accacia) used engine oil maintained at pH-7 was prepared and autoclaved at 121°c for 15 minutes. After cooling, the media was inoculated with 500µl of liquid culture of the purified bacterial isolates to be screened for oil degradation potential. Culture was incubated for a period of 7days 37° C in shaker incubator at 120rpm. Growth of the culture was tracked throughout the incubation and oil degradation was quantified by the procedure explained below. A control flask without inoculation was also maintained.

Quantification of Oil Degradation Potential

Quantification of oil degradation was done by -

- **Viable Cell Count on NAM Plates:** In order to quantify the oil degradation potential 10 µl of culture was spread on solidified NAM plates and the growth of bacteria was indicative of the ability of the bacteria to utilize the oil as its carbon and energy source as the MSM was having very less amount of carbon source which might have exhausted after some time.

- **Determination of % Oil Degradation**: Weight of oil added on zero day was measured similarly the weight of oil recovered from the flask after seven days incubation was measured and the values so obtained were put in the formula given below in order to get the percentage oil degradation in each case.

$$\% \text{ Oil Degradation} = \frac{\text{Weight of Oil on Zero Day - Weight of Oil on 7}^{th}\text{ Day}}{\text{Weight of Oil on Zero Day}} \times 100$$

Identification of the Isolate Showing Maximum Oil Degradation

Culture showing maximum oil degradation was identified based on the key of Bergey's manual given in the book of **[9]** by performing various staining (Gram's staining, Endospore staining) and biochemical activities (Catalase Test, Mannitol fermentation Test, Voges Proskeurs Test).

RESULTS

Isolation of Microorganisms from Oil Contaminated Soil Sample

Oil degrading bacteria were isolated by serial dilution agar plating technique by diluting the soil upto 10^{-5} dilutions and four different bacterial species namely MJAN1101, MJAN1102, MJAN1103 & MJAN1104 differing on the basis of colony morphology were chosen for further studies and were purified by quadrant streaking.

Screening of Purified Cultures for Oil Degradation

Oil degradation studies of the purified cultures (MJAN1101, MJAN1102, MJAN1103 and MJAN1104) isolated from oil contaminated sites were carried out in Mineral Salt Media supplemented with emulsified used engine oil and the percentage oil degradation by the cultures was quantified by the methods explained earlier. The Table 1 and 2 below show the results of quantification of oil degradation, Table 1 shows that the culture was growing throughout the incubation period, and Table 2 shows the percentage oil degradation calculated after recovering the oil left after completion of incubation period.

Table 1: Viable Cell Count During Incubation.

S.NO	CULTURE NO.	NO.OF COLONIES ON 4th DAY	NO.OF COLONIES ON 7th DAY
1	MJAN1101	LAWN	LAWN
2	MJAN1102	LAWN	LAWN
3	MJAN1103	LAWN	LAWN
4	MJAN1104	LAWN	LAWN
5	CONTROL FLASK	NO GROWTH	NO GROWTH

Table 2: Percentage Oil Degradation.

S.NO	CULTURE	WT.OF OIL ON ZERO DAY	WT OF OIL ON 7TH DAY	% OF OIL DEGRADATION
1.	MJAN1101	1.980	0.726	63.37%
2.	MJAN1102	1.980	0.895	54.79%
3.	MJAN1103	1.980	1.181	40.35%
4.	MJAN1104	1.980	1.297	34.49%
5.	CONTROL FLASK	1.980	1.980	0%

Identification of the Culture (MJAN1101) Showing Maximum Oil Degradation potential during screening:

Table 3 below shows the results of Gram staining, Catalase test, Endospore staining, Mannitol fermentation test and Voges proskeurs Test of the culture MJAN1101 showing maximum oil degradation potential.

Table 3: Staining and Biochemical Activities of MJAN1101.

S.NO.	TEST	RESULT
1.	GRAM STAINING	POSITIVE
2.	CELLULAR MORPHOLOGY	BACILLUS
3.	CELLULAR ARRANGEMENT	STREPTO BACILLUS
4.	CATALASE	POSITIVE
5.	ENDOSPORE	POSITIVE
6.	MANNITOL FERMENTATION TEST	POSITIVE
7.	VP TEST	POSITIVE

Based on Bergey's manual **[9]** the isolated strain was tentatively identified as ***Bacillus subtilis.***

DISCUSSION

Soil sample was collected from mechanic workshop as previously done by [10;4]. Microorganisms were isolated by serial dilution agar plating method as done earlier by [5]. Streaking was done to purify the microorganism and purity was tested by gram's staining.

Percentage Oil degradation by purified cultures was calculated based on the formula given by [8]

Purified culture was identified by comparing the results of staining and biochemical activities of the culture showing maximum oil degradation to the bergey's manual as done earlier by [2].
Bacillus spp. Has been reported earlier by few researchers [6; 4; 11; 2] for oil degradation potential. 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 [2].

CONCLUSION

Based on the above study it can be concluded that *Bacillus subtilis* can be a good source for the remediation of oil contaminated sites and it will prove to be a very cost effective method for bioremediation.

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