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Degradation of Discarded used Engine Oil by *Pseudomonas aeruginosa* DP-1 and its Optimization

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A B S T R A C T

Oil degrading organisms were enriched in MSM medium supplemented with used engine oil. Organisms showed zone of clearance of 7-24 mm on TBA and 3-16 mm on used engine oil supplemented MSM plate. The % degradation of used engine oil was 14 to 37%. Isolate DP-1 showed highest activity, it was identified as *Pseudomonas aeruginosa* DP-1 by 16S r-RNA gene sequencing analysis. The organism gave 2, 6-dichlorophenol indophenol test positive. After optimization the used engine degradation was as high as 68% in 32 days of incubation. Selection and optimization resulted in 1.8 fold increase in used engine oil degradation potential of the organism.

Introduction

Petroleum hydrocarbons and its derivatives, such as engine oil, diesel oil, mineral oil, and heavy oil residues have a serious threat on the environment. They have been classified as most hazardous pollutants (Su *et al.,* 2011, Udeani *et al.,* 2009). Engine oil is a mixture of base polycyclic aromatic hydrocarbons (PAHs) having saturated and unsaturated long-chain with C16–C36 carbon length, cyclic alkanes and additives such as anticorrosive, antiwearing and antitearing agents (Koma *et al.,* 2003). On the other hand, used oil contain additive, some metals such as lead, zinc, barium and magnesium resulting from engine wear and tear and higher percentages of alkyl benzenes, naphthalene, methyl naphthalene and higher PAHs due to pyrosynthesis (Dominguez-Rusado *et al.*,2003; Lu S-T *et al.,* 2008). Used engine oil contains more amounts of carcinogenic PAHs as well as heavy and toxic metals compare to the fresh engine oil. Thus, it founds a potential threat to humans, animals, and vegetation (Adelowo *et al.,* 2006). Used engine oil contaminated soil and water resources have been treated by chemical and physical

methods however, these methods are transfer pollution from one phase to another like soil to water or air and also they are cost intensive (Huang *et al.,* 2009).

Bioremediation is found to be the best alternative especially microbial degradation, in which microorganisms are used to degrade pollutants (Akio *et al.,* 2006). Transformation, polymerization and mineralization are the potential mode to detoxify hazardous pollutant by microbes. Many times single strain are capable of degrading a limited components of the pollution component, whereas complete biodegradation require mutual activities of several strains called consortium (Wang *et al.,* 2007; Lal and Khanna, 1996). However, there is increasing research on the isolation of individual organisms that can not only degrade the major components of engine oils but also demonstrate versatility for other recalcitrant hydrocarbons, as these pollutants are mostly found together in the same environmental compartments.

The study was carried out for the assessment of biodegradation of used engine oil by *Pseudomonas aeruginosa* DP-1 and optimization of some parameters, as the organism is previously reported as a hydrocarbon degrader, but not used for discarded used engine oils.

Materials and Methods

Collection of soil sample

Used engine oil contaminated soil samples were collected in polythene bag from 3 locations of Ahmedabad Municipal Transport Service (AMTS) workshop Jamalpur, Ahmedabad. The collected samples were mixed to prepare composite soil sample. The composite sample was brought to lab and preserved in refrigerator.

Collection of used engine oil

Discarded used engine oils 2T and 4T were collected from garage in sterile plastic bottle and they were mixed.

Isolation of used engine degrading bacteria

The used engine oil contaminated soil sample 10 g was added in 100 mL MSM medium. MSM medium was supplemented with 1% (v/v) sterile (0.2 mm Millipore membrane filter) used engine oil as carbon source. Inoculated flask was incubated at 35±2 °C and 100 rpm.

After 1 week of incubation 1 mL of enriched sample was serially diluted to 10^{-4}, 10^{-5} and 10^{-6}, from the diluted sample 0.1 mL sample was spreaded on sterile MSM and nutrient agar plates and both the plates were over layered with 0.1 mL of sterile used engine oil and plates were incubated at 35±2 $^{\circ}$C for three days.

Well isolated colonies with distinct colony morphology on MSM agar were selected and purified on MSM agar by streak plate method. Procedure was repeated for three times and well isolated colonies were selected and preserved on nutrient agar slants

Screening of isolates

From all the 15 selected isolates 1mL (v/v) inoculum (3×10^{7} cells mL^{-1}) was inoculated in 50 mL MSM medium supplemented with 0.5 mL sterile used engine oil all the flask were incubated at 35±2°C 100 rpm. Degradation of used engine oil was estimated with gravimetric method (Black CA 1965).

To confirm the degradation of tributyrine, 50 µL (3×10^{7} cells) of all the isolated cultures were spot inoculated on tributyrine agar (TBA) plates in triplicates and plates were

incubated at 35±2 °C. Zone of clearance were measured and average of 3 observations were recorded. The culture which showed highest degradation of used engine oil and largest zone of clearance on TBA was selected for further study.

Detection of hydrocarbon utilization by 2, 6-DCPIP

To detect oxidative utilization of hydrocarbon from used engine oil 1mL (3×10^7 cells) of isolate DP-1 was mixed with 15 µL of sterile used engine oil and sterile 40µL of 0.01% 2, 6-dichlorophenol indophenol. Inoculated tubes were incubated for 48h 35±2°C. Oxidation of hydrocarbon was determine based on decolorization of added 2, 6-DCPIP (Hanson *et al.,* 1993).

Optimization of used engine oil biodegradation

If otherwise mention the experiment condition were pH 7 incubation temperatures 35±2°C used engine oil concentration 1% v/v and inoculum 1mL(v/v) having 3×10^7cells. All inoculated and control flasks were incubated for 32 days and amount of used engine oil degradation was measured by gravimetric method.

Influence of pH 5, 6, 7, 8, temperature 20, 30, 35, 50°C, concentration of engine oil 1, 2, 3, 4%, and amount of inoculum size 1, 2, 3, 4%, were studied in MSM by modifying these parameters as required under the mentioned conditions.

Results and Discussion

Isolation and screening

Enriched sample of used engine oil in MSM broth showed 42 morphological different colonies on nutrient agar plates and 15 different types of colonies on MSM agar plates (Fig. 1). Used engine oil degradation zones of 15 colonies on used engine oil supplemented MSM agar plates were in the range of 3 to 16 mm and on TBA plates the zones were 7 to 24 mm. Isolates showed 14 to 37% of used engine oil degradation in 32 days of incubation. Isolate DP-1, DS-7, DP-4 showed 30% and above degradation whereas isolate DS-6, DS-5 and OP gave less than 20% of degradation (Table 1).

Confirmation of oxidative utilization test

Isolate DP-1 which showed highest zone of used engine oil degradation, highest zone on TBA plate and highest percentage of used engine oil degradation. The isolate also showed decolorization of 2, 6-DCPIP in 48 h of incubation (fig. 2), indicated hydrocarbon oxidative test positive, thus this isolate was selected for further study.

Identification

Potential isolate DP-1 was identified as *Pseudomonas aeruginosa* based on 16S r-RNA gene sequence analysis. The sequence was deposited in NCBI with accession number MG976750.

Optimization

Influence of pH on oil degradation was studied and results are shown in figure 3 (a). Highest degradation was observed at pH 7. The acidic pH was less influencing compare to alkaline pH. Increases in pH on alkaline side (pH 7 to 8) was found to exert more inhibition of used engine oil degradation compare to same decrease on acidic side (pH 7 to 6).

Influence of inoculum size is shown in figure 3 (b). Inoculum size of 3×10^7 cells, 6×10^7 cells, 9×10^7 and 1.2×10^8 cells in the system show marginal variation, and degradation was in the range of 59 to 60±2%.

These could be due to growth of used engine oil degrading organisms which could have resulted in almost equal cell numbers after 1 week of incubation, and the results reported were of 32 days of incubation.

Table.1 Screening of isolates for degradation of used engine oil (UEO) and tributyrin (TB)

Isolate	Zone of clearance (mm)		Degradation of UEO
	TB	UEO	%
Control	0	0	0
DP1	24	16	37
DS1	22	12	31
DS2	19	12	30
DS3	16	11	20
DS4	15	12	26
DS5	12	5	17
DS6	9	3	14
DS7	21	8	36
MSMa	19	12	28
MSMb	14	9	24
YP	19	13	25
OP	7	3	18
Z1	20	14	26
Z2	15	11	29
Z3	20	15	27

Fig.1 Zone of degradation on MSM overlayered with UEO

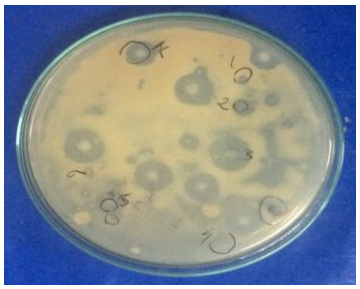
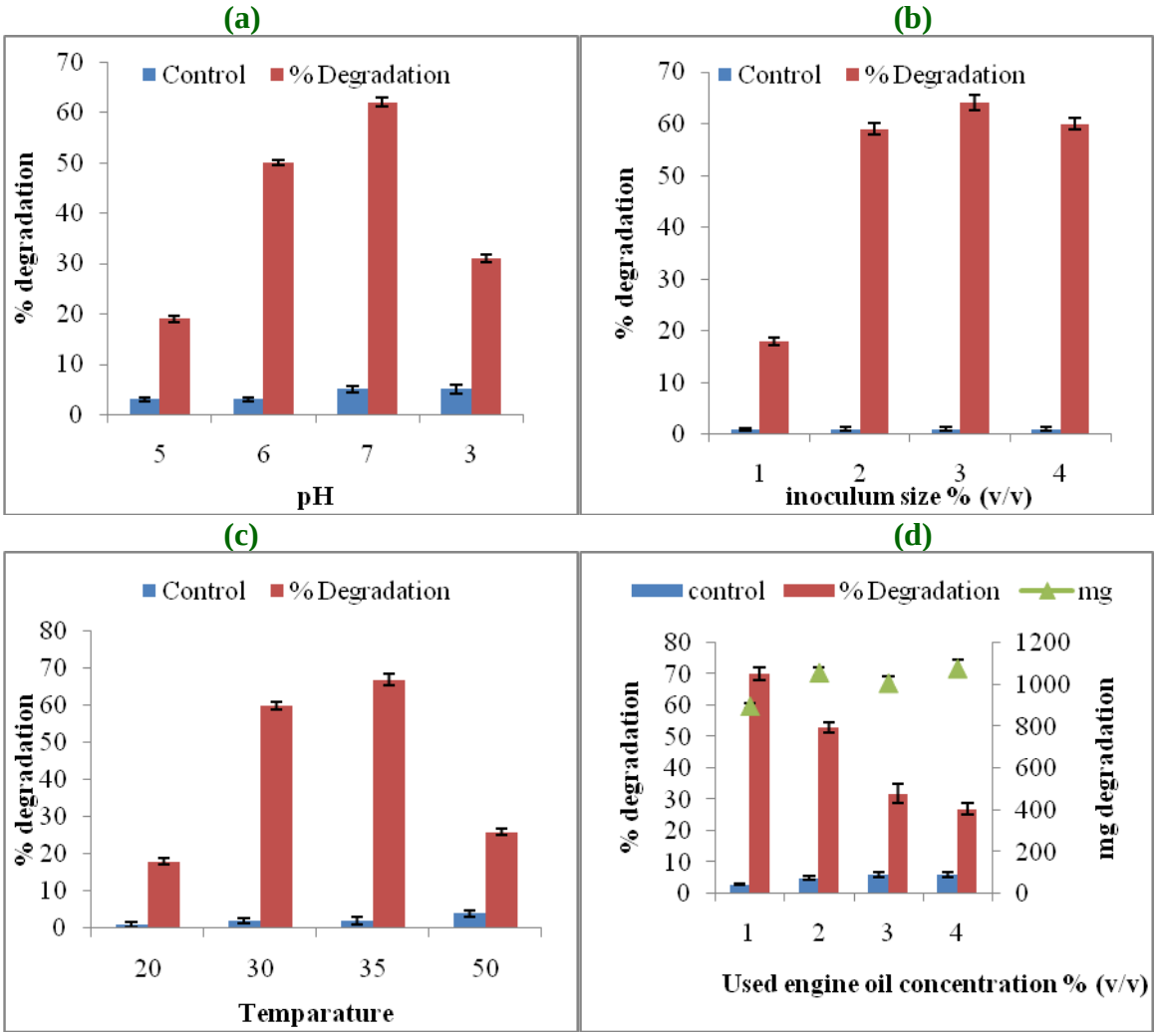


Fig.2 Hydrocarbon utilization by 21, 6-DCPIP oxidation test
a) No oxidation b) oxidation positive

a) **b)**

Fig.3 Effect of different parameters; (a) pH, (b) innoculum size, (c) temparature and (d) UEO concentration



Influence of incubation temperature is shown in figure 3 (c), the optimum temperature was found to be 35±2 °C temperature and the degradation was 2.5 to 3.7 fold lower on either side of the optimum temperature compare to the highest degradation. This could be due to the mesophilic nature of the organism and the place of sampling, which had temperature around 35±2 °C.

Influence of used engine oil concentration is shown in figure 3 (d), as the concentration of used engine oil increased percentage of used engine oil degradation was found to decrease. The % degradation was decreases in the range of 67 to 27% for 1, 2 3 and 4% of used engine oil added. However as the amount of the added used engine oil was different, if the degradation was considered in terms of milligram of used engine oil degraded they were 900, 1060, 1010 and 1080 ±50 mg.

Screening test showed degradation of used engine oil by as many as 15 isolates. Out of these 15 isolates DP-1 showed the highest activity. It was identified as strain *Pseudomonas aeruginosa* DP-1. It showed oxidation 2, 6-DCPIP test. Optimization of oil degradation procedure resulted in as high as 69% used engine oil degradation.

Developed culture can be used for the degradation of discarded used engine oil as well as for the cleaning of used engine oil contaminated soil.

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