Isolation, Characterization and Identification of Bacterial isolates from Auto-mechanic Workshop contaminated with Hydrocarbon

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ABSTRACT

This work focused on screening of hydrocarbon and heavy metal degrading microorganisms from the soil contaminated with hydrocarbon in auto-mechanic workshop using selective enrichment techniques. It resulted in the collection of 9 distinct species. All strains were cultivated in liquid media with engine oil as a sole carbon and energy source. Bacterial strains capable of degrading hydrocarbons belong to the genera *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Micrococcus letus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Clostridium*spp, *Streptococcus*spp and *Pseudomonas aeruginosa*.

Microorganisms were identified using cultural and morphological characteristics as well as biochemical tests. Rate of biodegradation depends greatly on the composition, state, and concentration of the hydrocarbons. Temperature and oxygen and nutrient concentrations were important variables in the environment. The total hydrocarbon (THC) content on day 2 stood at 139.30 and the amount degraded by *P. aeruginosa* was 0.089 mg/kg. The percentage degraded by the same organism was 8.89 %, compared to THC on day 2 for *B. subtilis* which was 145.70. The amount degraded was 0.047 mg/kg and percentage was 4.71 %. However, *B. subtilis* and *P. aeruginosa* both showed varying level of degradation capabilities.

(Keywords: engine oil, hydrocarbon, microorganisms, degradation, bioremediation, bacteria)

INTRODUCTION

Engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organ-metallic constituents (Butler and Mason, 1997). It is used to lubricate the parts of automobiles engine, in order to keep everything running smoothly (Hagwell *et al*., 1992). New motor oil contains a higher percentage of fresh and lighter (often more volatile and water soluble) hydrocarbons that would be more of a concern for acute toxicity to organisms. Used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic hazards including mutagenicity and carcinogenicity (Boonchan*et al*., 2000).

PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment (Van Hamme *et al*., 2003). The illegal dumping of used motor oil is an environmental hazard with global ramifications (Blodgett, 2001). The release of oil into the environment causes environmental concern and attracts the public attention (Roling*et al*., 2002). Engine Oil at minimal concentration in soil stimulates growth (Anoliefo and Edegbai, 2000). When microorganisms in the soil come in contact with oil, the initial reaction is a reduction of the activities as a result of reduced air availability (Odu, 1981).

Soil polluted with waste engine oil becomes water logged; inducing several stresses on the plant and microbial community; ranging from changes in structure and configuration of enzymes. Polluted soil could also become unsuitable due to increase in the toxic levels of elements (Udo and Fayemi, 1975).

The most widely used procedures to degrade waste engine oil in soil are the chemical and physical methods. These methods are however not favourable as they introduce harmful materials into the environment (Davis and Wilson, 2005). The most suitable technology for cleaning spills is the bioremediation method, which must be specific for a particular site; haven met some conditions like the type, quantity and toxicity of contaminant chemicals present and the indigenous microbial population (Ikhajiagbe and Anoliefo, 2011; Osarumwense and Igiebor, 2018).

Other remediation technologies include the addition of nutrient to stimulate the activities of host microbial community. In the presence of favourable environmental condition, there is an increase in the growth of microbial population which results in faster degradation of poisonous materials (Igiebor*et al*., 2017). Some other technologies (phytoremediation and fungal remediation) have been used to clean up polluted soils and underground water (Ikhajiagbe and Anoliefo, 2011).

Bioremediation makes use of indigenous oil–consuming microorganisms, called petrophiles, by enhancing and fertilizing them in their natural habitats. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a food source (Harder, 2004). Microorganisms degrade these compounds by using enzymes in their metabolism and can be useful in cleaning up contaminated sites (Alexander, 1999).

Microbial remediation of a hydrocarbon–contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil (Osarumwense and Igiebor, 2018). These microorganisms can degrade a wide range of target constituents present in oily sludge (Barathi and Vasudevan, 2001; Mishra *et al*., 2001). A large number of Pseudomonas strains capable of degrading PAHs have been isolated from soil and aquifers (Johnson *et al*., 1996). Harder (2004) estimated that bioremediation accounts for 5 to 10 percent of all pollution treatment and has been used successfully in cleaning up the illegal dumping of used engine oil.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from an auto-mechanic workshop opposite the University of Benin, Benin City and were packed in sterile polybags and transferred to the laboratory for analysis.

Dilution of Sample

1g of soil sample was weighed and added into 9ml of distilled water. Then, 1ml from the sample was taking out and added into 9ml of distilled water. This step was continuously repeated until third dilution.

Isolation of Bacteria

Bacterial species were isolated from the collected soil samples by serial dilution and agar plating method wherein the soil sample was diluted from 10^{-1} to 10^{-3} dilutions, and the diluted soil samples were spread on sterile Nutrient agar plates. The inoculated plates were incubated at 37^0C for 24 hours. Mixed cultures were obtained after incubation, labelled accordingly and purified by streaking on sterile nutrient agar plates. The purity of cultures was cross checked by gram staining procedures (Saroj and Keerti, 2013).

Staining and Biochemical Activities of Purified Cultures

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n order to identify the purified cultures tentatively on the basis of Bergey's manual (Aneja, 2003), various staining and biochemical tests were performed namely Gram staining, Catalase test, Indole test, citrate utilization test, Urease test, Motility test, oxidase test, coagulase test, Glucose fermentation, fructose fermentation, and Lactose fermentation (Aneja, 2003).

Screening of Petroleum Degraders

The screening of petroleum degraders was done using the method of Osarumwense and Igiebor (2018).

RESULTS AND DISCUSSION

Table 1 shows the optical density (OD) at 600nm of two microorganisms (*P. aeruginosa* and *B. subtilis*) from day 0 to 14.

Table 1: Optical Density of *P. aeruginosa* and *B. subtilis* at 600nm.

Days	*P. aeruginosa*	*B. subtilis*
0	0	0
1	2.034	2.012
2	2.036	2.085
3	1.987	2.044
4	1.844	1.842
5	1.866	1.979
6	1.925	1.989
7	2.063	2.045
8	2.005	1.928
9	1.899	1.898
10	1.871	1.774
11	1.805	1.786
12	1.644	1.43
13	1.231	1.036
14	1.107	1.021

Table 2 above shows the total amount of hydrocarbon content (THC) degraded and their percentage degradation by the two microorganisms from day 0 – 14. The total amount of hydrocarbon degraded on day 2 by *P. aeruginosa* was 0.088947 compared to the amount degraded by *B. subtilis* which was 0.04709, on the same day. The percentage degrade on day 6 by both organisms were significantly different, as percentage degraded by *P. aeruginosa* was 35.90582 and that degraded by *B. subtilis* was 29.82341. However, there were no significant differences between the amounts and percentages degraded by both organisms on day 10.

DISCUSSION

There are many reports on the degradation of hydrocarbon pollutants by different bacteria. Several bacteria are even known to feed exclusively on hydrocarbons (Yakimov *et al*., 2007). Bacteria with the ability to degrade hydrocarbons are named hydrocarbon-degrading bacteria. Kafilzadeh *et al*. (2011) isolated 80 bacteria strains which belonged to 10 genus, which are; *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Alcaligenes*, *Acinetobacter*, *Escherichia*, *Klebsiella* and *Enterobacter* and *Bacillus* were the best hydrocarbon degrading bacteria.

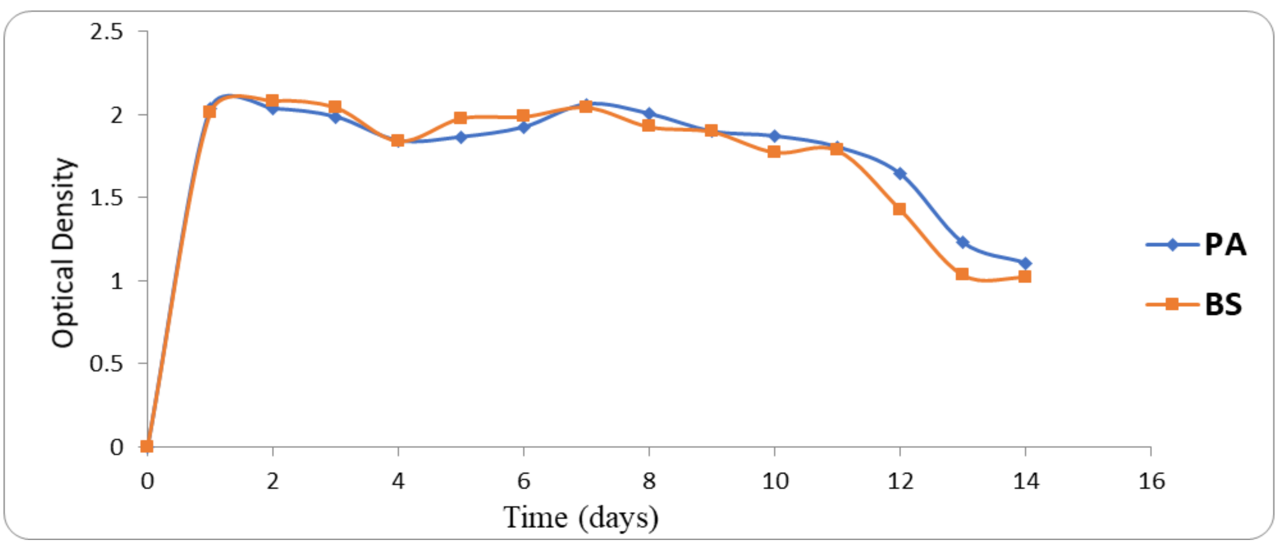

Figure 1: Variation of Optical Density with Time During the Degradation of Used Oil by *P. aeruginosa* (PA) and *B. subtilis* (BS).

Table 2: Total Amount of Hydrocarbon Content Degraded by *P. aeruginosa* and *B. subtilis*.

AMOUNT OF TOTAL HYDROCARBON CONTENT DEGRADED						
DAYS	*P. aeruginosa*			*B. subtilis*		
	THC (mg/kg)	Amount degraded (mg/kg)	% degraded	THC (mg/kg)	Amount degraded (mg/kg)	% degraded
0	152.90	0	0	152.90	0	0
2	139.30	0.088947	8.894702	145.70	0.04709	4.70896
4	125.60	0.178548	17.85481	118.10	0.2276	22.75997
6	98.00	0.359058	35.90582	107.30	0.298234	29.82341
8	86.00	0.437541	43.75409	77.60	0.492479	49.24787
10	72.50	0.525834	52.58339	73.20	0.521256	52.12557
12	71.80	0.530412	53.0412	58.80	0.615435	61.54349
14	71.30	0.533682	53.36821	57.30	0.625245	62.52453

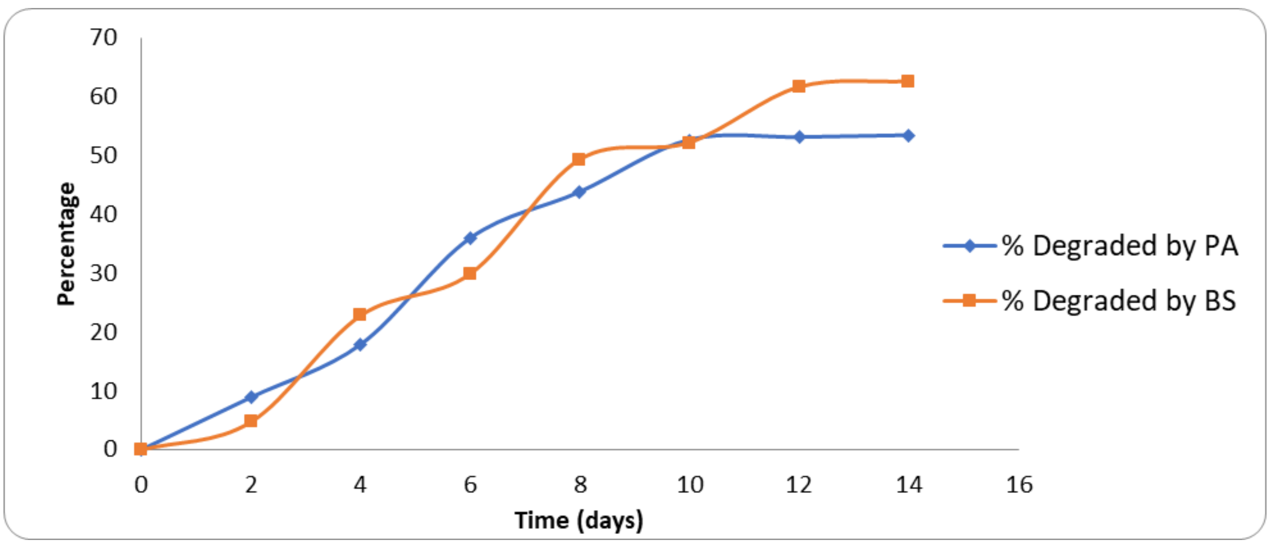

Figure 2: Percentage Degradation of Used Oil by *P. aeruginosa* (PA) and *B. subtilis* (BS).

Bacteria strains that are able to degrade aromatic hydrocarbons have been repeatedly isolated, mainly from soil. These are usually gram-negative bacteria most of them belong to the genus Pseudomonas. The biodegradative pathways have also been reported in bacteria from the genera *Mycobacterium*, *Corynebacterium*, *Aeromonas*, *Rhodococcus* and *Bacillus* (Mrozik *et al*., 2003). Although many bacteria are able to metabolize organic pollutants, a single bacterium does not possess the enzymatic capability to degrade all or even most of the organic compounds in a polluted soil.

Mixed microbial communities have the most powerful biodegradative potential because the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas (Fritsche and Hofrichter, 2005). The polluted soil samples were enriched with the hydrocarbon degrading bacteria and a total of 9 bacteria were isolated from the oil contaminated soil. These isolates were purified from the soil sample on the basis of colony, morphology, texture, growth. Biochemical tests like starch hydrolysis, urease production, citrate, coagulase and catalase test.

The indigenous bacteria isolated from hydrocarbon polluted soil were similar to the ones isolated by Osarumwense and Igiebor (2018) which include; *Streptococcus* spp., *Escherichia coli*, *Micrococcus letus*, *Clostridium* spp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Proteus* spp. and *Staphylococcus aureus*. From the studies, bacteria identification using biochemical test revealed that *S. aureus* was mostly present in the soils polluted with hydrocarbons. The growth of the organism may be attributed to the fact that the enzymes are stable and metabolically active at 37^0C.

Pseudomonas and *Bacillus* species are the most common bacteria hydrocarbon–degraders reported in the literatures (Barathi and Vasudevan, 2001; Bhattacharya *et al*., 2002; Pokethitiyook *et al*., 2003; Van Hamme *et al*., 2003). This is also supported by the present studies that *P. aeruginosa* was still able to thrive well at 45^0C. Changes in soil colour were observed after a period of time and may be as a result of the biodegradation potentials of the organisms.

Christopher and Christopher (2004) reported a sequential change of the composition of the oil degrading bacteria over a period of time in oil contaminated soil samples. Komukai-Nakamura *et al*. (1996) reported the sequential degradation of Arabian light crude oil by *Acinetobacter* sp T4 and *Peudomonas putida* PB4. Successful removal of hydrocarbon by the addition of bacteria had been reported by Osarumwense and Igeibor (2018). Microorganisms have developed the capabilities to protect themselves from toxicity by various mechanisms, such as adsorption, uptake, methylation, oxidation and reduction.

CONCLUSION

Microbial activities are very important for the renewal of our environment and maintenance of the global carbon cycle. These activities amidst other substances can be degraded or transformed by microorganisms. However, in most cases these degradabilities which were estimated in the laboratory by using selected cultures and under ideal growth conditions, have shown that *P. aeruginosa* and *B. subtilis* have the potentials to degrade hydrocarbon polluted soil.

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