



Research Article

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A Comparative Study of Oil Degradation with Used and Unused Engine Oil by Microbes Isolated From Water Sample of Mechanic Workshops



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Abstract

Recently spills of used and unused engine oil and petroleum products into the surrounding are major contaminants of the ecosystem; removal of those contaminants is becoming a very crucial problem. The current study was aimed to isolate the oil-degrading bacteria and compare their tendency to degrade used and unused engine oil. Five bacterial isolates capable of utilizing used engine-oil as a carbon source were isolated from contaminated water sample from various mechanic workshops and confirmed oil degrading capacity by using Bushnell Haas Media, on the basis of quantity of oil measured in both the used and unused engine oil, it was found that the consumption of used engine oil was very low or negligible in comparison to unused engine oil, *Pseudomonas spp.* 3 shows 28% of oil degradation was best of remaining isolates and in unused engine oil *Acinetobacter spp.* depicted higher capacity to degrade it.

Keywords: Bioremediation; Polycyclic aromatic hydrocarbons; Oil eating bacteria

Introduction

Oil dumping and petroleum products can cause severe damage to the ecosystem and pose threats of fire, ground water pollution due to percolation and air pollution due to evaporation [1]. Environmental pollution with petroleum and petroleum products has been recognized as one of the most serious current problems especially when associated with accidental spills, tank failure, pipeline ruptures on large-scale. If this occurs, hydrocarbons may reach the water label before becoming immobilized in the soil [2].

Unused engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organo metallic constituents that is used to lubricate the parts of an automobiles engine, in order to keep everything running smoothly while used engine oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic hazards including mutagenicity and carcinogenicity. In addition,

PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment [3]. The illegal dumping of used motor oil is an environmental hazard with global ramifications [4].

Bioremediation processes have been shown to be effective methods that stimulate the biodegradation of contaminated soil and water [5,6] may restore contaminated soils through the broad biodegradative capabilities evolved by microorganisms towards undesirable organic compounds [7].

In the present work microorganism isolated from water samples of various mechanic workshops where used and unused engine oil were illegally thrown in the locations that is not addressed for their disposal, And to compare their capability in percent of degrading used and unused engine oil by bacterial isolates which were confirmed to had tendency of degrading oil within duration of incubation (Table 1 & 2).

Table 1: Morphological characteristics of the six bacterial isolates cultured on nutrient agar medium.

Colony Color	Colony Size	Colony Form	Colony Elevation	Colony Margin	Gram Stain
Light Yellow	Medium	Circular	Slightly raised	Entire	Gram negative coccobacilli

Blue green	Large	Irregular	Raised	Undulated	Gram negative cocco bacilli
Yellow	Medium	Circular	Slightly raised	Entire	Gram negative cocco bacilli
Orange	Medium	Irregular	Slightly elevated	Convex	Gram negative coccobacilli
Beige-brown	Medium	Irregular	Raised	Undulated	Gram positive Rod

Table 2: Biochemical test result.

Name of Test	Isolate 1	Isolate 2	Isolate3	Isolate 4	Isolate 5
Glucose fermenting	Non fermenting	Non fermenting	Non fermenting	Non fermenting	Fermenting
Catalase test	Positive	Negative	Positive	Positive	Positive
Citrate test	Negative	Negative	Negative	Negative	Negative
Indole test	Negative	Negative	Negative	Negative	Negative
Motility test	Positive	Negative	Negative	Negative	Positive
Methyl red test	Negative	Positive	Positive	Negative	Positive
Voges Praskauer test	Negative	Positive	Positive	Negative	Positive
Nitrate test	Negative	Negative	Positive	Negative	Positive

Materials and Methods

Water samples were taken from mechanic workshops, nutrient agar media, nutrient broth, MacConkey agar media, used and unused engine oil and reagents for performing biochemical tests.

Collection of Samples

Various water samples were collected from mechanic workshops located at Rewa District.

Serial dilution was done in 0.8% NaCl solution: 5ml NaCl was then transferred in the test tube labeled and 4-5ml in the test tube labeled 10-1, 10-2, 10-3, 10-4 and 10-5. 50µl of inoculums from the serially diluted test tube (10-1, 10-3, and 10-5) was spread on solidified Nutrient Agar plates. Finally the plates were incubated in 37 °C for overnight.

Primary screening: After overnight incubation next day eight types of colonies were found on nutrient medium plates, Colonies of all plates were selected for secondary screening. By sub culturing and pure culture was formed. After this eight bacterial isolates were again grown on MacConkey agar media with pH 7.2 after incubation of one at 37 °C isolates were put for in broth media for further investigation in incubator at 37 °C.

Confirmatory test for oil degrading microbes by using bushnell haas media: (Bushnell, L.D. and Haas, 1941) Bushnell Haas Agar which was used for microbiological examination of fuels. Composition-Magnesium sulphate 0.200gm, Calcium chloride 0.020gm, Monopotassium phosphate 1.000gm, Dipotassium phosphate 1.000gm, Ammonium nitrae 1.000gm, Ferric chloride 0.050gm, Agar 20.000gm, Final pH (at 25 °C) 7.0±0.2. Inoculate the isolated cultures into the agar plate. After inoculation engine oil layered was made in inoculated agar plate and then put for the incubation at 37 °C for 7 days. Positive result

will observed by measuring the quantity of oil degraded by the bacteria. Staining and biochemical characterization was done for identification of isolated strains of Bacteria.

Inoculation of pure culture isolates into oil containing nutrient broth: After the confirmation of oil degrading capacity by five bacterial isolates, ten conical flasks were taken in which nutrient broth were taken. Then took 20ml of used and unused engine oil in each nutrient broth containing conical flask, put the conical flask for autoclave at 15lb on 120 °C for 30 minutes. Inoculate the bacterial pure culture isolates with the help of sterile inoculation loop under laminar in each conical flask. Covered the mouth of conical flask with aluminium foil and put for incubation at 37 °C in incubator and also put the conical flasks in shaker in regular 2-4 days intervals within duration of incubation for creating proper agitation of bacterial isolates.

Measurement of degraded oil: After the period of 25 days incubation amount of oil degraded were measured through separation funnel method by putting the nutrient broth along with the oil and bacterial isolates of each conical flasks in separation funnel for 30-40 minutes then the oil amount were measured in measuring cylinder by transferring the oil with the help of micropipette.

Results and Discussion

Eight bacterial strains were isolated from water sample of mechanic workshops by Nutrient and MacConkey agar medium, in which five bacterial isolates are capable of utilizing used engine oil as a carbon source confirmed by using Bushnell has enrichment media.

By interpreting results of biochemical tests of entire oil degrading bacterial isolates five strains are Pseudomonas sp.1, Pseudomonas sp.2, Pseudomonas sp.3, Acinetobacter

sp., *Bacillus* sp respectively. By comparing the above value of oil degradation we found that degradation of unused engine oil is higher by *P. spp.*, *Acinetobacter* spp. and *B. spp.*, while in case of used engine oil metals and heavy polycyclic aromatic hydrocarbons were higher hence their degradation was low in percent by the bacterial isolates, only *Acinetobacter* spp. Shows high degrading tendency of used engine oil. The substance present in used engine oil could contribute to chronic hazards including mutagenicity and carcinogenicity [8-10], in this way for degradation of it mixed microbial isolates which have high tendency to degrade used engine oil substance could be used.

Pseudomonas species are the most common bacterial hydrocarbon-degraders reported in the literature [11-14]. *Acinetobacter* spp. is widespread in nature and can remove or degrade a wide range of organic such as phenol [15,16] and inorganic compounds such as phosphates and metal [17-19]. However, individual organisms often prefer to metabolize a limited range of hydrocarbon substrates [20]. Consequently, a mixed population of fungi and bacteria is usually required to provide all the metabolic capabilities for complete degradation of complex mixtures of hydrocarbons [21-23].

Conclusion

This study was done to overcome the problem of illegally dumping of petroleum hydrocarbon products of used and unused engine oil which creates environmental hazard with global ramifications. The release of oil into the environment causes environmental concern and attracts the public attention. Hence bioremediation provide an effective cheap and efficient strategy to speed up the clean-up processes and their treatment with the oil eating bacteria should be done before dumping of used engine oil into the environment.

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