

## Isolation, Biochemical Characterization and Identification of Oil Degrading Bacteria Occurring in Oil Contaminated Sites of Mechanical Workshops

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### ABSTRACT

Used engine oil is one of the major pollutants which causes serious problems to the environment. The source of used engine oil is mechanical workshops. There are some potent microbial strains which can degrade used engine oil by utilizing the hydrocarbons present in used engine oil as nutrient sources. This process is known as biodegradation. The present study deals with the isolation, characterization and identification of oil-degrading bacteria from oil-contaminated sites of mechanical workshops. The soil samples from five mechanical workshops of Karimnagar town were processed in the laboratory. Twenty-four bacterial isolates were obtained. They were identified to the genus level. The identified bacterial isolates were thirteen *Bacillus*, four *Acinetobacter*, three *Pseudomonas* and three *Micrococcus* species. The *Bacillus* species are widely spread in the soils of mechanical workshops.

**Key words:** Oil-contaminated sites, hydrocarbons, biodegradation and bacterial isolates

### INTRODUCTION

Used engine oil is responsible for the contamination of soils with huge levels of various aliphatic, polyaromatic and heavy metals. The mechanical workshops where motor vehicles are serviced are the sources of used engine oils. From motor vehicles used engine oil is disposed into the surrounding soils. Used engine oil has high amounts of heavy polyaromatic compounds, hydrocarbons and heavy metals when compared to unused engine oil. These compounds present in used

engine oil alter the physical & chemical conditions in soil and immobilize the nutrients making them non-available to plants. The health hazards of these compounds include mutagenicity and carcinogenicity. The naphthalene, one of the chemical components of used engine oil, causes hazardous effects to kidneys, heart, lungs and nervous system<sup>1,2,3</sup>. There are microorganisms in nature which can degrade these hydrocarbons occurring in used engine oils. This process is called biodegradation.

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These microorganisms utilize these hydrocarbons as their nutrient sources. Such prominent isolated microbes include *Mycobacterium*, *Streptococcus*, *Corynebacterium* etc., Bioremediation employs biodegradation process to clean up the environment<sup>4</sup>. Bioremediation process is the efficient and genuine technique for treating oil contaminated soils. Microbes isolated from oil contaminated sites are efficient in treatment of oil polluted soils when compared to microbes isolated from normal soil samples. The present study deals with the isolation, characterization, identification (till genus level) and abundance of predominantly growing oil degrading bacteria occurring in oil contaminated sites of Kaman area of Karimnagar town, Telangana state, India.

## MATERIALS AND METHODS

### Sample collection

Soil samples were collected from four different mechanical workshops. They were designated as

A, B, C & D. All the four mechanical workshops were more than ten years old. In A & B workshops light motor vehicles and in workshops C & D two wheelers are serviced respectively. The soil from 10 cm depth was collected using a spatula and sealed in sterile polythene bags and carried to laboratory<sup>3</sup>.

### Isolation and identification of oil degrading bacteria

Sterilized Bushnell Haas Agar medium with 1% used engine oil was used to isolate the bacteria. The used engine oil serves as carbon source. 50µg of nystatin per ml was added to the medium to prevent the fungal growth<sup>5</sup>. Each soil sample's ten fold serial dilutions from  $10^{-1}$  to  $10^{-4}$  were prepared. Then 0.02 milliliter of  $10^{-4}$  dilution was added on the above agar medium taken in petriplates and spread evenly using a sterile spreader. The plates were incubated at 35<sup>0</sup>C for 48 hours. After the incubation period in each plate colony number was recorded with the aid of a colony counter. Each bacterial isolate was examined microscopically after gram staining & endospore staining, colony characteristics

were observed and biochemical tests were performed to identify till genus level. The biochemical tests performed were IMViC tests, Phyenylalanine, Hydrogen sulfide, Mannitol salt, Urease, Oxidase, Catalase, Starch hydrolysis, Gelatin hydrolysis and Casein hydrolysis tests. The occurrence of each isolate was also observed<sup>1</sup>.

## RESULTS AND DISCUSSION

In the present work, used engine oil contaminated soil samples of four mechanical workshops were processed in the laboratory to isolate oil degrading bacteria using Bushnell Haas Agar medium with 1% used engine oil. The bacterial colony forming units (CFU) on the Bushnell Haas Agar of mechanical workshops A, B, C & D were  $6 \times 10^4$ ,  $7 \times 10^4$ ,  $5 \times 10^4$  and  $6 \times 10^4$  respectively (Table1). Twenty four bacteria were isolated from the soils of four mechanical workshops. The bacterial isolates of soil of workshop A were designated as A1, A2, A3....., workshop B as B1, B2, B3....., workshop C as C1, C2, C3..... and workshop D as D1, D2, D3.....The colony morphology was observed (Table 2), gram staining, endospore staining and motility tests were performed followed by biochemical tests (Table 3). Based on the morphological & microscopic observation and biochemical tests all the bacteria were identified till genus level (Table 3). Thirteen bacteria were identified as *Bacillus* species, four were *Acinetobacter* species, three were *Pseudomonas* species and remaining three were *Micrococcus* species. Ogunbayo *et al.*,<sup>4</sup> isolated *Bacillus*, *Pseudomonas* and *Micrococcus* from oil contaminated sites of mechanical workshops located in Lagos city. Shahida *et al.*,<sup>6</sup> have isolated *Acinetobacter* species from oil contaminated sites of mechanical workshops in Sokoto metropolis. In the present study *Bacillus* species were present in all the mechanical workshops soils indicating that the *Bacillus* species can exist predominantly in extreme conditions like in high concentrations of hydrocarbons. This may be due to their ability to form endospore<sup>7</sup>. The *Bacillus* species had shown highest percentage of

occurrence followed by *Acinetobacter*, *Pseudomonas* and *Micrococcus*. Manzoor *et al.*,<sup>8</sup> have isolated fifteen bacterial species from oil contaminated soils of drilling oil site of Oil and Gas development company limited, Nashpa Karak and out of fifteen bacterial

isolates seven were identified as *Bacillus* species. Mbachu *et al.*,<sup>2</sup> reported that *Bacillus* species isolated from mechanical workshops at Mgbuka-Nkpor, Anambra State, Nigeria were found to be the efficient oil degraders when compared to other bacterial isolates.

**Table1: CFU of bacteria isolated from oil contaminated sites of mechanical workshops**

S. No	Workshop	CFU
1	A	6 x 10 <sup>4</sup>
2	B	7 x 10 <sup>4</sup>
3	C	5 x 10 <sup>4</sup>
4	D	6 x 10 <sup>4</sup>

**Table 2: Colony Characteristics and Microscopic observation of bacterial isolates**

S.No.	Bacterial isolate	Colony characteristics					Microscopic observation		
		Shape	Surface	Elevation	Margin	Colour	Gram staining	Endospore staining	Motility test
1	A1	Round	Smooth	Flat	entire	Green	Gram positive cocci	Negative	Negative
2	A2	Round	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive
3	A3	Round	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive
4	A4	Round	Smooth	Slightly raised	Entire	Milky white	Gram positive rods	Positive	Positive
5	A5	Circular	Smooth	Flat	Entire	Cream	Gram positive rods	Negative	Positive
6	A6	Circular	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive
7	B1	Round	Smooth	Slightly raised	Entire	Milky white	Gram positive rods	Positive	Positive
8	B2	Circular	Smooth	Flat	Entire	Cream	Gram negative rods	Negative	Negative
9	B3	Circular	Smooth	Flat	Entire	Green	Gram negative rods	Negative	Negative
10	B4	Round	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive
11	B5	Circular	Smooth	Flat	Entire	Cream	Gram positive rods	Negative	Positive
12	B6	Round	Smooth	Flat	entire	Green	Gram positive cocci	Negative	Negative
13	B7	Round	Smooth	Flat	entire	Green	Gram positive cocci	Negative	Negative
14	C1	Round	Smooth	Slightly raised	Entire	Milky white	Gram positive rods	Positive	Positive
15	C2	Round	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive
16	C3	Circular	Smooth	Flat	Entire	Cream	Gram positive rods	Negative	Positive
17	C4	Round	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive
18	C5	Round	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive
19	D1	Round	Smooth	Slightly raised	Entire	Milky white	Gram positive rods	Positive	Positive

20	D2	Round	Smooth	Slightly raised	Entire	Milky white	Gram positive rods	Positive	Positive
21	D3	Round	Smooth	Flat	entire	Green	Gram positive cocci	Negative	Negative
22	D4	Round	Smooth	Flat	entire	Green	Gram negative rods	Negative	Negative
23	D5	Round	Smooth	Flat	entire	Green	Gram negative rods	Negative	Negative
24	D6	Round	Smooth	Flat	Entire	Milky white	Gram positive rods	Positive	Positive

Table 3: Biochemical tests and identification of bacterial isolates till genus level

S.No	Bacterial isolates	Indole test	Methyl Red test	Voges Proskauer test	Citrate test	Phenylalanine test	Hydrogen sulfide test	Mannitol salt test	Urease test	Oxidase test	Catalase test	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Identified bacterium
1	A1	-	+	-	+	+	+	-	+	+	+	-	+	+	<i>Micrococcus</i> species
2	A2	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
3	A3	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
4	A4	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
5	A5	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i> species
6	A6	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
7	B1	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
8	B2	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
9	B3	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
10	B4	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
11	B5	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i> species
12	B6	-	+	-	+	+	+	-	+	+	+	-	+	+	<i>Micrococcus</i> species
13	B7	-	+	-	+	+	+	-	+	+	+	-	+	+	<i>Micrococcus</i> species
14	C1	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
15	C2	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
16	C3	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i> species
17	C4	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
18	C5	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
19	D1	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
20	D2	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species
21	D3	-	+	-	+	+	+	-	+	+	+	-	+	+	<i>Micrococcus</i> species
22	D4	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
23	D5	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
24	D6	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i> species

**Indication of symbols: + = Positive result & - = Negative result**

### CONCLUSION

In the present study the isolated bacterial species had grown by utilizing hydrocarbons present in the used engine oil as sole source of carbon. The *Bacillus* species are widely spread in the oil contaminated sites of mechanical workshops. Further the most efficient oil degrading strain among the four isolated and identified bacteria can be determined. Hence, these bacterial species can be further studied, improved and can be used for bioremediation.

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