The Bioscientist Vol. 3(1): 1-13, January 2015
Available online at http://www.bioscientistjournal.com

Full Length Research Paper

INVESTIGATIONS ON BIODEGRADATIVE POTENTIALS OF BACTERIA FROM OIL-POLLUTED SOILS ON BIODIESEL, BIODIESEL BLEND AND PETRODIESEL.

*[1]Balogun,K.I., [1]Arekemase, M.O., [1]Oyeyiola, G.P.and[2]Ameen, M.O.
[1]Department of Microbiology,University of Ilorin, Ilorin,Nigeria
[2]Department of Chemistry,University of Ilorin, Ilorin, Nigeria.
*Corresponding author's email: Abdulkabiru@gmail.com

ABSTRACT
Soil samples collected from engine oil dump sites, diesel polluted sites and uncontaminated plots (controls) were analyzed for oil-degrading and heterotrophic bacteria following standard microbiological and biochemical methods. The pH readings of the different bacterial isolates grown in Mineral Salt Media (MSM) for 16 days, Optical density readings, enumeration of bacterial isolates in the different MSM were used as indices for comparison of the rate of biodegradation of the diesel types.Nine indigenous microorganisms were isolated from both the engine oil and petrodiesel contaminated soils using the enrichment technique. Oil degraders isolated include: *Pseudomonas aeruginosa, Pseudomonas cepacia, Staphylococcus schleiferi, Pseudomonas fluorescens, Bacillus subtilis, Klebsiellapneumoniae, Staphylococcus chromogenes, Escherichia coli and Micrococcus varians.*Heterotrophic bacterial counts were significantly higher ($P < 0.05$) in non-impacted than in impacted soils. Conversely, the population of oil degraders was significantly lower in non-impacted than in impacted soils. The pH range was 3.90-5.70 while the absorbance at 600nm range was 0.10 -1.17. The bacterial plate counts in MSM supplemented with biodiesel were higher than in those supplemented with biodiesel blend and petrodiesel. Results show that biodiesel is more easily and faster biodegraded than petrodiesel. This finding could be exploited in case of oil-spill clean-up campaigns.

Key words: Absorbance, Biodegradation,Biodiesel, Oil-spill, Petrodiesel.

INTRODUCTION

Petroleum products continue to be used as the principal source of energy; however, despite its important usage, petroleum hydrocarbons also act as a global environmental pollutant. Since, the petroleum hydrocarbons are used widely, oil spills are inevitable even in virtually uninhabited areas like Antarctica. Hydrocarbons are biopersistent, bioaccumulative and can cause deleterious effects to aquatic fauna and flora as well as to humans (Benson *et al*., 2007; Dorn *et al.*, 1998).

Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. This can cause water and soil contamination (Edgara*et al.*, 2000; Namkoong *et al.*, 2002). The main causes of oil spill in Nigeria, according to Okpokwasili (1996) include criminal damage (sabotage and crude theft, bunkering), equipment failure e.g. wellhead blow out, valve and flanges failure etc,

corrosion either through chemical or biological agents, human error and technical failure.

Engine oil is a complex mixture of hydrocarbons and other organic compounds including some organometallic constituents (Butler and Mason, 1997). As it is inevitable for the efficient and effective functioning of the automobile engines, soil contamination with used engine oil is becoming one of the major environmental problems (Mandri and Lin, 2006), mainly due to uncontrollable disposal, particularly in developing economies.

Petroleum diesel, also called petrodiesel or fossil diesel is produced from the fractional distillation of crude oil between 200^{o}C (392^{o}F) and 350^{o}C (662^{o}F) at atmospheric pressure, resulting in a mixture of carbon chains that typically contain between 8 and 21 carbons atoms per molecule (Chris, 2007).

Biodiesel or fatty-acid methyl ester (FAME), according toRefat (2009), is a renewable, biodegradable, environmental benign, energy efficiency substitution fuel which can fulfill energy security needs without sacrificing engines' operational performance. Thus, it provides a feasible solution to the twin crises of fossil fuel depletion and environmental degradation. It is obtained from vegetable oil or animal fats (biolipids) which have been transesterified with methanol (Robert, 2004). The exhaust emissions of particulate matter (soot) and of total hydrocarbons (a contributing factor in the localized formation of smog and ozone) from biodiesel have been found to be respectively 30% and 93% lower than that from petrodiesel (AFDC, 2012).

Biodiesel can be blended and used in many different concentrations, including B100 (pure biodiesel), B20 (20% biodiesel, 80% diesel), B5 (5% biodiesel, 95% diesel) and B2 (2% biodiesel, 98% diesel). B20 is the most common biodiesel blend in the United States because it represents a good balance of cost, emissions, cold-weather performance, materials compatibility, and ability to act as a solvent. B20 and lower-level blends generally do not require engine modification (AFDC, 2012).

Bioremediation uses biodegradation to achieve its goal as it is defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Bioremediation technologies can be classified as in situ or ex-situ. In situ bioremediation involves treating the contaminated material at the site, while ex-situ involves the removal of the contaminated materials to be treated elsewhere.

Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment. Hydrocarbon degrading bacteria can metabolize both biodiesel and petrodiesel at the same time. It is known that greater degradation of oil pollutants is carried out in situ by a consortium of microorganisms and more than 200 species of bacteria, fungi and even algae can biodegrade hydrocarbons (Onifade and Abubakar, 2007). Many physical, chemical and environmental factors like temperature, nutrients, oxygen, biodegradability, photo-oxidation, bioavailability, soil moisture, soil acidity and alkalinity etc affects the process of biodegradation of hydrocarbons (Rahman*et al*., 2003; Venosa and Zhu, 2003; Delille*et al.*, 2004; Pelletier *et al*., 2004; Trindade*et al*., 2005).

The most prevalent bacterial hydrocarbon degraders belong, in descending order, to the genera *Pseudomonas, Achromobacter, Flavobacterium, Nocardia, Arthrobacter, Vibrio, Bacillus, Micrococcus,* and *Acinetobacter.* Other genera of bacteria capable of degrading hydrocarbons include *Actinomyces, Aeromonas, Alcaligenes, Corynebacterium, Erwinia, Klebsiella, Lactobacillus, Leucothrix, Moraxella, Mycobacterium, Peptococcus, Sarcina, Sphaerotilus, Spirillum, Streptomyces,* and *Xanthomonas* (Atlas, 1995). According to

Bhattacharya *et al*. (2002), a wide range of hydrocarbon utilizers (HCUs) found to be useful in the soil include the following genera: *Pseudomonas, Rhodococcus, Mycobacterium, Bacillus, Acinetobacter, Providencia, Flavobacter, Corynebacterium and Streptococcus*.

One question that has been debated for a decade according to Zhang *et al*. (1998); DeMello*et al*. (2007); Mariano *et al*. (2008a) and Owsianiak*et al*. (2009) is if diesel blended with biodiesel is more biodegradable than neat diesel.

The clean-up of contaminated areas can be achieved with bioremediation, a technique based on the action of microorganisms, which turn hazardous contaminants into non-toxic substances such as CO_2, water and biomass.

This study seeks to isolate and identify bacteria from diesel and engine oil contaminated soils capable of effectively degrading and cleaning up waste biodiesel, petrodiesel and engine oil with a view of comparing the rates of biodegradability of petrodiesel, biodiesel (from *Jatrophacurcas*) and biodiesel blend

MATERIALS AND METHODS

Sampling sites

The study was carried out in Ilorin, Kwara State using 6 sampling sites each for the engine oil and diesel polluted soils.

Collection of diesel and engine oil contaminated soil samples

Samples were collected from contaminated sites and reference areas (control soil) about 100 m from contaminated sites. At each sampling point, two samples (100g each) were collected with aluminium foil at depths of 0 -15 cm using a hand auger/sterile trowel followed by bulking. Samples were immediately transported to the laboratory for analysis.

Source of petrodiesel and biodiesel.

The petrodiesel was brought from Nigerian National Petroleum Corporation (NNPC) mega station in Ilorin and stored in the dark at ambient temperature throughout the study while the biodiesel was prepared locally.

The bench scale trans-esterification reactions to produce methyl esters (biodiesel) from the *Jatropha*seed oil was carried out in a 500 ml conical flask, following the modified method of Benjamin *et al*. (2007).

Preparation of culture media

The culture media used during the research were Nutrient Agar (NA) and Modified Mineral Salts Media (MSM). These culture media were prepared as follows:

- **Preparation of Nutrient Agar**

This medium was prepared according to manufacturer's specification.

- **Preparation of MSM using 2ml of biodiesel as carbon source.** The method of Okpokwasili and Ananchukwu (1988) was used. One litre of

The following salts were weighed into a flask:

K_2HPO_4 (Potassium phosphate dibasic anhydrous)	- 1.4g
$(NH_4)_2PO_4$	- 0.2g
KH_2PO_4 (Potassium dihydrogenortho phosphate)	- 0.6g
$MgSO_4.7H_20$	- 0.6g
Agar-Agar	- 4.0g

The method of Okpokwasili and Ananchukwu (1988) was used. One litre of sterilized distilled water was added to the mixture and mixed thoroughly. It was then

autoclaved at 121^0C for 15 mins and allowed to cool to about 50^0C before adding 2ml of biodiesel.

- **Preparation of Modified MSM using 2ml of petrodiesel as carbon source.**

The same medium was prepared as (b) above except that 2ml of biodiesel was replaced with 2ml of petrodiesel.

- **Preparation of Modified MSM using 0.4ml (or 20%) biodiesel and 1.6ml (or 80%) of petrodiesel (i.e**
- **biodiesel blend) as carbon source.**

The same medium was prepared as (b) above except that 2 ml of biodiesel was replaced with biodiesel/petrodiesel blend.

Bacterial counts

One millilitre from serial dilutions of 10^{-3}, 10^{-4} and 10^{-5} were inoculated by spread plate unto appropriate medium for the enumeration of bacterial population. Heterotrophic bacteria were enumerated on Nutrient agar (Biotec) plate after 48-hour inoculated at room temperature (28 ± 2^0C) and counted as colony-forming units (cfu/g) of soil sample. Diesel-utilizing bacterial counts were determined on Mineral Salts Medium (MSM) using vapour phase transfer technique after 7 days incubation at 28 ± 2^0C. Engine oil-utilizing bacterial counts were also determined on Mineral Salts Medium (MSM) after 7 days incubation at 28 ± 2^0C.

Isolation and characterization of diesel oxidizing bacteria (DOB)

The isolation ofdiesel oxidizing bacteria was determined by the method of Asitok and Antai (2006).

Isolation and characterization of used engine oil degrading bacteria

This was also carried out as described above.

Identification of bacterial isolates

Identification was based on the colonial & cellular morphologies and biochemical characteristics of each isolate.

The method of Fawole and Oso (2007) was used in carrying out catalase, urease, Methyl Red &VogesProskaeur, indole, starch hydrolysis, sugar fermentation, and triple sugar iron agar tests while the method of John and Lansing (2002) was used in carrying out citrate utilization,oxidase, haemolysis and coagulase tests.

Screening test on the isolates for utilization of the three types of diesels

The isolates were screened for their ability to utilize the various diesel types as their sole source of carbon and energy for growth as described by Okpokwasili and Okorie (1988).

Statistical analysis

The data obtained were subjected to descriptive statistics and Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Enumeration of bacteria isolated from soil samples

Microorganisms play important roles in the natural environment; they contribute to the geological cycle of elements and transformation of natural chemicals (Watanabe, 2002). Microorganisms are extremely diverse and can adapt to survive in inhospitable environments. Microbes are capable of breaking down many complex molecules by adaptation of their degradative enzyme system (Sohal and Srivastava, 1994). Contaminated sites often harbour a vast array of microbial flora that is capable of utilizing the contaminant as an energy and carbon source (Watanabe, 2002; Das and Mukherjee, 2006).

The total count of bacteria (heterotrophic and hydrocarbon utilizers) isolated from the diesel contaminated soil and engine oil contaminated soil samples are shown in Tables 1 and 2 respectively.

The heterotrophic bacterial counts for the diesel contaminated soil ranged from 1.17×10^6 to 2.7×10^6 cfu/g and hydrocarbon utilizing bacterial plate counts ranged from 1.12×10^5 to 2.8×10^5 cfu/g while the heterotrophic bacterial counts for the engine oil contaminated soil ranged

from 8.7 X 10^5 to 2.05 X 10^6 cfu/g and hydrocarbon utilizing bacterial plate counts ranged from 1.4 X 10^5 to 5.9 X 10^5 cfu/g.

Generally, the heterotrophic bacteria counts were higher in non-impacted soils than in impacted soils and the statistical analysis revealed that the difference in counts between the sample sites was significant. There were higher counts of degraders of diesel and engine oil in impacted soils than in non-impacted soils and there was also significant difference (p=0.05) between the sites.

The relatively low heterotrophic bacterial counts observed in oil contaminated soils can be attributed to the toxic or unfavourable effect of oil contamination (Jensen, 1975). The finding of the presence of higher oil-degrading bacterial populations in contaminated soils corroborates the results of Hubert *et al.* (1997) andMichalcewick (1995) that attributed these high microbial populations to the stimulatory effect of additional carbon and energy source in the form of petroleum products.

The results of heterogeneous, diesel and engine oil utilizing bacterial counts in all the soil samples as presented in Tables 1 and 2 suggest that both the engine oil and diesel utilizing bacteria were adapted to the quantity of hydrocarbons in the environment, hence the high counts of petroleum- utilizing bacteria in heavy oil polluted areas.

Obire and Nwaubeta (2001 and 2002) in a related study on bacteria have reported similar findings, they asserted that percentage of both diesel (hydrocarbon) and engine oil utilizers in a particular environment appears to be an index of the presence of hydrocarbonsinthat environment and environmental exposure to petroleum hydrocarbons. The results also agrees with the works of Mulkins-Phillips and Stewart (1974), they found out that a higher number of microorganisms were found tohave potential to degrade diesel fuel than that found to have biodegradative potential for engine oil. This might be due to higher viscosity of engine oil than that of diesel.

Table 1: Counts of heterogeneous bacteria and diesel utilizing bacteria in diesel polluted soil (x 10^4cfu/g

Type of Bacteria	Generator site, Chemistry Department, Unilorin	Generator house, Bamed Printers, Tanke	Sampling site Generator site near Faculty of Law lecture rooms, Unilorin PS Counts (x 10^4cfu/g)	Truck mechanic workshop, Pipeline road	Base of tank containing diesel, Tanke	Bababeji automobile workshop, Tanke
Heterotrophic	184.00	235.00	212.00	117.00	270.00	270.00
Hydrocarbon utilizers	13.00	18.00	26.00	28.00	24.00	24.00
Percentage Hydrocarbon utilizers	7.10	7.70	12.00	23.70	8.90	8.90

Values represented in the table are means of three replicates (n=3).

Table 2: Counts of heterogeneous bacteria and engine oil utilizing bacteria in engine oil polluted soil (x 10^4cfu/g soil).

Type of Bacteria	Generator house, Bamed Printers, Tanke	Generator repairer's shop,NNPC, Tanke	Sampling site Motorcycle mechanic workshop, Ahmadiyya Mosque, Tanke	Automechanic workshop, Sanrab	Engine oil selling point near the Bus terminus	Bababeji automobile workshop, Tanke
			Counts (x 10^4cfu/g)			
Heterotrophic	90.00	205.00	147.00	87.00	134.00	120.00
Hydrocarbon utilizers	14.00	23.00	19.00	23.00	24.00	59.00
Percentage Hydrocarbon utilizers	15.60	11.20	12.90	26.40	17.90	49.20

Values represented in the table are means of three replicates (n=3)

Characterization of bacterial isolates

A total of nine isolates were identified as follows: *Pseudomonas aeruginosa, Pseudomonas cepacia, Staphylococcus schleiferi, Pseudomonas fluorescens, Bacillus subtilis, Klebsiellapneumoniae, Staphylococcus chromogenes, Escherichia coli and Micrococcus varians.*

Biodegradative ability of bacterial isolate. pH readings of different bacterial isolates grown in Mineral Salt Media for 16 days.

Table 3 showed that after 16 days of biodegradation, *Staphylococcus schleiferi*and *Micrococcus varians* in mineral salt medium supplemented with biodiesel had the least pH of 3.9 and 4.0 respectively. *Micrococcus varians* and *Pseudomonas aeruginosa*in mineral salt medium supplemented with biodiesel/diesel blend had the least pH of 4.1 and 4.4 respectively. *Staphylococcus schleiferi*and *Micrococcus varians* in mineral salt medium supplemented with petrodiesel blend had the least pH of 4.0 and 4.1 in that order.

*Staphylococcus schleiferi*and *Micrococcus varians*exhibited highest biodegradability in mineral salt medium supplemented with biodiesel while *Bacillus subtilis*(pH 5.0) and *Pseudomonas cepacia*(pH 5.0) exhibited lowest biodegradability in mineral salt medium supplemented with biodiesel. *Micrococcus varians, Pseudomonas aeruginosa*exhibited highest biodegradability in mineral salt medium supplemented with biodiesel/diesel blend while *Klebsiellapneumoniae*(pH 5.2) and *Escherichia coli* (pH 5.2) exhibited lowest biodegradability in mineral salt medium supplemented with biodiesel. *Staphylococcus schleiferi*and *Micrococcus varians*exhibited highest biodegradability in mineral salt medium supplemented with biodiesel while *Escherichia coli* (pH 5.4) and *Klebsiellapneumoniae* (pH 5.4) exhibited lowest biodegradability in mineral salt medium supplemented with biodiesel.

There is growing evidence that *B. subtilis*could be effective in clearing oil spills (Ghazali*et al*., 2004). Khan *et al.* (2011) also reported that *Bacillus subtilis*is more tolerant to high levels of oils due to its resistant endospores. According to Atlas *et al*. (1995), the most prevalent bacterial hydrocarbon degraders belong, in descending order, to the genera *Pseudomonas, Achromobacter, Flavobacterium, Nocardia, Arthrobacter, Vibrio, Bacillus, Micrococcus,* and *Acinetobacter*. Other genera of bacteria capable of degrading hydrocarbons include *Actinomyces, Aeromonas, Alcaligenes, Corynebacterium, Erwinia, Klebsiella, Lactobacillus, Leucothrix, Moraxella, Mycobacterium, Peptococcus, Sarcina, Sphaerotilus, Spirillum, Streptomyces,* and *Xanthomonas.*

Recent studies have shown that some species of microorganisms including members of the genus *Pseudomonas, Bacillus, Micrococcus* and *Aspergillus*have potentials of biodegrading hydrocarbon contaminants (Santhini*et al*., 2009).

The preliminary biodegradation assay was carried out to determine the various types of diesel degradation capabilities of the nine indigenous microbial cultures that were isolated from contaminated environments. Preliminary investigation of these cultures in the modified mineral salts media together with agitation and aeration for 16 days allowed microbial degradation of the diesel oil as shown in (Tables 3 and Figure 1).

It was observed that the pH of all the cultures decreased while the control recorded

Table 3: pHand absorbance readings of different bacterial isolates grown in Mineral Salt Media (MSM) for 16 days.

Bacterial isolates code	Bacterial isolates	pH readings			Absorbance at 600nm		
		MSM (+ Biodiesel)	MSM (+ Biodiesel/ Diesel Blend)	MSM (+Diesel)	MSM (+ Biodiesel)	MSM (+ Biodiesel/ Diesel Blend)	MSM (+Diesel)
B_{D1}/B_{E5}	*P. aeruginosa*	4.10	4.40	4.60	1.01	1.02	1.08
B_{D2}	*P. cepacia*	5.00	5.10	5.30	0.38	0.31	0.19
B_{D3}	*S. schleiferi*	3.90	5.00	4.00	0.59	0.78	0.16
B_{D4}	*P. fluorescens*	4.60	4.90	5.20	0.41	0.44	0.60
B_{D5}	*B. subtilis*	5.00	5.10	5.10	0.30	0.53	0.41
B_{E1}	*K. pneumoniae*	5.00	5.20	5.40	0.20	0.29	0.54
B_{E2}	*S. chromogenes*	4.70	4.60	4.60	0.83	1.05	1.12
B_{E3}	*E. coli*	4.20	5.20	5.40	0.36	0.68	0.15
B_{E4}	*M. varians*	4.00	4.10	4.10	0.92	0.97	1.17
Control	*No inoculums*	5.70	5.60	5.70	0.05	0.27	0.11

Legend: B_{D1}-B_{D5} -Bacteria isolated from diesel polluted soil.
B_{E1}- B_{E5} -Bacteria isolated from engine oil polluted soil.

no change (Table 3). This may be due to the production of acidic metabolites in the medium (Moro *et al.*, 2001). It was also observed that the higher the drop in pH the greater the degradation thus suggesting the production of more acidic metabolites (Ogunbayo, 2012).

Optical Density Readings

Table 3 also showed the result of variation in the absorbance at 600 nm of mineral salt media (MSM) exhibited by different bacterial isolates after 16 days. *Pseudomonas aeruginosa*and *Micrococcus varians*in mineral salt medium supplemented with biodiesel had the highest absorbance of 1.01 and 0.92 respectively. *Staphylococcus chromogenes*and *Pseudomonas aeruginosa*in mineral salt medium supplemented with biodiesel/diesel blend had the highest absorbance of 1.05 and 1.02 respectively. *Micrococcus varians*and *Staphylococcuschromogenes*in mineral salt medium supplemented with diesel showed the highest absorbance of 1.17 and 1.12 respectively.

The absorbances of the controls are 0.05, 0.27 and 0.11 for the biodiesel, biodiesel/petrodiesel blend and diesel respectively. The absorbance ranged from 0.20 to 1.01, 0.29 to 1.02 and 0.15 to 1.17 for the mineral salt media supplemented with biodiesel, biodiesel/petrodiesel blend and diesel in that order. *Pseudomonas aeruginosa*and *Micrococcus varians*in mineral salt medium supplemented with biodiesel had the highest absorbance of 1.01 and 0.92 respectively. *Staphylococcus chromogenes*and *Pseudomonas aeruginosa*in mineral salt medium supplemented with biodiesel/diesel blend had the highest absorbance of 1.05 and 1.02 respectively. *Micrococcus varians*and

*Staphylococcus chromogenes*in mineral salt medium supplemented with diesel showed the highest absorbance of 1.17 and 1.12 respectively. Smita*et al.* (2012) andAsitok and Antai (2006) reported similar findings that the absorbance of broth medium changed according to degradation extent in each tube. The values were significantly different (p=0.05).

Enumeration of Bacterial isolates in the different mineral salt media

The bacterial plate count after 16 days is shown in Figure 1. It was observed that tubes with isolates inoculated into the mineral salts medium containing biodiesel had the highest bacterial counts with *Pseudomonas aeruginosa*and *Pseudomonas cepacia*having the highest counts while tubes with isolates inoculated into the mineral salts medium containing petrodiesel had the lowest bacterial counts with *Staphylococcus chromogenes* and *Escherichia coli* having the lowest count.

In the present study, all isolates proved to be good degraders of all the types of diesel used. This effect was consistent with increased cell growth. Figure 1 showed the highestbacterial counts in tubes containing mineral salts medium supplemented with biodiesel and lowest bacteria count in tubes containing mineral salts medium supplemented with diesel.

The values were significantly different (p=0.05).This corroborates the findings in the works of Zhang *et al*. (1998); Makareviciene and Janulis (2003); Pasqualino*et al*. (2006) andLapinskiene*et al*. (2006) who asserted that biodiesel is more easily biodegraded than fossil diesel and can promote and speed up the biodegradation of hydrocarbons by means of co-metabolism. Also according to the work of Adriano (2008), statistically (Anova, p=0.05) theblend B20 and the pure biodiesel (B100) differed from B0 (petrodiesel) in terms of increment in CO_2 production, a factor of biodegradability test.

Zhang *et al*. (1998) explain that biodiesel is more easily metabolized than diesel because the former is a natural product consisting of pure fatty acids that are hydrocarbon chains with two oxygen atoms attached at one end, which are very biologically active, beingrecognized and attacked immediately by enzymes such as acetyl-CoA dehydrogenase. The biodegradation of diesel, which consists of a large amount of alkanes (hydrocarbon chains from C_{10}-C_{20}) without oxygen attached, requires microorganisms that are adapted to produce enzymes that recognize these molecules. Moreover, the presence of aliphatic cyclic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and alkylbenzenes, as well as their

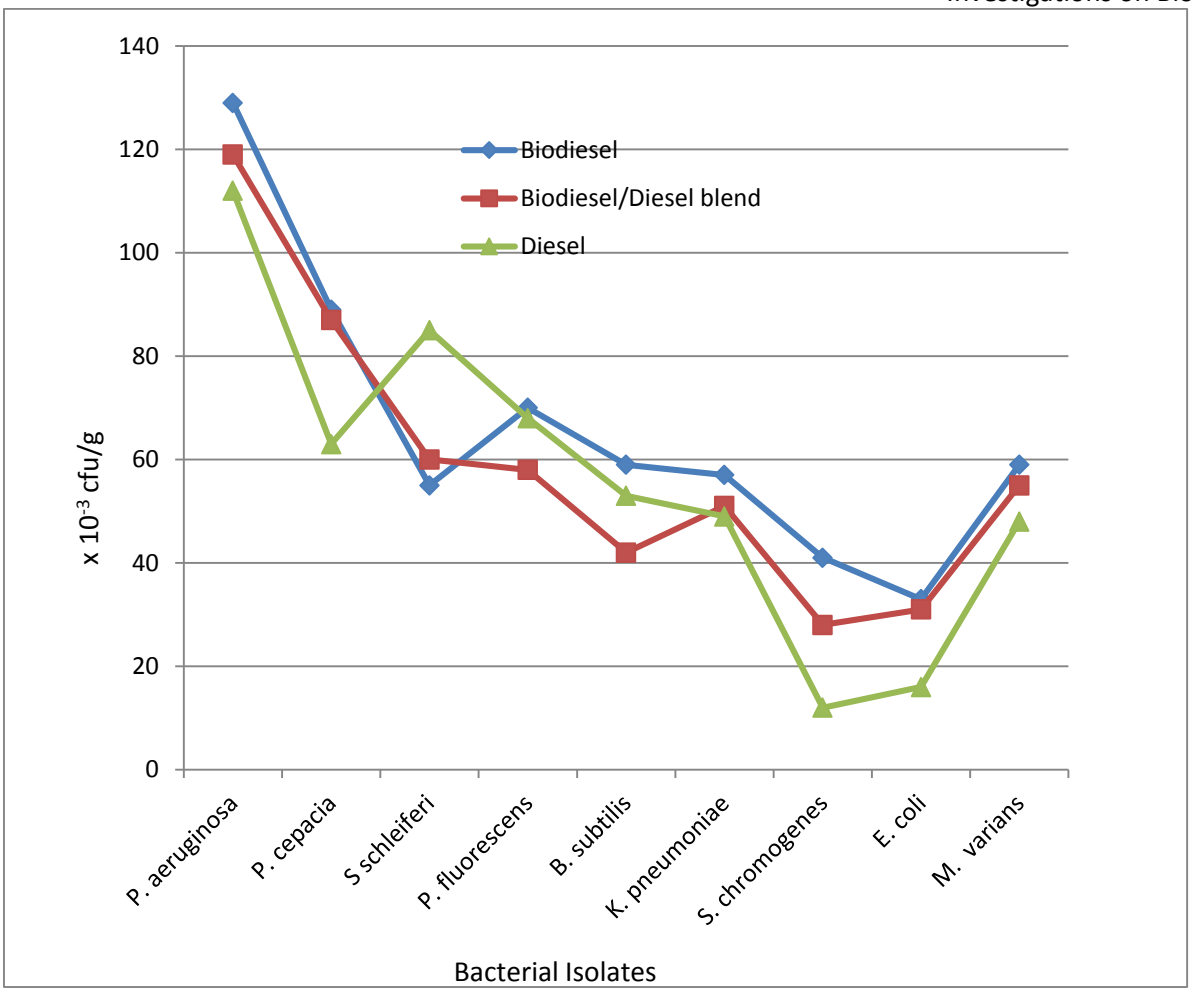

Figure 1: Comparison of bacterial counts ($x10^3$ cfu/g) in Mineral Salts Media supplemented with the different types of diesel after 16 days.

derivatives such as toluene, xylenes and PCBs (phenyl and biphenyls) gives the diesel a composition much more chemically complex.

Furthermore, Zhang *et al.* (1998) and Pasqualino*et al.* (2006) found out that biodiesel can promote and speed up the biodegradation of diesel by means of co-metabolism, that is a term used to describe the process in which microorganisms use a second substrate (readily degradable) as the carbon (energy) source to degrade the first substrate which otherwise is scarcely attacked by the microorganisms when it is the sole carbon source. Based on this concept, Taylor and Jones (2001); Obbard *et al.*(2004) andFernández-Álvarez*et al.* (2006 & 2007) found out that in some cases biodiesel can be applied in contaminated areas as an enhancement agent to bioremediation processes.

CONCLUSION

The practice of bioremediation for the withdrawal of environmental hydrocarbon contaminants is coming into view as an important cost-effective treatment that can be applied for the treatment of marine oil spills and contaminated sites.

The biodegradation of biodiesel, biodiesel/diesel blend and petrodiesel by bacteria isolated from both diesel and engine oil contaminated soils was investigated. Based on this work, bioremediation is a cost-effective technique for remediating all types of diesel contaminated sites as the bacteria capable of degrading these pollutants are in abundance

in nature. However, a number of factors must be taken into consideration before in situ bioremediation can be used. These include: type and concentration of oil contaminated; prevalent climatic conditions; type of environment that has been contaminated; and nutrient content as well as pH of the contaminated site.

Further research will be directed towards understanding the specific constituents of biodiesel, biodiesel/diesel blend and petrodiesel that are biodegraded by the isolates, the impacts of individual isolate in influencing the effectiveness of a microbial association, improving the biodegradability potentials and studying environmental conditions that would encourage in situ bioremediation.

Acknowledgment: The authors would like to express their deepest appreciation to the Management of Department of Microbiology, University of Ilorin for their unflinching support in the course of this project

REFERENCES

Energy efficiency and renewable energy. Alternative Fuels Data Centre (AFDC).www.afdc.energy/fuels/biodiesel_blends.html. Accessed on 23 May 2013.

Asitok, A.D. and Antai, S.P. (2006).Petroleum hydrocarbon utilization and biosurfactant production by Pseudomonas and Bacillus species. Research Journal of Microbiology, 20: 824-831.

Atlas, R.M. (1981). Microbial-degradation of petroleum-hydrocarbons – an environmental perspective.Microbiological Reviews, 45, 180-209.

Atlas, R.M. and Cerniglia, C.E. 1995. Bioremediation of petroleum pollutants.Bioscience, 45(5): 332-338.

Benjamin, I.U., David, O.P., Haruna, M.K. and Emmanuel, O.O. (2007).Determination of optimal catalyst concentration for maximum biodiesel yield from Tiger nut oil.Leonardo Journal of Science, 10: 131-136.

Benson, N.U., Essien, J.P., Williams, A.B. and Ebong, G.A. 2007. Petroleum hydrocarbons accumulation potential of shellfishes from littoral waters of the bight of bonny, Niger Delta, Nigeria.Research Journal of Environmental Science, 1: 11-19.

Bhattacharya, D., Sarma P.M., Krishnan, S., Mishra, S. and Lal, B. (2002). Evaluation of genetic diversity among Pseudomonas citronellolis strains isolated from oily sludge-contaminated sites. Applied Environmental Microbiology, 69 (3): 1435-1441.

Butler, C.S. and Mason, J.R. (1997).Structure-function analysis of the bacterial aromatic ring-hydroxylatingdioxygenases.Advanced Microbial Physiology, 38: 47-84.

Chris, C. (2007). Implementing phytoremediation of petroleum hydrocarons, Method I in Biotechnology, 23: 99-108.

Das K. and Mukherjee, A.K. (2006). Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis*and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India.Bioresource Technology, 1-7.

Demello, J.A., Carmichael, C.A., Peacock, E.E., Nelson, R.K.., Arey, J.S. and Reddy, C.M. (2007). Biodegradation and environmental behavior of biodiesel mixtures in the sea: an initial study. Marine Pollution Bulletin, 54: 894-904.

Dorn, P.B., Vipond, T.E., Salanitro, J.P. and Wisniewski, H.L. (1998).Assessment of the acute toxicity of crude oils in soils using earthworms, Microtox (R) and plants.Chemosphere, 37: 845-860.

Edgara, G.J. and Barret, N.S. (2000).Marine Pollution Bulletin, 40: 36.

Fawole, M.O. and Oso, B.A. (2007).Laboratory Manual of Microbiology. Spectrum Books Limited Ibadan, pp 1-77.

Fernández-Álvarez, P., Vila, J., Garrido-Fernández, J.M., Grifoll, M. and Lema,

J.M. (2006).Trials of bioremediation on a beach affected by the heavy oil spill of the Prestige. Journal of Hazardous Materials, 137: 1523–1531.

Fernández-Álvarez, P., Vila, J., Garrido-Fernández, J.M., Grifoll, M. and Lema, J.M. (2007). Evaluation of biodiesel as bioremediation agent for the treatment of the shore affected by the heavy oil spill of the Prestige . Journal of Hazardous Materials, 147 (3): 914-922.

Ghazali, F.M., Rahman, R.N.Z.A., Salleh, A.B. and Basri, M. (2004).Biodegradation of hydrocarbons in soil by microbial consortium. InternationalBiodeterioration and Biodegradation, 54; 61-67.

Hubert, C., Shen. Y. and Voordouw, G. (1997).Comparison of toluene – degrading microbial communities from soil at different concentrations of toluene.Applied and Environmental Microbiology, 63: 3064-3070.

Jensen, V. (1975). Bacterial flora of soil after application of oily waste .Oikos, 26: 152-158.

John, P.H. and Lansing, M.P. (2002).Laboratory exercises in Microbiology.5thedn.The McGraw-Hill Companies, New York, pp 321-361.

Khan, J.A. and Rizvi, S.H.A. (2011). Advances in Applied Science Research 2 (3): 455.

Lapinskiene, A., Martinkus, P. andRebzdaite, V. (2006). Eco-toxicological studies of diesel and biodiesel fuels in aerated soil.Environmental Pollution, 142: 432-437.

Makareviciene, V. andJanulis, P. (2003). Environmental effect of rapeseed oil ethyl ester.Renewable Energy, 28: 2395-2403.

Mandri, T. and Lin, J. (2006). Isolation and characterization of engine oil degrading indigenous microorganism in Kwazulu-Natal, South Africa. African Journal of Biotechnology, 6 (1): 023-027.

Mariano, A.P., Tomasella, R.C., Oliveira, L.M., Contiero, J. and Angelis, D.F. (2008). Biodegradability of diesel and biodiesel blends. African Journal of Biotechnology, 7: 1323-1328.

Michalcewicz, W. (1995).The influence of diesel fuel oil on the number of bacteria, fungi, actinomycetes and soil microbial biomass.RoczPanstw Zak lHig., 46: 91-97.

Moro, D.D., Opere, B.O., Latunji, C.M., Nwakaudu, A. and Famurewa, O. (2001). Bioremediation of engine oil contaminated site. Journal of Research and Review in Science, 2: 82-86.

Mulkins-Phillips, G.J. and Stewart, J.E. (1974).Distribution of hydrocarbon utilizing bacteria in North Western Atlantic waters and coastal sediments.Canadian Journal of Microbiology, 20:955-962.

Namkoong, W., Hwang, E.Y., Park, J.S. and Choi, J.Y. (2002). Bioremediation of diesel contaminated soil with composting. Environmental Pollution, 119 (1): 23-31.

Obbard, J.P. and Xu, R. (2004). Bioremediation of petroleum contaminated beach sediments: use of crude palm oil and fatty acids to enhance indigenous biodegradation. Water, Air, &Soil Pollution, 157: 149-161.

Obire, O. and Nwaubeta, O. (2001).Biodegradation of refined petroleum hydrocarbons in Soil.Journal of Applied Sciences and Environmental Management, 5: 43-46.

Obire, O. and Nwaubeta, O. (2002).Effect of refined petroleum hydrocarbons on Soil physicochemical and bacteriological characteristics.Journal of Applied Sciences and Environmental Management,6:39-44.

Ogunbayo, A.O., Bello, R.A. and Nwagbara, U. (2012). Bioremediation of Engine Oil Contaminated Site. Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS), 3 (3): 483-489.

Okpokwasili, G.C. (1996). Microbial Degradation of Petroleum Hydrocarbon by Brackish Water Isolates in Nigerian

Wetlands.Akpata, T.V.I and AvenOkoli (ed), The Nigerian Man and The Biosphere. (M.AB-5) National Committee, pp 138-146.

Okpokwasili, G.C. and Amanchukwu, S.C. (1988).Petroleum hydrocarbons degradation by *Candida* species.Environment International, 14: 243-247.

Okpokwasilli, G.C. and Okorie, B.B. (1988).Biodeterioration potentials of organisms isolated from car-engine lubricating oil. Tribology International, 21: 215-220.

Onifade, A.K. and Abubakar, F.A. (2007). Characterization of hydrocarbon-degrading microorganisms isolated from crude oil contaminated soil and remediation of the soil by enhanced natural attenuation. Research Journal of Microbiology, 2: 149-155

Owsianiak, M., Chrzanowski L, Szulc, A., Staniewski, J., Olszanowski, A., Olejnik-Schmidt, A.K. and Heipieper, H.J. (2009). Biodegradation of diesel/biodiesel blends by a consortium of hydrocarbon degraders: Effect of the type of blend and the addition of biosurfactants.Bioresource Technology, 100: 1497-1500.

Pasqualino, J.C., Montané, D. and Salvadó, J. (2006). Synergic effects of biodiesel in the biodegradability of fossil-derived fuels.Biomass Bioenergy, 30: 874-879.

Pelletier, E., Delille, D. and Delille, B. (2004). Crude oil bioremediation in sub-Antarctic intertidal sediments: Chemistry and toxicity of oiled residues. Marine Environmental Research, 57: 311-327.

Rahman, K.S.M., Rahman, T.J., Kourkoutas, Y., Petsas I Marchant., R and Banat, I.M. (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients.Bioresource Technology, 90: 159-168.

Refaat, A.A. (2009). Correlation between the chemical structure of biodiesel and its physical properties. International Journal of Environmental Science and Technology, 6 (4): 694.

Robert, B. (2004). Bosch Automotive Handbook, 6th edn, pp 327-328.

Santhini K, M.Y.L.A. J., Sajani, S. and Usharani, G. (2009). Screening of *Micrococcus* sp. from oil contaminated soil with reference to Bioremediation. Botany Research International, 2 (4): 248-252.

Sohal S.H. and Srivastava, A.K. (1994).Environment and Biotechnology.Role of biotechnology in pollution control.Ashish Publishing House. New Dehli, pp 163-170.

Taylor, L.T. and Jones, D.M. (2001). Bioremediation of coal tar PAH in soils using biodiesel. Chemosphere, 44: 1131-1136.

Trindade, P.V.O., Sobral, L.G., Rizzo, A.C.L., Leite, S.G.F. and Soriano, A.U. (2005). Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: A comparison study. Chemosphere, 58: 515-522.

Venosa, A.D. and Zhu, X. (2003).Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands.Spill Science&Technology Bulletin, 82: 163-178.

Vidali, M. (2001). Bioremediation: An overview. Pure and Applied Chemistry, pp 1163.

Watanabe, K. (2002). Linking genetics, physiology and ecology: an interdisciplinary approach for advancing bioremediation.Journal of Bioscience and Bioengineering, 94 (6): 557-562.

Zhang, X., Peterson, C.L., Reece, D., Haws, R. and Moller, G. (1998).Biodegradability of biodiesel in the aquatic environment.Trans. ASAE, 41:1423-1430.