African Journal of Microbiology Research Vol. 4(21), pp. 2257-2264, 4 November, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Determination of the ability of microorganisms isolated from mechanic soil to utilise lubricating oil as carbon source

A. Sebiomo[1]*, S. A. Bankole[2] and A. O. Awosanya[1]

[1]Department of Biological Sciences, Tai Solarin University of Education, Ijagun, Ijebu-Ode, Ogun State, Nigeria.
[2]Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Accepted 20 August, 2010

Ten bacteria and five fungi capable of utilising lubricating oil as carbon source were isolated from mechanic soil. The growth profiles were determined by monitoring the optical density, total viable counts, dry weights and pH of the culture utilizing lubricating oil as sole carbon and energy source. The total viable counts increased significantly with optical density and dry weights of fungi as the days of incubation progressed until the 14th day ($P < 0.001$). There was significant decrease in pH ($P < 0.001$) as fungal cells metabolised lubricating oil, *Pseudomonas aeruginosa* produced the highest optical density and viable count values of 0.441 and 8.31 to the end of 14th day of incubation among all bacterial isolates used in this study. *P. aeruginosa* also presented lowest pH value of 6.53 after 14 days of incubation between all bacterial isolates. *Bacillus licheniformis* and *Enterobacter aerogenes* recorded the lowest optical density and viable count values of 0.19 and 8.1 of all bacterial isolates after 14th day of incubation, *Enterobacter aerogenes* had the highest pH value of 6.8. Of all the fungal isolates used in this study *Aspergillus niger* and *Aspergillus terreus* recorded the highest dry weight and viable count values of 19.3 and 6.41. *Trichoderma* sp. had the lowest pH, dry weight and viable count values of 5.1, 17 and 6.3, respectively, *Aspergillus flavus* had the highest pH of 5.6. Correlation analysis indicated negative correlation between optical density and pH (correlation coefficient = +0.174) and between viable counts and pH (correlation coefficient = +0.159), there was positive correlation between viable counts and optical density (correlation coefficient = 0.735). For the fungal isolates there was negative correlation between dry weights and pH (correlation coefficient = +0.958) and between viable counts and pH (correlation coefficient = -0.830). The correlation analysis between fungal dry weights and viable counts showed positive correlation (0.786). The utilization of hydrocarbon in lubricating oil resulted in gradual reduction in the oil layer and complete disappearance of the oil with prolonged incubation. This study has shown that the microorganisms isolated from mechanic soils have potential application in the bioremediation of sites polluted with lubricating oil.

Key words: Bacteria, fungi, lubricating oil, biodegradation.

INTRODUCTION

Lubricating oil (engine oil) is a common contaminant in water and soils. Generally, lubricating oil comprises 80% of hydrocarbon lubricant, with the remainder being additives, which consists partly of zinc diaryl, molybdenum disulfide, zinc dithiophosphate, metal soaps and other organometallic compounds (Lu and Kaplan, 2008). Large amounts of engine oil are liberated into the environment when the motor oil is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by motor mechanics and generator mechanics (Odjeda and Sadiq, 2002). In addition the oil is also released into the environment from the exhaust system during engine use and due to engine leaks (Anoliefo and Edegbai, 2000; Osubor and Anoliefo, 2003). The illegal dumping of used motor oil is an

*Corresponding author. E-mail: rev20032002@yahoo.com. Tel: +2348077675121, +2347039334401.

environmental hazard with global ramifications (Blodgett, 2001). The release of oil into the environment causes environmental concern and attracts the public attention (Rolling et al., 2002).

With an ever increasing world's population, there is a concomitant increase in demand for petroleum derivatives, which apparently constitutes a source of environmental pollution (Raven et al., 1993). Oil pollution is a major environmental concern in many countries, and this has led to a concerted effort in studying the viability of using oil-degrading microorganisms for bioremediation. The discharge of used engine oil from vehicles is a major source of oil pollution in Ijebu-ode and its environs. The soil is habitat to many living organisms, any change in their number or form may upset or cause a total collapse of the ecosystem. The effect of oil spills on soil can lead to an enrichment of the oil-degrading microbial population (Akoachere et al., 2008).

Microbial remediation of hydrocarbon-contaminated site is performed with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. These microorganisms can degrade a wide range of target constituents present in oily sludge (Barathi and Vasudevan, 2001; Mishra et al., 2001; Eriksson et al., 1999). A large number of *Pseudomonas* strains capable of degrading PAHs (Poly Aromatic Hydrocarbons) have been isolated from soil and aquifers (Jhonson et al., 1996; Kiyohara et al., 1992; Fall et al., 1979). Other petroleum hydrocarbon degrading bacteria include *Yokenella* spp., *Alcaligenes* spp., *Roseomonas* spp., *Flavobacter* spp., *Acinetobacter* spp., *Stenotrophomonas* spp., *Corynebacterium* spp., *Streptococcus* spp., *Providencia* spp., *Sphingobacterium* spp., *Capnocytophaga* spp., *Moraxella* spp. and *Bacillus* spp., (Rusansky et al., 1987; Antai, 1990; Bhattacharya et al., 2002). Besides, other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent. However they take longer periods of time to grow as compared to their bacterial counterparts (Prenafeta-Boldu et al., 2001).

Due to the increasing mechanic workshops and their indiscriminate disposal of waste lubricating oil into the environment there is need to consider options for their removal from the environment due to the environmental risk presented by them to the environment. The aim of this work is to determine the ability of the bacteria and fungi isolated from soil which had been previously exposed to oil pollution in a mechanic site to utilise lubricating oil as carbon source via monitoring growth indices such as viable counts, pH, optical density and dry weight.

MATERIALS AND METHODS

Sample collection

Lubricating oil contaminated soil was collected from a mechanic workshop in Ago-Iwoye, Ogun State Nigeria. Non-contaminated soil was also collected from a distance not less than 120 m from contaminated sites. All soil samples soil samples were collected in triplicates.

Isolation and characterisation of lubricating oil utilizing bacteria and fungi

The bacterial species indigenous to the mechanic soil samples were isolated by pour plate technique using 0.1 ml aliquots of appropriate dilution into nutrient agar plates. Individual cultures were identified by morphological and biochemical techniques using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

The fungi species indigenous to the mechanic soil were isolated using Potato Dextrose Agar (PDA) into which streptomycin (50 mg/ml) in order to suppress bacterial growth. Fungal isolates were characterized as described by Barnett and Hunter (1972).

The isolated cultures of bacteria and fungi were screened for the ability to utilise lubricating as sole carbon source.

Determination of the ability of bacterial and fungal isolates to utilise lubricating oil

A known volume of 150 ml of the basal medium (minimal salt medium, composition 10 g NaCl, 0.29 g KCl, 0.42 g $MgSO_4$, 0.83 g KH_2PO_4, 0.42 g $NaNO_3$, 1.25 g $NaHPO_4$, 100 ml distilled water, pH 7.2), was dispensed into 250 ml conical flasks and lubricating oil was introduced separately into flasks at 1.0% v/v after sterilization (Okpokwasili and Okorie, 1988). Overnight broth cultures (20 g/l of nutrient broth for bacteria and 35 g/l of malt extract broth for fungi) of each organism was seeded into each flask and incubated in a gyratory shaker incubator (New Brunswick Scientific Incubator Shaker) at 150 rev/min and 30°C. Utilisation of lubricating oil was monitored at two days interval for 14 days by monitoring bacterial and fungal growth measured by viable count on nutrient agar. The optical density was determined at 600 nm wavelength with PG T70 U.V/VIS spectrophotometer and changes in ionic concentration, pH, was determined with pH meter (Model Hama microprocessor P211 pH meter). Fungi was harvested on the filter paper by filtration and dried in the oven, the weight was then determined using the digital mettler balance P 163.

RESULTS AND DISCUSSION

10 bacterial species which include: *Pseudomonas stutzeri, P. putida, P. aeruginosa, P. mallei, Bacillus licheniformis, B. cereus, B. Subtilis, Corynebacterium* sp.*, Alcaligenes eutrophus* and *Enterobacter aerogenes* and five fungal species which include: *Aspergillus flavus, A. niger, A. terreus, A. ochraceus,* and *Trichoderma* sp. were isolated from contaminated soil in this study and capable of utilizing lubricating oil as carbon source. In addition, three bacterial species which include: *P. putida, B. cereus* and *B. subtilis* and five fungal species which include: *A. niger, A. flavus, Rhizopus* sp., *Penicillum* sp., and *A. terreus* were isolated from control soil samples. Lin and Mandari (2007) reported that three isolates with best oil degradation ability were identified as *Flavobacterium* sp., *A. calcoaceticum* and *P. aeruginosa*, using biochemical tests and confirmed by partial

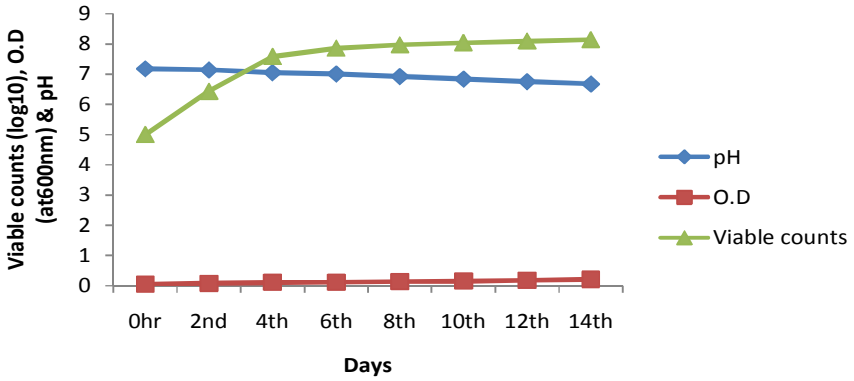

Figure 1. Changes in viable counts, optical density and pH (*Pseudomonas stutzeri*).

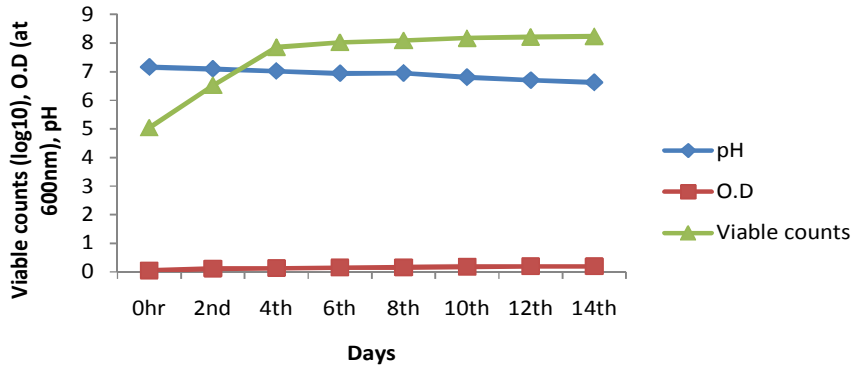

Figure 2. Changes in viable counts, optical density and pH (*Bacillus licheniformis*).

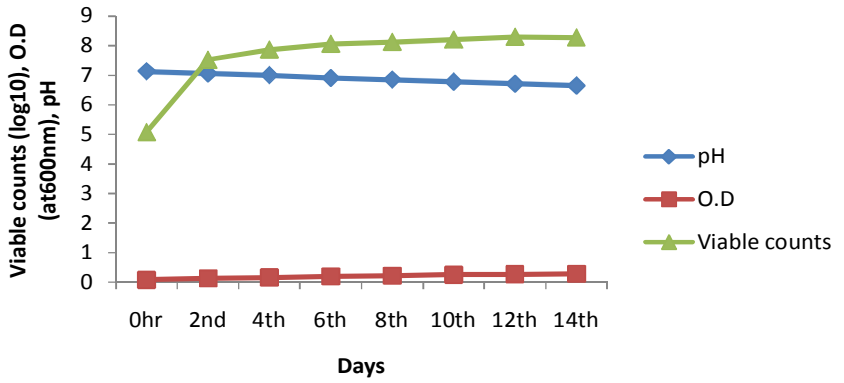

Figure 3. Changes in viable counts, optical density and pH (*Pseudomonas putida*).

sequencing of 16S rDNA. Oil degraders isolated from contaminated and uncontaminated soils included *P. fluorescence*, *Serratia marcescens* and *Bacillus mycoides* (Akochere et al., 2008). Oboh et al. (2006) reported that the fungal isolates obtained in their study were mainly *Aspergillus* species, while others were *Trichoderma*, *Penicillum*, *Rhizopus* and *Rhodotorula* species which were all able to utilise hydrocarbon as carbon source. In this study all the bacterial and fungal isolates were able to grow on lubricating oil as sole source of carbon and energy when screened for ability to utilise lubricating oil as sole carbon source, this results are similar to the results obtained by Oboh et al (2006).

The growth profiles determined by monitoring the optical density, total viable counts, dry weights and pH of the culture utilizing lubricating oil as sole carbon and energy source are shown in Figures 1 - 15. The total viable counts increased significantly with optical density

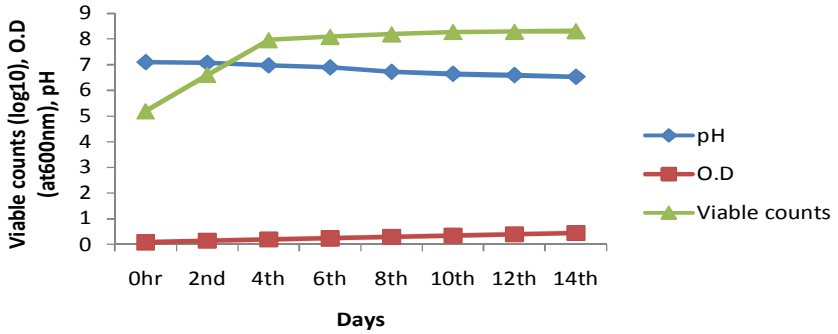

Figure 4. Changes in viable counts, optical density and pH (*Pseudomonas aeruginosa*).

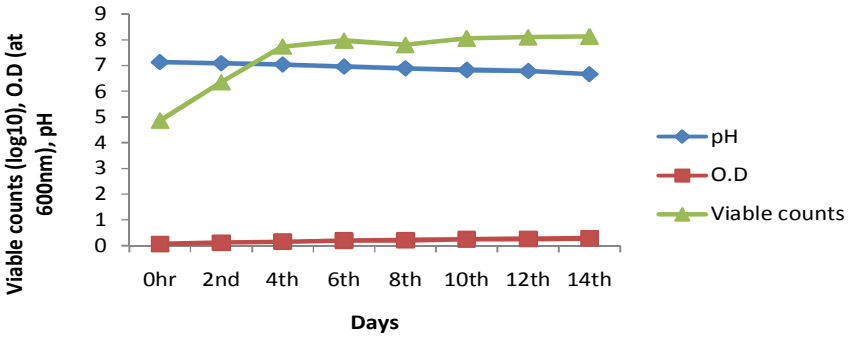

Figure 5. Changes in viable counts, optical density and pH (*Bacillus cereus*).

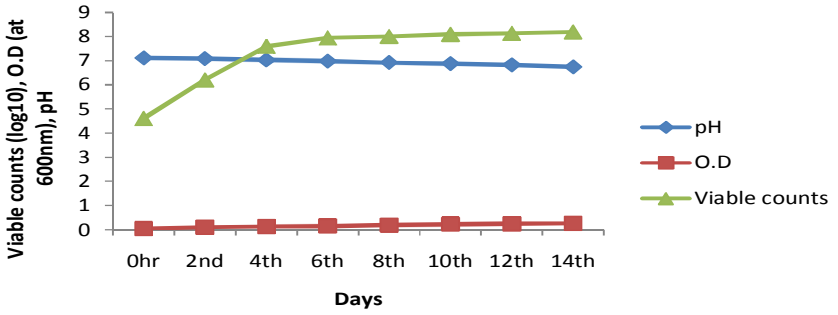

Figure 6. Changes in viable counts, optical density and pH (*Corynebacterium* sp.).

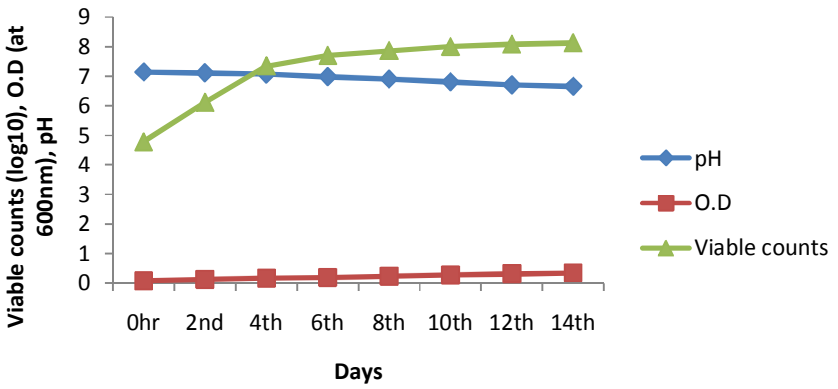

Figure 7. Changes in viable counts, optical density and pH (*Alcaligene eutrophus*).

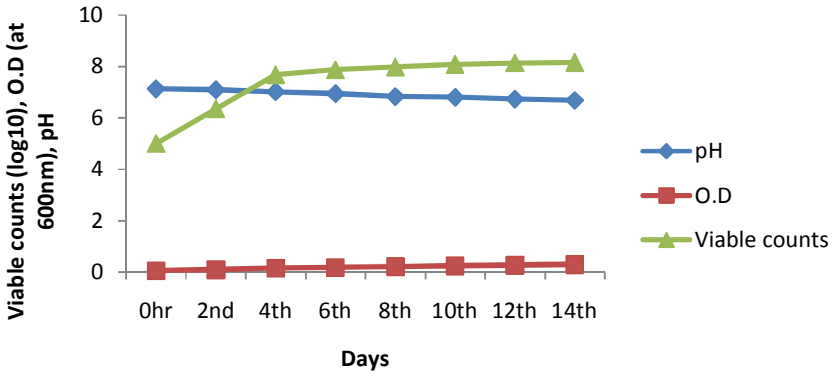

Figure 8. Changes in viable counts, optical density and pH (*Pseudomonas mallei*).

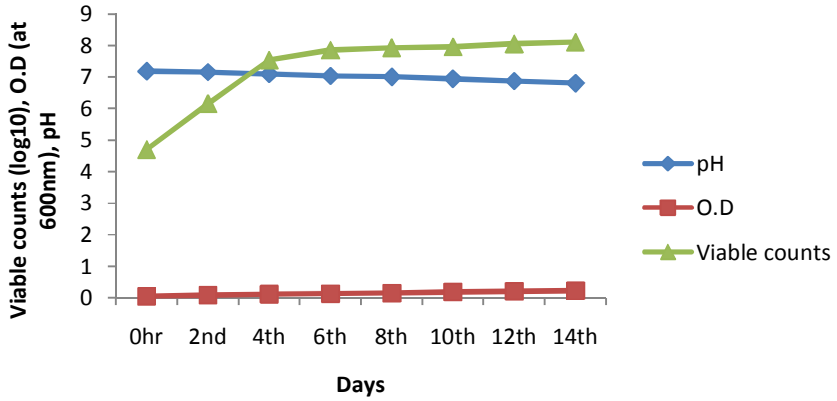

Figure 9. Changes in viable counts, optical density and pH (*Enterobacter aerogenes*).

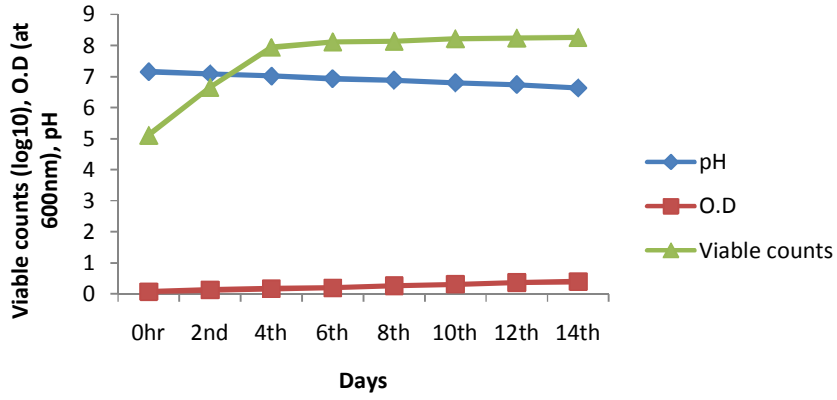

Figure 10. Changes in viable counts, optical density and pH (*Bacillus subtilis*).

and dry weights of fungi as the days of incubation progressed from the 0 h to the 14th day ($P < 0.001$) (Tables 1, 2,3, 4 and 5).There was significant decrease in pH ($P < 0.001$) (Table 5) as fungal cells metabolised lubricating oil (Figures 1 - 15), meanwhile there was no significant changes in pH as growth of the bacterial isolates progressed from the 0 h to the 14th day of incubation ($P = 0.391$) and similarly between each bacteria ($P = 0.532$) (Table 3). There was no significant difference in the changes in viable counts between each fungi ($P = 0.995$) (Table 5). Similar trend was observed for the difference in changes in fungal dry weights between each fungi ($P = 0.986$) (Table 4). The changes in pH could be attributed to the production of acidic

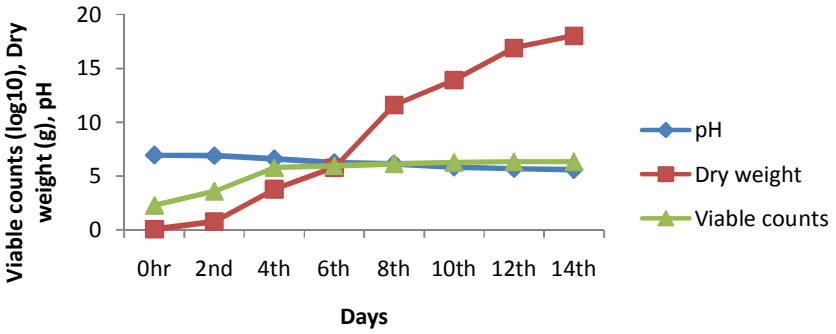

Figure 11. Changes in viable counts, dry weght and pH (*Aspergillus flavus*).

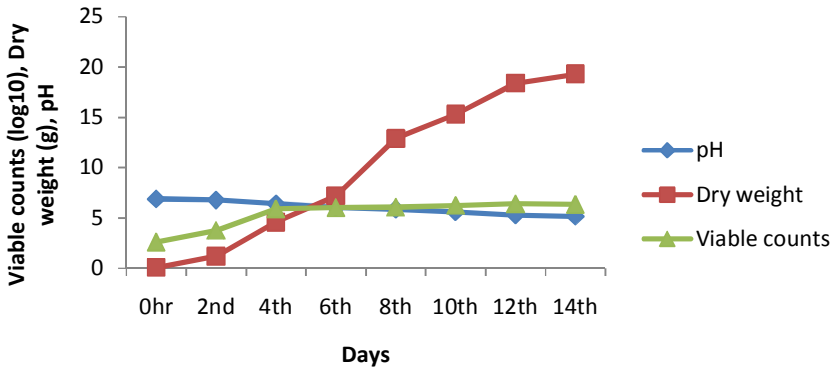

Figure 12. Changes in viable counts, dry weight and pH (*Aspergillus niger*).

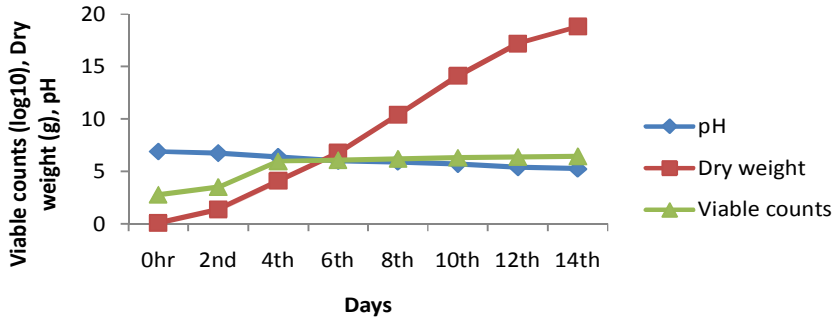

Figure 13. Changes in viable counts, dry weight and pH (*Aspergillus terreus*).

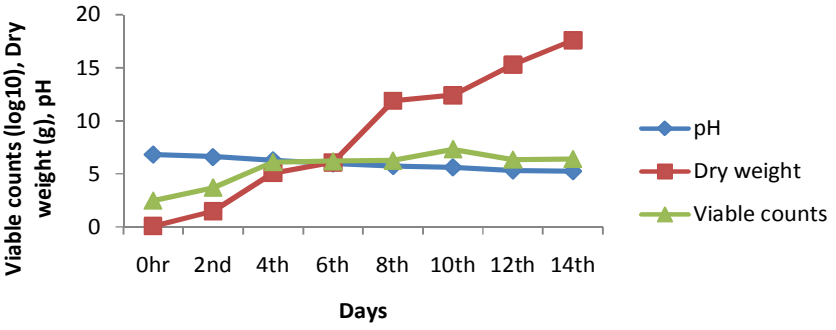

Figure 14. Changes in viable counts, dry weight and pH (*Aspergillus ochraceus*).

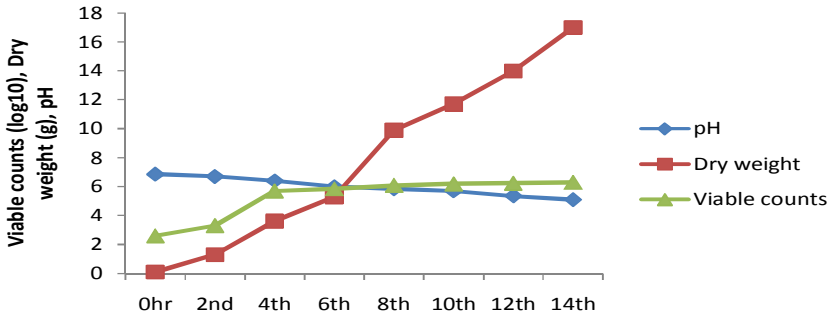

Figure 15. Changes in viable counts, dry weight and pH (*Trichoderma* sp.).

Table 1. ANOVA: Effect of bacterial growth on changes in optical density.

Factors	P-value
Bacteria	0.031
Days of incubation	<0.001

Table 2. ANOVA: Effect of bacterial growth on changes in viable counts.

Factors	P-value
Bacteria	0.999
Days of incubation	<0.001

Table 3. ANOVA: Effect of bacterial growth on changes in pH.

Factors	P-value
Bacteria	0.532
Days of incubation	0.391

Table 4. ANOVA: Effect of fungal growth on changes in dry weight.

Factors	P-Value
Fungi	0.986
Days of incubation	<0.001

Table 5. ANOVA: Effect of fungal growth on changes in viable counts.

Factors	P-value
Fungi	0.995
Days of incubation	<0.001

Table 6. ANOVA: Effect of fungal growth on changes in pH.

Factors	P-value
Fungi	0.870
Days of incubation	<0.001

metabolites. Similar observations were made by Nweke and Okpokwasili (2003). *P. aeruginosa* showed the fastest growth rate on lubricating oil producing the highest optical density and viable count values of 0.441 and 8.31, respectively after the 14th day of incubation of all bacterial isolates used in this study (Figure 4). *P. aeruginosa* was responsible for producing lowest pH conditions of all bacterial isolates, value of 6.53 after 14 days of incubation. In contrast, *E. aerogenes* had the highest pH value of 6.8 (Figure 9). *B. licheniformis* and *E. aerogenes* recorded the lowest optical density and viable count values of 0.19 and 8.1 of all bacterial isolates after 14th day of incubation (Figures 2 and 9).

The results obtained in this study showed that the bacterial isolates grew maximally on lubricating oil especially *P. aeruginosa* which showed the best bacterial growth utilizing lubricating oil as carbon source. The growth profiles have shown that none of the bacterial isolates exhibited lag phases. This observations have been reported previously (Amund, 1984; Okeretungba and Ezeronye, 2003; Oboh et al., 2006) and can be attributed to the constitutive expression of hydrocarbon catalyzing enzymes or physiological owing to previous exposure to exogenous hydrocarbons in lubricating oil content of the mechanic soils. Other microorganisms isolated in this study have been reported as hydrocarbon utilizers, for example, *Pseudomonas putida* was found by Britton (1988) to produce enzyme mono-oxygenase linked to electron carrier rubredoxin during hydroxylation of n-alkane to produce n-alkane-1-ol. A species of *P. fluorescence* capable of producing dehydrogenase enzyme that attacks aromatic naphthalene to produce cathecol had been reported by Mark (1990).

The utilization of hydrocarbon in lubricating oil resulted in gradual reduction in the oil layer and complete disappearance of the oil with prolonged incubation. The reduction in pH of the culture fluids in the experimental

flasks within the 14 day incubation period further confirmed chemical changes of the lubricating oil substrate which must have been precipitated by microbial enzymes. Similar observations were recorded by Atlas and Bartha (1972).

The correlation analysis for bacterial isolates indicated negative correlation between optical density and pH (correlation coefficient = +0.174), and between viable counts and pH (correlation coefficient = +0.159). Meanwhile there was positive correlation between viable counts and optical density (correlation coefficient =.735). For the fungal isolates there was also negative correlation between dry weights and pH (correlation coefficient = +0.958) and between viable counts and pH (correlation coefficient =-0.830). The correlation analysis between fungal dry weights and viable counts showed positive correlation (0.786).

A. niger and *A. terreus* of all the fungal isolates used in this study showed the best and fastest growth on the substrate lubricating oil recording the highest dry weight and viable count values of 19.3 and 6.41, respectively (Figures 12 and 13). While *Trichoderma* sp. (Figure 15) had the lowest pH, dry weight and viable count values of 5.1, 17 and 6.3, respectively. Meanwhile *A. flavus* had the highest pH of 5.6 (Figure 11). The fungal species have been able to produce the most acidic conditions in culture from the 0 h to the 14th day of incubation (Figures 11 - 15) this might be due to the ability to produce large quantities of acidic metabolites. The ability of fungal isolates which include, *Aspergillus fumigatus, Aspergillus oryzae, Aspergillus wentii, Aspergillus flavus, Aspergillus niger, Trichoderma* sp*., Penicillum notatum, Rhizopus stolonifer* and *Rhodotorula* sp. to utilize hydrocarbon substrate as carbon and energy source have been reported by Oboh et al. (2006). Some strains of *Bacillus* sp. and species of fungi including *Aspergillus* spp. and *Fusarium* sp. capable of initiating the degradation of n-alkanes by sub terminal oxidation have been reported (Watkinson and Morgan, 1990).

It is evident from this study that lubricating oil degrading microorganisms could readily be isolated from mechanic soils without need for time consuming enrichment procedures. This study has shown that the microorganisms isolated from mechanic soils have potential application in the bioremediation of sites polluted with lubricating oil.

REFERENCES

Akoachere TK, Akenji NT, Yongbai NF, Nkwelang G, Ndip NR (2008). Lubricating oil-degrading bacteria in soils from filling stations and auto-mechanic workshops in Buea, Cameroon:occurrence and characteristics of isolates. Afr. J. Biotechnol., 7(11): 1700-1706.

Amund OO, Adebiyi AG (1999). Effect of viscosity on the biodegradability of automobile lubricating oils. Tribol. Intern., 24: 235-237.

Anoliefo GO, Edegbai BO (2000). Effect of spent engine oil as a oil contaminant on the growth of two egg elephant species; *Solanum melongena* L. and *S. incanum*. J. Agric. For. Fish., 1: 21-25.

Antai SP (1990). Biodegradation of Bonny light crude oil by *Bacillus* sp. and *Pseudomonas* sp. Waste Manage., 10: 61-64.

Atlas RM, Bartha R (1972). Degradation and mineralisation of petroleum by two bacteria isolated from coastal waters. Biotechnol. Bioeng., 14: 297-308.

Barathi S, Vasudevan N (2001). Utilisation of petroleum hydrocarbons by *Pseudomonas fluorescens* isolated from petroleum contaminated soil. Environ. Int., 26: 413-416.

Bhattacharya D, Sarma PM, Krishran S, Mishra S, Lal B (2002). Evaluation of genetic diversity among *Pseudomonas catrorellois* strains isolated from oily sludge-contaminated sites. Appl. Environ. Microbial., 69(3): 1435-1441.

Blodgett WC (2001). Water-soluble mutagen production during the bioremediation of oil-contaminated soil. Florida Scientist, 60(1): 28-36.

Eriksson M, Dalhammer G, Borg-Karlson AK (1999). Aerobic degradation of a hydrocarbon mixture in natural uncontaminated potting soil by indigenous microorganisms at 20^{0}C and 6^{0}C. Appl. Microbiol. Biotechnol., 51: 532-535.

Fall RR, Brown JL, Schaeffer TL (1979). Enzyme recruitment allows the biodegradation of recalcitrant-branched hydrocarbons by Pseudomonas citronellosis. Appl. Environ. Microbiol., 38: 715-722.

Heitkamp M, Cerniglia CE (1988). Mineralisation of polyaromatic hydrocarbons by a bacterium isolated from sediment below an oil field. Appl. Environ. Microbiol., 54: 1612-1614.

Holt JG, Kreig NR, Sneath PHA, Stanley JT, William ST (1994). Bergey's manual of Determinative Bacteriology. Baltimore, USA: William and Wikins.

Johnson K, Anderson S, Jacobson CS (1996). Phenotypic and genotypic characterisation of Phenanthrene-degrading fluorescent Pseudomonas biovars. Appl. Environ. Microbial., 62: 3815-3825.

Kiyohara H, Takizawa N, Nagao K (1992). Natural distribution of bacteria metabolizing many kinds of polyaromatic hydrocarbons. J. Ferment. Bioeng., 74: 49-51.

Mandari T, Lin J (2007). Isolation and characterisation of engine oil degrading Indigenous microorganisms in Kwazulu-Natal, South Africa. Afr. J. Biotechnol., 6(1): 23-27.

Mark SR (1990). The bodegradation of aromatic hydrocarbons by bacteria. Biodegradation, 1: 191-206.

Mishra S, Joyt J, Kuhad RC, Lal B (2001). Evaluation of inoculum addition tostimulate in-situ bioremediation of oily-sludge contaminated soil. Appl. Environ. Microbiol., 67(4): 1675-1681.

Nweke CO, Okpokwasili GC (2003). Drilling fluid based oil biodegradation potential of a soil *Staphylococcus* species. Afr. J. Biotechnol., 2(9): 293-295.

Oboh OB, Ilori OM, Akinyemi OJ, Adebusoye AS (2006). Hydrocarbon degrading Potentials of bacteria isolated from a Nigerian Bitumen (Tarsland) deposit. Nature Sci., 4(3): 51-57.

Odjegba VJ, Sadiq AO (2002). Effect of spent engine oil on growth parameters, Chlorophyll and protein levels of *Amaranthus hybridus* L. The Environmentalist, 22: 23-28.

Okerentugba PO, Ezenronye OU (2003). Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. Afr. J. Biotechnol., 2: 288-292.

Okpokwasili GC, Okorie BB (1988). Biodeterioration potentials of microorganisms isolated from car engine lubricating oil. Tribol. Int., 21: 215-220.

Osubor CC, Anoliefo GO (2003). Inhibitory effect of spent lubricating oil on the growth and respiratory function of *Arachis hypogea* L. Benin Sci. Dig., 1: 73-79.

Prenafeta-Boldu XF, Kuhn AD, Ankle H, Van Groenestijn JW (2001). Isolation and characterisation of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. Mycological Res., 4: 477-484.

Rusansky S, Avigad R, Michaeli S, Gutnick DL (1987). Involvement of a plasmid In growth and dispersion of crude oil by *Acinetobacter calcoaceticus* RA57. Appl. Environ. Microbiol., 53: 1918-1923.