

## Assessment of Spent Motor Oil Degradation Potential of Some Bacteria Isolated from Soil of Ohiya Mechanic Village Umuahia, Abia State

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**Abstract:** Indiscriminate release of hydrocarbon squander such as, spent motor oil (SEO) in the environment is becoming worrisome due to its likely effect on the soil ecosystem. The study assessed the capacity of some bacterial isolates to utilize spent motor oil. A total of fifteen (15) bacteria isolate comprising 4 distinct species of *Bacillus*, 3 *Pseudomonas*, 3 *Citrobacter* species, 3 *Monococcus* species and 2 *Acinetobacter* species were isolated. The ability of the pure culture and mixed bacterial consortium to degrade spent motor oil was tested in a shake- flask culture containing mineral salt medium (MSM) supplemented with 2% (V/v) spent motor oil and 5% (V/v) bacteria isolate and the residual hydrocarbon content was measured gravimetrically after 28 days of incubation. The percentage utilization of the pure isolates were 78.20%, 74.82%, 56.28%, 61.28% and 50.66% after 28 days of incubation for *Bacillus*, *Pseudomonas*, *Monococcus*, *Acinetobacter* and *Citrobacter* species respectively while the consortium of bacteria degraded 89.27% of spent motor oil under similar conditions. Thus, the consortium of bacteria was more effective in the degradation of SMO in soil. Based on our findings, we recommend the use of bacterial isolates for remediation of spent oil contaminated soil since it is eco-friendly.

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### I. Introduction

Pollution of soil by utilized greasing up oil is quickly expanding because of global increment in the utilization of oil based commodities in various kinds of auto-mobiles and equipment. Release of utilized engine oils pollutes our natural habitat with hydrocarbon. Hydrocarbon contamination of the air, soil, and freshwater by PAHs pulls in open consideration on the grounds that numerous PAHs are harmful, mutagenic, and cancer-causing (Mandri and Lin, 2007).

Used/Spent motor oil is characterized as utilized greasing up oils expelled from the crankcase of inward ignition motors (Jain et al., 2009). Before they are utilized, they comprise of hydrocarbons, (80 to 90% by volume) and performance upgrading added substances (10 to 20% by volume). Motor oils are adjusted during use by vehicles, engine bicycles, generators and other apparatus due to the breakdown of added substances, pollution with the results of burning and the products of metals from the mileage of the engine.(Jain et al., 2009).

Colossal amount of spent motor oils are generated every day from the processes of changing the oils of vehicles and different engines in the mechanical workshops. The remediation processes utilizing microorganisms and different living beings are relying upon the capacity of these organisms to debase or remove the hydrocarbon contaminants totally (Harder 2004). The utilization of the bioremediation technique that involves employing the use of microorganisms to detoxify or degrade pollutants depending to their varied metabolic capabilities is an evolving technique for the elimination and degradation of many environmental pollutants including the products of petroleum industry (Ulrici, 2000). Bacteria are seen as the most vigorous agents in hydrocarbon degradation, and they act as prime degraders or utilizers of spilled oil in environment (Brooijmans et al., 2009; Yakimov et al., 2007). It is, therefore, the aim of this research to investigate the utilization potentials of some bacterial isolates from Ohiya mechanic village in Umuahia, Abia State, Nigeria.

## **II. Materials and Methods**

### **Study Area and Sample Collection**

The soil samples were collected from Ohiya mechanic Village one of the largest functioning mechanic villages in Umuhia, Abia State (Latitude: 5°26' and 5°35'N, Longitude: 7°03' and 7°05'E). Sample was collected from five different locations within the study area. These selected sites were polluted with spent engine oil for a long time (more than 5 years). The soil samples were collected using auger-boring instrument from a depth of 0-20cm into a polythene bag container which was then transported to the laboratory for analysis.

### **Determination of Total Heterotrophic Count**

For the determination of total heterotrophic count (THC), a 10 fold serial dilution of soil sample was prepared. Aliquots 0.1ml of appropriate dilution was placed in triplicate on nutrient agar plates. The plates were incubated at 28°C for 24-48hrs. then the colonies were counted and average counts recorded, and used in the calculation of the colony forming unit per gram (CFU/g) of soil. (Ebuehi et al., 2005)

### **Determination of Hydrocarbon Utilizing Bacteria Count**

A 10 fold serial dilution of soil sample was prepared. Aliquots 0.1ml of appropriate dilution of soil sample suspensions was placed in duplicate onto the mineral salt agar (MSA) Zajic and Supplison, (1972). The medium contains 1.8g K<sub>2</sub>HPO<sub>4</sub>, 4.0g NH<sub>4</sub>CL, 0.2g MgSO<sub>4</sub>.7H<sub>2</sub>O, 1.2g KH<sub>2</sub>PO<sub>4</sub>, 0.01g FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.1g NaCL, 20g agar per litre of distilled water. Sterile filter paper (Whatman No.1) saturated with diesel oil were aseptically placed onto the covers of the inoculated inverted plates and then incubated for 5-7 days at 30°C, colonies were counted from triplicate plates and the average counts were recorded and used for the calculation of colony forming units per gram (CFU/g) of soil (Adieze, 2012).

### **Identification of the Bacteria Isolates**

The bacteria isolates from hydrocarbon utilizing bacteria plate on MSA were characterized based on colonial characteristics and cell morphology such as, colonies differing in size, shape and colour on differential/selective media and biochemical tests which include Gram's reaction, indole tests, methyl red, Voges-Proskauer, citrate utilization, urea test, utilization of different types of sugars, oxidase and catalase tests. Pure cultures of bacterial isolates were identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Salam et al., 2011)

### **Spent motor oil degradation studies**

The ability of isolates to degrade spent engine oil was evaluated using the gravimetric method demonstrated in terms of reduction in the amount of crude oil used. The rate of utilization was monitored on the first day (day zero) of the study and subsequently at 4-day interval for 28 days; n-hexane was employed as the solvent for extraction. On each day, three samples per single treatment were analyzed for the quantity of residual crude oil using the method described by Nwaogu et al. (2008) with slight modifications. Five percent (v/v) of standardized inoculums was inoculated into test tubes containing MSA supplemented with two percent (v/v) of spent motor oil and incubated in an orbital shaker for 28 days. The negative control in these tests was MSM without inoculation.

### **Extraction and Determination of Residual oil**

The residual oil was extracted by using liquid-liquid solvent extraction method. The organic solvent used was n-hexane. This was done by measuring 50ml of n-hexane into the bottles containing OIL-MSM. The contents were later transferred into separating funnel. A funnel fitted with filtered paper (Whatman No1), anhydrous sodium sulphate spread on the filter paper was employed to remove any moisture in the mixture, this was used to collect the layer containing the organic solvent and residual oil in a pre-weighed 50ml Pyrex beaker. The organic solvent was allowed to evaporate in an oven, after the evaporation, the amount of residual oil was calculated as thus:

Weight of oil degraded = original weight of oil – weight of residual oil obtained after evaporating the extractant.

## **III. Results**

### **Total Heterotrophic and Hydrocarbon Utilizing Bacterial Count.**

The results obtained from the microbial isolation and identification indicates that the soil sample contained relatively high total heterotrophic and hydrocarbon utilizing bacteria with colony forming unit per gram (Cfu/g) of  $1.80 \pm 0.42 \times 10^5$  and  $5.25 \pm 0.25 \times 10^6$  cfu/g respectively.

**Table 1: Total heterotrophic bacterial and hydrocarbon utilizing bacteria counts**

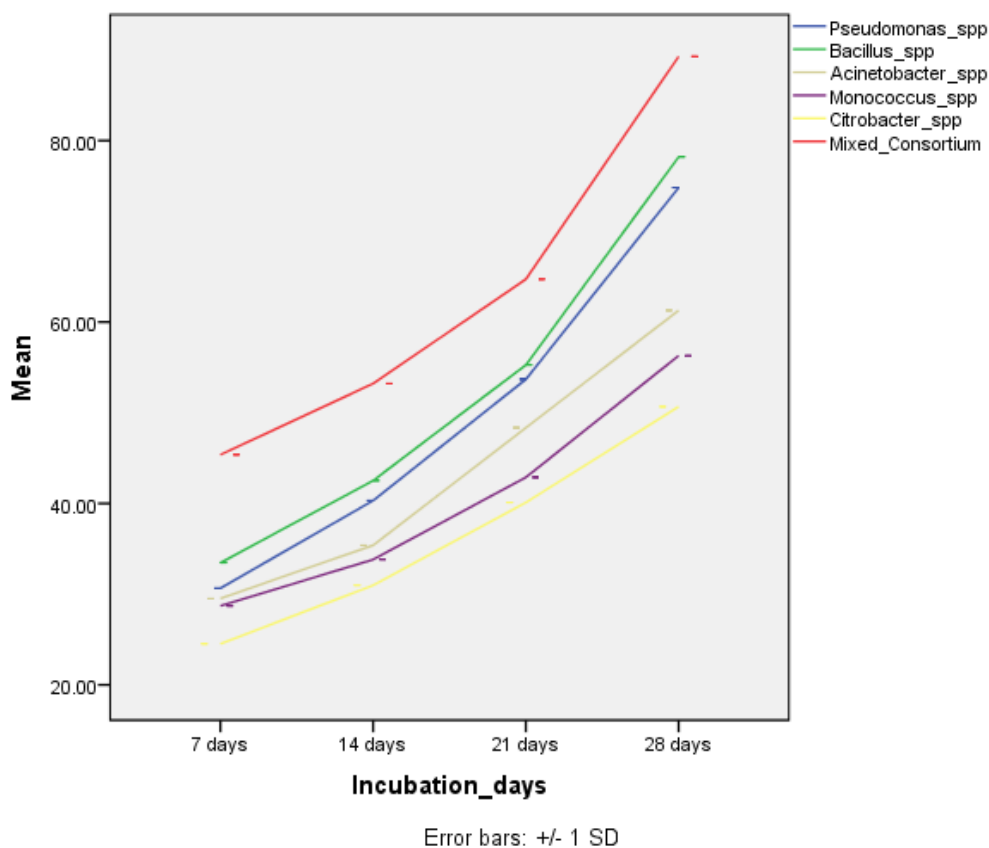
Sample	Total heterotrophic bacteria count (cfu/g)	Total hydrocarbon utilizing bacterial count(cfu/g)
Soil (0-20cm)	1.80 ±0.42× 10 <sup>5</sup>	5.25 ±0.25× 10 <sup>6</sup>

**Table 2: Biochemical tests and identification of hydrocarbon degrading bacteria**

s/n	Bacteria isolate	Glucose	Lactose	Gas	H <sub>2</sub> S	Indole	Motility	Oxidase	Citrate	Urease	Catalase	VP	Methyl red	Organism identified
1	A2	-	-	-	-	-	+	-	-	-	+	-	+	<i>Bacillus spp</i>
2	B1	-	-	-	-	-	+	+	+	-	+	-	+	<i>Pseudomonas spp</i>
3	B2	+	-	-	-	-	-	-	+	-	+	-	-	<i>Acinetobacterspp</i>
4	A1	-	-		+	-	-	+	+	+	+	-	+	<i>Micrococcus spp</i>
5	D1	+	-		-	+	+	+	+	+	+	-	+	<i>Citrobacterspp</i>
6	B1	-	-	-	-	-	+	+	+	-	+	-	+	<i>Pseudomonas spp</i>
7	E1	+	-		-	+	+	+	+	+	+	-	+	<i>Citrobacterspp</i>
8	A3	-	-	-	-	-	+	-	-	-	+	-	+	<i>Bacillus spp</i>
9	C2	-	-		+	-	-	+	+	+	+	-	+	<i>Micrococcus spp</i>
10	B3	+	-	-	-	-	-	-	+	-	+	-	-	<i>Acinetobacter</i>
11	E2	+	-		-	+	+	+	+	+	+	-	+	<i>Citrobacterspp</i>
12	D3	-	-		+	-	-	+	+	+	+	-	+	<i>Micrococcus spp</i>
13	C1	-	-	-	-	-	+	+	+	-	+	-	+	<i>Pseudomonas spp</i>
14	C3	-	-	-	-	-	+	-	-	-	+	-	+	<i>Bacillus spp</i>
15	D2	-	-	-	-	-	+	-	-	-	+	-	+	<i>Bacillus spp</i>

### Spent Motor Oil Utilization Studies

The ability of pure and a consortium culture of *Bacillus sp*, *Acinetobactersp*, *Citrobactersp*, *micrococcus sp* and *Pseudomonas sp* were used for the utilization studies and result is presented in figure 1. after 28 days of incubation, the highest oil utilization was observed in the consortium culture (89.27%), this was followed by *Bacillus sp* (78.20%), *Pseudomonas specie* (74.82%), *Acinetobacter specie* (61.28%), *Monococcus specie* (56.29%) and *Citrobacter specie* ( 50.67%).



**Figure1:** Biodegradation Potentials of Isolated pure Bacteria Strain and a Mixed Bacteria Consortium

#### IV. Discussion

Total heterotrophic and hydrocarbon utilizing bacterial counts from the soil sample were  $1.80 \pm 0.42 \times 10^5$  and  $5.25 \pm 0.25 \times 10^6$  cfu/g respectively. There were reasonably high counts of total hydrocarbon utilizing bacterial population (THUB) soil sample compared with the total heterotrophic bacteria population (THB). This was in line with the reports of Michalcewicz (1995) and Herbert *et al.*, (1997) and may be attributed to the supposed stimulatory effect of engine oil as carbon/energy source. The high count oil degraders observed at depth 0 – 20cm (top soil) is an indicative that most engine oil degrading bacteria are aerobic (Walker and Crawford, 1997; Malatova, 2005; Akoachere, *et al.*, 2008). Bacterial species with potential ability to utilize hydrocarbons exist ubiquitously in the environment ( Onugbolu and Adieze, 2016).

Results from the biochemical characteristics of the isolates shows that a total of Fifteen (15) isolates were obtained belonging to five genera (Table 2). Four isolates were identified as Bacillus species, two were Acinetobacter species, three were Pseudomonas species, three were Citrobacter species while three were Micrococcus species. This is in harmony with the findings of Ogunbayo *et al.*, (2012) that isolated Bacillus spp, Pseudomonas spp and Micrococcus spp from oil contaminated sites of mechanical workshops in Lagos state Nigeria and the result of Shahida *et al.*, (2015) that isolated Acinetobacter species from oil contaminated sites of mechanical workshops in Sokoto metropolis, Nigeria.

#### Spent Motor Oil Utilization Studies

The application of microorganisms to utilize crude oil have been reported in previous studies. (Atlas, 1981; Gerson, 1985; Hidebrandt and Wilson, 1991). In this study, the consortium culture had higher percentage utilization of spent motor oil than pure culture. This result is in conformity with the reports of Obire, 1988; Amund *et al.*, 1994; Facundo *et al.*, 2001; Kulwadee *et al.*, 2001) that affirmed microbial consortia as preferable degraders over pure culture. In a mixed culture, some species utilize intermediates of degradation of the original hydrocarbon produced by other members of the culture prompting a total debasement of the oil (Atlas, 1981; Facundo *et al.*, 2001). This can also be as a result of synergistic relationship among individual bacteria of the consortium that supported the degradative capability of the bacterial consortium (Ghazali *et al.*, 2004).

After 28 days of incubation, it was seen that all the pure isolates utilized spent motor oil at various degrees. The highest oil utilization for pure isolates was observed in Bacillus spp (78.20%), followed by Pseudomonas spp (74.82%), Acinetobacter spp (61.26%), micrococcus spp (56.29%), and Citrobacter spp

(50.67%). This is in accordance with the discoveries of Mbachu et al. (2014) who opined that *Bacillus* species isolated from mechanical workshops at Mgbuka-Nkpor, Anambra State, Nigeria were efficient oil degraders when contrasted with other bacterial isolates. This might be because of their resistance endospores. This shows that *Bacillus* species are effectively engaged in the natural debasement of oil polluted environment. The result of this study is not in concurrence with the report that spore-forming microbes have insignificant role in oil biodegradation (Bossert and Bartha, 2006). *Bacillus* sp fit for debasing hydrocarbons has additionally been accounted for by Nwaogu et al., 2008., Esin and Antai, (2002).

*Pseudomonas* species has been reported to be a potent hydrocarbon utilizer in previous studies (Udotong, 1995; Essien et al., 1997; Ezeji et al., 2005). The high debasing capacity of *Pseudomonas* spp recorded in this study is in agreement with the report that *Pseudomonas* species has the ability to debase hydrocarbon such as biphenyl (Ohta et al., 2001), toluene, P-xylene (Yu et al., 2001), benzenes (Munoz et al., 2007). The result of this study aligned with the hypothesis that individual organisms in a consortium of microorganism performs ample roles and rely upon the presence of various species and strains to successfully debase raw petroleum in the ecosystem (Ghazali et al., 2004).

In this study, the Total Petroleum Hydrocarbons (TPH) decreased as the time (weeks) of incubation increased. This is in conformity with the findings of Adenipekun and Isikhuemhen (2008) who reported that TPH removal consistently increase as the time of incubation increases, as well as Shukar et al. (2009) who opined that debasement rate of *Pseudomonas* spp in soil polluted with diesel oil increased as the time of incubation increases. Abdulsalam et al. (2012) also observed TPH increased in utilized motor oil polluted soil as the time of incubation is increases.

## V. Conclusion

The study indicated that spent motor oil polluted soil harboured bacteria that degraded and emulsified spent motor oil but the mixed culture were the most efficient oil degraders. some oil degrading bacteria identified were species of *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Citrobacter* and *Monococcus*. consequently, we recommend the use of these bacteria for the remediation of spent motor oil contaminated soil since it is eco-friendly.

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