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

*Balogun, K.I., 1Arekemase, M.O., 1Oyeyiola, G.P. and2Ameen, M.O.
1Department of Microbiology, University of Ilorin, Ilorin, Nigeria
2Department 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, Klebsiella pneumoniae, 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 (Edgaraet 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°C (392°F) and 350°C (662°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 to Refat (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; Trinidade 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 Owianiak 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 *Jatropha curcas*) and biodiesel blend 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.

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 sterilized distilled water was added to the mixture and mixed thoroughly. It was then

**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.

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 sterilized distilled water was added to the mixture and mixed thoroughly. It was then

**Preparation of culture media**

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

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
- $MgSO_4.7H_2O$ - 0.6g
- Agar-Agar - 4.0g

The method of Okpokwasili and Ananchukwu (1988) was used. One litre of
autoclaved at 121°C for 15 mins and allowed to cool to about 50°C 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°C) 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°C. Engine oil-utilizing bacterial counts were also determined on Mineral Salts Medium (MSM) after 7 days incubation at 28 ± 2°C.

**Isolation and characterization of diesel oxidizing bacteria (DOB)**

The isolation of diesel 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 & Voges-Proskauer, 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 X 10⁶ to 2.7 X 10⁶ cfu/g and hydrocarbon utilizing bacterial plate counts ranged from 1.12 X 10⁵ to 2.8 X 10⁵ cfu/g while the heterotrophic bacterial counts for the engine oil contaminated soil ranged...
from $8.7 \times 10^5$ to $2.05 \times 10^6$ cfu/g and hydrocarbon utilizing bacterial plate counts ranged from $1.4 \times 10^5$ to $5.9 \times 10^5$ 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) and Michalcewick (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 hydrocarbons in that 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 to have 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 10^4 cfu/g)

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Sampling site</th>
<th>Counts (x 10^4 cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrophic</td>
<td>Generator site near Faculty of Law lecture rooms, Unilorin PS</td>
<td>184.00</td>
</tr>
<tr>
<td>Hydrocarbon utilizers</td>
<td>Generator site, Chemistry Department, Unilorin</td>
<td>235.00</td>
</tr>
<tr>
<td>Percentage Hydrocarbon utilizers</td>
<td>Base of tank containing diesel, Tanke</td>
<td>212.00</td>
</tr>
<tr>
<td></td>
<td>Generator site near Faculty of Law lecture rooms, Unilorin PS</td>
<td>270.00</td>
</tr>
<tr>
<td></td>
<td>Base of tank containing diesel, Tanke</td>
<td>117.00</td>
</tr>
<tr>
<td></td>
<td>Base of tank containing diesel, Tanke</td>
<td>270.00</td>
</tr>
<tr>
<td></td>
<td>Bababeji automobile workshop, Tanke</td>
<td>270.00</td>
</tr>
</tbody>
</table>

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 10^4 cfu/g soil).

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Sampling site</th>
<th>Counts (x 10^4 cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrophic</td>
<td>Generator house, Bamed Printers, Tanke</td>
<td>90.00</td>
</tr>
<tr>
<td>Hydrocarbon utilizers</td>
<td>Generator repairer’s shop, NNPC, Tanke</td>
<td>205.00</td>
</tr>
<tr>
<td>Percentage Hydrocarbon utilizers</td>
<td>Automechanic workshop, Ahmadiyya Mosque, Tanke</td>
<td>147.00</td>
</tr>
<tr>
<td></td>
<td>Engine oil selling point near the Bus terminus</td>
<td>87.00</td>
</tr>
<tr>
<td></td>
<td>Bababeji automobile workshop, Tanke</td>
<td>134.00</td>
</tr>
<tr>
<td></td>
<td>Bababeji automobile workshop, Tanke</td>
<td>120.00</td>
</tr>
<tr>
<td></td>
<td>Bababeji automobile workshop, Tanke</td>
<td>90.00</td>
</tr>
<tr>
<td></td>
<td>Bababeji automobile workshop, Tanke</td>
<td>112.00</td>
</tr>
<tr>
<td></td>
<td>Bababeji automobile workshop, Tanke</td>
<td>120.00</td>
</tr>
</tbody>
</table>

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*, *Klebsiella pneumoniae*, *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 aeruginosain* 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 *Klebsiella pneumoniae* (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 *Klebsiella pneumoniae* (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
Bacteria isolated from diesel polluted soil.

Bacteria isolated from engine oil polluted soil.

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 aeruginosain* 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 aeruginosain* mineral salt medium supplemented with biodiesel/diesel blend had the highest absorbance of 1.05 and 1.02 respectively. *Micrococcus variansand*
Staphylococcus chromogenes in mineral salt medium supplemented with diesel showed the highest absorbance of 1.17 and 1.12 respectively. Smita et al. (2012) and Asitok 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 highest bacterial 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) and Lapinskiene 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) the blend B20 and the pure biodiesel (B100) differed from B0 (petrodiesel) in terms of increment in CO₂ 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, being recognized 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 C₁₀-C₂₀) 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
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) and Fernández-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.

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