INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

BIODETERIORATION OF PREMIUM MOTOR SPIRIT (PMS) BY FUNGAL SPECIES

[a]SANYAOLU, adeniyi a.a., [b]SANYAOLU, victoria t., [a]KOLAWOLE-JOSEPH, omowunmi s. & [a]JAWANDO, sunday s.
[a]Environmental Biology Section, Department of Biological Sciences, School of Science, Yaba College of Technology, pmb 2011, yaba- lagos, Nigeria
[b]Environmental Biology unit, Department of Science Laboratory Technology, School of Technology, Lagos state polytechnic, Ikorodu Main Campus, Lagos state , Nigeria.

ABSTRACT
This study investigated and compared some indices in samples of PMS that were kept sterile and also some pre-sterilized samples that were inoculated with fungal species. These fungi were previously isolated from the unsterilized samples of the PMS. All samples of the PMS were sourced from two Filling Stations in Lagos metropolis. Using both types of sample, some parameters that served as indicators of deterioration in the inoculated samples of the PMS by the fungal species were determined. Four species of fungi were isolated from the PMS samples. Of all the four fungal isolates namely *Aspergillus niger, Aspergillus flavus, Trichoderma sp* and *Aspergillus terreus* obtained in this study, *Aspergillus terreus,* was found to be the least predominant in the samples as it was found only in 1 location out of the 2 locations. Using the growth profiles of these fungal isolates in nutrient medium, the hydrocarbon utilizing ability of each of these fungal isolates were found to be different, one from the other, thus providing some evidence that all the four species were capable of deteriorating PMS in storage. The ability of *A. flavus* in particular to degrade the Polyaromatic hydrocarbon (PAH) components of PMS was further investigated at days 0 and 21 using Gas chromatographic techniques. These results were contrasted with the control (sterile PMS) which had no *A. flavus*. The concentration of naphthalene for the control PMS (with no *A. flavus*) remained 5.67124mg/L at both days 0 and 21 while the concentration of Acenaphthylene remained at 1.49312mg/L for both days 0 and 21 in the same sample. In the PMS sample containing *A. flavus*, at day 0, naphthalene concentration was 3.12313mg/L, and at day 21 naphthalene had been reduced to 0.976152mg/L. At day 0, Acenaphthylene was 0.870552mg/L, while at day 21 the concentration had reduced to 0.102997mg/L. The control was also found to contain fluorene at 0.00021941mg/L at both days 0 and 21. Fluorene however was not found in the treatment samples of PMS at both days 0 and 21. This result suggests that the three main fractions of PAH found in PMS are Naphthalene, Acenaphthalene, and fluorine, and that *A.flavus* is capable of causing a deterioration in PMS in storage by utilizing these fractions of PAH for its metabolic processes.

KEY WORDS: fungi, biodeterioration, PMS, and PAH.

INTRODUCTION

Petroleum like all fossil fuels primarily consists of a complex mixture of molecules called hydrocarbons with minor impurities such as nitrogen, oxygen and sulphur. In large concentrations, the hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many organisms, including humans (Alexander, 1994). The ability to actively decompose specified fractions of petroleum oil is expressed by many micro organisms (Bartha and Atlas, 1977). Problems associated with the biodeterioration of crude oil and oil derived products have been of immense interest to experts and scientists for a long time. Biodeterioration according to Hueck (1965) is defined as any undesirable change in the quality of a material caused by the vital activities of organisms. Biodeterioration of petroleum hydrocarbon is a biochemical assimilatory process i.e. the organism uses the hydrocarbon in the petroleum as a source of food and energy. Most of the research devoted to this phenomenon was carried out between the 50's and the 70's of the last century (Vishnyakova *et al*., 1970; Odier 1976), when the comprehension of the dangers associated with this microbial activity was realized.

However, the problem of hydrocarbon material biofouling is an urgent issue at the present time as well (Wilhelms *et al*., 2001; Watanabe *et al*., 2002; Roling *et al*., 2003), as it affects various aspects of the society. Moreover, the majority of applied microbiological methods of enhanced oil recovery also deteriorates oil and appears to be a source of microorganisms in natural reservoirs and oil pipelines (Vishnyakova *et al*., 1970).

Interestingly, almost the same microorganisms are responsible for oil deterioration in natural reservoirs, storage tanks, oil pipelines, Industrial systems of water cooling, systems of water preparation for pumping into oil fields as well as in the processes related to the biocorrosion of metal pipes and cement constructions (Chesneau, 2000; Watanabe *et al*., 2002; Muthukumar *et al*., 2003). A long-term storage of oils and oil products in industrial tanks for strategic purposes still leads to its deterioration despite efforts such as the application of biocides undertaken to solve this problem (Vishnyakova *et al*., 1970; Chesneau 2000). The main

products of hydrocarbon microbial metabolism are carbon dioxide, water and in smaller quantities fatty acids and surfactants participating in stabilization of inverted water-oil emulsion (Olliver and Magot 2005).

Microbiological Contamination of aviation fuel is a major concern as the deterioration of kerosene and rocket fuels often lead to accidents (Yang *et al*., 1992; Ferrari *et al*., 1998; Chesneau 2000). Applications of various chemical compounds for crude oil and oil products disinfection often resulted in pollution of the environment due to the slow decomposition of these xenobiotics, many of which possess mutagenic and carcinogenic properties (Yang *et al*., 1992; Ferrari *et al*., 1998; Zhiglecova *et al*., 2000).

Many years ago, methods for the determination of microbiological contamination of oil and oil products as well as monitoring of its disinfection were expensive and time-consuming. At the present time, methods and the test kits allowing a quick and reliable determination of microbial infection in fuels and crude oil are being developed. The identification and application of the most effective biocides and inhibitors of oil fouling are also being pursued (Efremenko *et al*., 2002; Frundzhan *et al*., 2002; Bonch-Osmolovskaya *et al*., 2003).

In Nigeria however, there is a dearth of information on aspects of research connected with the biofouling of crude oil and its refined constituents in storage, and the attendant negative consequences of this phenomenon on the Nigerian people and economy.

In view of the following, the objectives of this research are:

- To isolate and identify the fungal species that are suspected to be involved in the biodeterioration of petrol (PMS) from 2 locations (that are remotely located from each other) in Lagos state, Nigeria.
- To evaluate the rate of growth of fungal species in PMS in storage.
- To evaluate the extent of the biodegradation of the PAH fraction of petrol by the fungal isolates.

MATERIALS AND METHODS

Sample location

Petrol and diesel samples were collected from Oando Filling Station in Shomolu and MRS Service Station in Badagry Local Government Areas in Lagos state. The coordinate for the Oando Filling Station in Shomolu is ±41Ft N.06° 31.453¹ E003° 22.069¹, while the coordinate for the MRS Service Station in Badagry is ±32Ft N06° 25.842¹ E002° 53.738¹. The MRS Service Station in Badagry is just some few kilometers away the Atlantic Ocean.

Collection of samples

Four sterile containers were used for the petrol. The containers were made sterile by first washing it with distilled water and detergent and thereafter rinsing it with sterile distilled water, after which it was soaked in absolute ethanol for 5mins.

Sample preparation

(a) Sterilization of petrol and diesel

The PMS was sterilized using the membrane filtration technique**.** The Membrane filter (Millipore 47mm-xx1104700) used was first sterilized by wrapping it in an aluminum foil and placing it in an autoclave at 121°C for 15mins. It was allowed to cool. Then the PMS was poured from the top lid, and allowed to drain through the filter into a pre-sterilized collecting container attached to the membrane filter. The whole entire exercise was done under an asceptic condition in a UV room. Prior to the sterilization however, isolation of the fungal species from both the PMS was done.

(b) Isolation and identification of hydrocarbon–utilizing fungi

The PMS was evenly distributed with the use of a sterile dropper on five sterile Petri dishes each (making up to 20 Petri dishes) with previously prepared sterile potato dextrose agar (PDA). Afterwards, the plates were incubated at a temperature of 28 – 31°C for 48hours or more depending on the growth rate.

To obtain pure cultures of the fungal isolates, developing fungal cultures were aseptically sub cultured into fresh PDA plates and incubated until the fungus begins to sporulate followed by subsequent sub culturing and incubation a number of times until pure cultures consisting of only one type of fungus was obtained. A part of the pure culture was then aseptically transferred into sterile agar slants which had previously been prepared in 14ml McCartney bottles. The bottles were then incubated till full growth of the fungus is observed. To identify the fungi, a small portion of the fungi was teased with a sterile inoculating loop into 2-3 drops of lactophenol in-cotton blue on a clean slide. First were the morphological studies as described by Talbot (1971) and Bryce (1992).

(c) Test for hydrocarbon utilization potentials of the isolated fungi

A modified method of the enrichment procedure as described by Nwachukwu (2000) and Adekunle and Adebambo (2005) was used in the estimation of hydrocarbon utilizers. A minimal salt solution containing 2.0g of NA_2HPO_4, 0.17g of K_2SO_4, 4.0g of NH_4NO_3, 0.53g of KH_2PO_4, 0.10g of $MgSO_4.7H_2O$ and 5.0g of yeast dissolved in 1000 ml of distilled water was prepared. The pH was adjusted to 4.6 before autoclaving at 121ºC for15 minutes. The solution was sterilized by autoclaving. Twenty one test tubes were sterilized and placed in test tube racks. Three out of the four fungal species isolated from the PMS (petrol) from both locations were inoculated into each of the test tubes using a sterile 2 mm cork borer into 2 ml of pre-sterilized PMS (from each of the two locations) in 10 ml MSS solution. Each of the test tubes containing each of the three fungal isolates (from each of the two locations) were replicated three times, making a total of eighteen test tubes for both locations i.e. nine test tubes per location. The remaining three test tubes which served as control contained only twelve ml of MSS and no fungus. Each of the test tubes were plugged with sterile cotton wool wrapped with Aluminum foil so as to ensure maximum aeration and prevent cross – contamination. All the test tubes were then incubated at room temperature (28 °C - 31 °C) for 21 days.

The test tubes were shaken constantly throughout the duration of the experiment to facilitate oil-cell interphase (contact).

The ability to degrade the petroleum products (based on the growth rate of the organisms in the MSS medium) was measured every 3 days, starting with day 0, using the visual method which is based on the turbidity of the (MSS). The turbidity was measured using the spectrophotometer (T80 UV/VIS -by PG Instruments Limited). The experiment was replicated three times. The statistical analysis was done using T – Test to compare the controls with the treatments (Parker, 1979).

(d) Gas chromatographic technique

The standard used for this test was first determined and known as seen in the calibration curves (appendix III). Petroleum product samples were analyzed at the beginning (day 0) and at the end (day 21) of the experiment as described in García de Oteyza *et al.* (2006). The concentration of the Poly-Aromatic Hydrocarbons was determined for day 0 and 21. The concentration of each constituents of PAH, which include Naphthalene, Acenaphthylene, Fluorene, Acenaphthene, Pyrene, Anthracene, Phenanthrene, Benz(a)Anthracene were determined for the day 0 and the day 21.

(i) **GC conditions for polyaromatic hydrocarbons**

GC MODEL: HP 6890 Powered with HP Chem Station Rev. A 09.01 [1206] Software
Injection Temperature: Split Injection
Split Ratio: 20:1
Column: HP-1 COLUMN
Column Length: 30m
Column ID: 0.25nm
Column Film thickness: 0.25nm
Injection Temp: 25°C
Detector Temp: 32°C
Detector: FID
Initial Temp: 60°C for 5min
First Rate: 15°C/min for15min and Maintained for 3min
Second Rate: 10°C/min for 4min and Maintained for 5min
Mobile Phase or Carrier: Nitrogen
Nitrogen Column Pressure:30psi
Hydrogen Pressure: 28psi
Compressed Air Pressure: 32psi

(i) Extraction of PAH for GC reading

5ml of the sample was transferred into a 50ml separatory funnel, and 5ml of the redistilled dichloromethane was added. The separatory funnel was shaken vigorously for about 2minutes with periodic venting to release vapour pressure. The organic layer was allowed to separate for 10minutes and was recovered into the 50ml tank. The aqueous layer was re-extracted twice with 2ml of the extractant. The combined extract was dried by passing through the funnel containing the anhydrous sodium sulphate. The dried extract was concentrated with a stream of nitrogen gas.

(ii) PAH separation for GC reading

The concentrated oil was separated into the aliphatic profiles and poly aromatic hydrocarbon profiles by packing the glass column with activated alumina, neutral and activity grade1. 10ml of the treated alumina was packed into the column and cleaned properly with redistilled hexane. The extract was poured onto the alumina and was allowed to run down with the aid of the redistilled hexane to remove the aliphatic profiles into a pre-cleaned 20ml capacity glass container. The aromatic fraction was recovered by allowing the mixture of hexane and dichloromethane in the ratio 3 to1 and finally removed the most polar PAH by removing with the dichloromethane into the pre-cleaned boro-silicate beaker. The mixture was concentrated to 1.0ml by stream of the nitrogen gas before the gas chromatography analysis.

RESULTS

The fungal isolate obtained in PMS from Badagary and Shomolu locations are *A. flavus, Trichoderma sp, A. terreus* and *A. niger* (**plates 1a and 1b – 4a and 4b**).

Plate 1a: culture plate of *A. niger* isolated from PMS. X 0.5

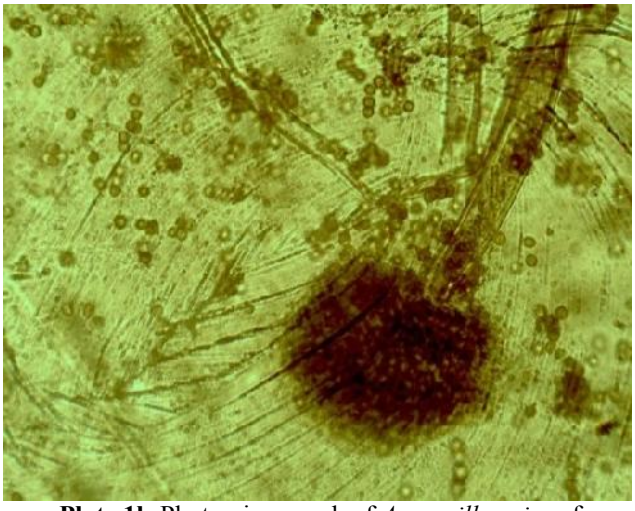

Plate 1b: Photomicrograph of *Aspergillus niger* from PMS. X100

Plate 2a: culture plate of *A.flavus* isolated From PMS. X 0.5

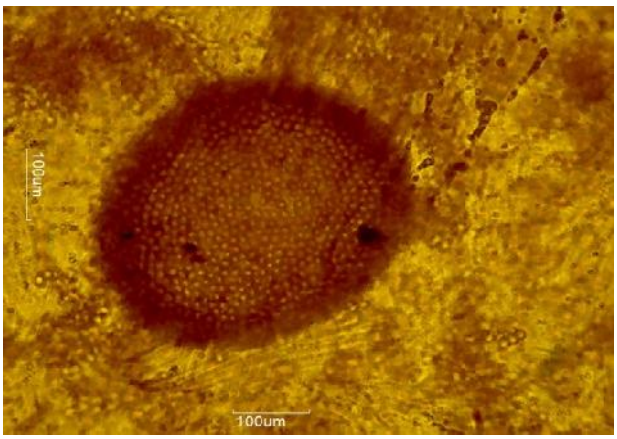

Plate 2b: Photomicrograph of *Aspergillus flavus* From PMS. X100

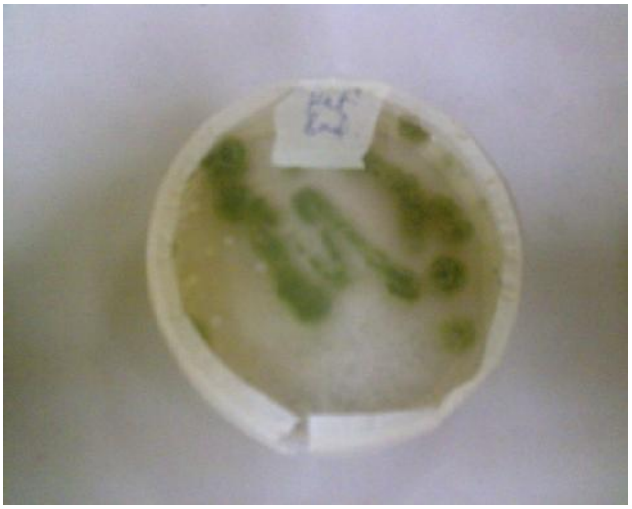

Plate 3a: culture plate of *Trichoderma sp* isolated from PMS. X 0.5

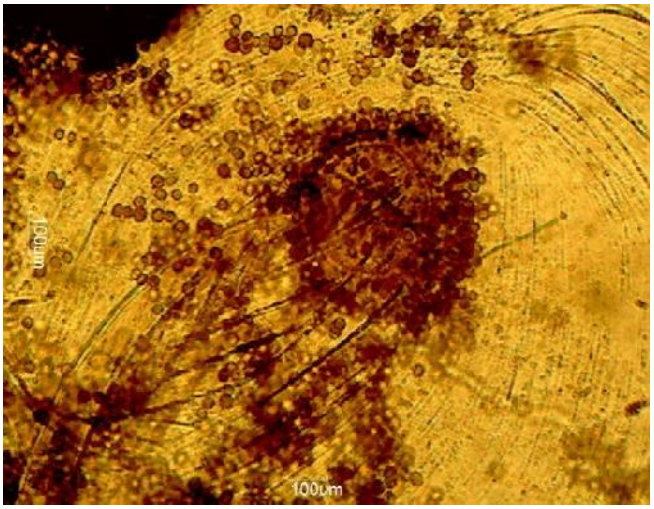

Plate 3b: Photomicrograph of *Trichoderma sp* from PMS. X 100

Plate 4a: culture plate of *A.terreus* isolated From Badagry PMS only. X 0.5

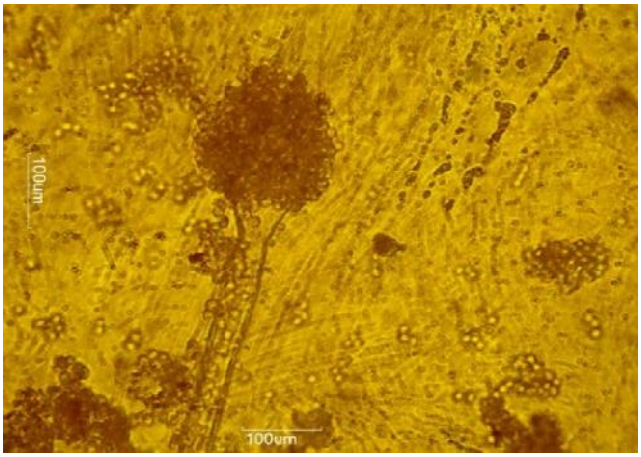

Plate 4b: Photomicrograph of *Aspergillus terreus* from Badagry PMS only. X 100

OPTICAL DENSITY MEASUREMENT USING SPECTROPHOTOMETER

The results for the mean optical density for both treatment samples of PMS from Shomolu (tables 1a and 1b – 4a and 4b) and also the PMS from Badagry (tables 5a and 5b – 8a and 8b) show that each of the petroleum product sample inoculated with each of the fungal isolate had an optical density that was significantly higher ($P < 0.05$) than their corresponding control (sterile) samples.

TABLE 1A: T-test for the Mean optical density of *A.flavus* at 520nm in treatment and control Samples of PMS from Shomolu at Day 0

	PMS (Shomolu) + *A.flavus*	STERILE PMS only (control)
Mean	1.573333333	1.52
Variance	3.33333E-05	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	16	
P(T<=t) one-tail	0.001941755	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.00388351	
t Critical two-tail	4.30265273	

TABLE 1B: T-test for the mean optical density of *A.flavus* at 520nm in treatment and control samples of PMS from Shomolu at Day 21

	PMS (Shomolu) + *A.flavus*	STERILE PMS only (control)
Mean	1.613333333	1.56
Variance	3.33333E-05	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	16	
P(T<=t) one-tail	0.001941755	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.00388351	
t Critical two-tail	4.30265273	

. **TABLE 2A:** T- test for the mean optical density of Trichoderma species at 520nm in the treatment and control samples of PMS from Shomolu at day 0

Day 0 (PMS)		
	PMS(Shomolu)+*Trichoderma sp*	STERILE PMS ONLY (control)
Mean	1.58	1.52
Variance	0.0001	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	10.39230485	
P(T<=t) one-tail	0.004566306	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.009132611	
t Critical two-tail	4.30265273	

TABLE 2B: T- test for the mean optical density of Trichoderma species at 520nm in the treatment and control samples of PMS from Shomolu at day 21

Day 21 PMS @ 520nm		
	PMS(Shomolu)+*Trichoderma sp*	STERILE PMS ONLY (control)
Mean	1.613333333	1.56
Variance	3.33333E-05	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	16	
P(T<=t) one-tail	0.001941755	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.00388351	
t Critical two-tail	4.30265273	

TABLE 3A: T- test for the mean optical density of *Aspergillus niger* at 520nm in the treatment and control samples of PMS from Shomolu at day 0

Day 0 (PMS) @ 520nm		
	pms(S)+Asper niger	STERILE PMS ONLY (control)
Mean	1.573333333	1.52
Variance	3.33333E-05	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	16	
P(T<=t) one-tail	0.001941755	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.00388351	
t Critical two-tail	4.30265273	

TABLE 3B: T- test for the mean optical density of *Aspergillus niger* at 520nm in the treatment and control samples of PMS from Shomolu at day 21

Day 21 PMS @ 520nm		
	PMS(Shomolu)+*Aspergillus niger*	STERILE PMS ONLY (control)
Mean	1.61	1.56
Variance	0	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	3.18453E+14	
P(T<=t) one-tail	4.93038E-30	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	9.86076E-30	
t Critical two-tail	4.30265273	

TABLE 4A: T-test for the Mean optical density of *A.flavus* at 520nm in treatment and control samples of PMS from Badagry at Day 0

Day 0 PMS @ 520nm		
	PMS (Badagry)+*A. flavus*	STERILE PMS ONLY (control*)*
Mean	1.55	1.52
Variance	0.0004	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	2.598076211	
P(T<=t) one-tail	0.060844967	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.121689934	
t Critical two-tail	4.30265273	

TABLE 4B: T-test for the Mean optical density of *A.flavus* at 520nm in treatment and control samples of PMS from Badagry at Day 21

Day 21 PMS @ 520nm		
	PMS(Badagry)+ *A. flavus*	STERILE PMS ONLY (control)
Mean	1.613333333	1.56
Variance	0.000133333	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	8	
P(T<=t) one-tail	0.007634036	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.015268072	
t Critical two-tail	4.30265273	

TABLE 5A: T-test for the Mean optical density of *Trichoderma sp*. at 520nm in treatment and control samples of PMS from Badagry at Day 0

Day 0 (PMS) @ 520nm		
	PMS(Badagry)+*Trichoderma sp*	STERILE PMS ONLY (control)
Mean	1.56	1.52
Variance	0.0004	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	3.464101615	
P(T<=t) one-tail	0.03708995	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.0741799	
t Critical two-tail	4.30265273	

TABLE 5B: T-test for the Mean optical density of *Trichoderma sp*. at 520nm in treatment and control samples of PMS from Badagry at Day 21

Day 21 PMS @ 520nm		
	PMS (Badagry)+*Trichoderma sp*	STERILE PMS ONLY (control)
Mean	1.61	1.56
Variance	0	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	3.18453E+14	
P(T<=t) one-tail	4.93038E-30	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	9.86076E-30	
t Critical two-tail	4.30265273	

TABLE 6A: T-test for the Mean optical density of *A. niger* at 520nm in treatment and control samples of PMS from Badagry at Day 0

Day 0 (PMS) @ 520nm		
	PMS(Badagry)+*A. niger*	STERILE PMS ONLY (control)
Mean	1.563333333	1.52
Variance	0.000433333	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	3.605551275	
P(T<=t) one-tail	0.034525332	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.069050664	
t Critical two-tail	4.30265273	

TABLE 6B: T-test for the Mean optical density of *A. niger* at 520nm in treatment and control samples of PMS from Badagry at Day 21

Day 21 PMS @ 520nm		
	PMS(Badagry)+*A. niger*	STERILE PMS ONLY (control)
Mean	1.616666667	1.56
Variance	3.33333E-05	7.39557E-32
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	17	
P(T<=t) one-tail	0.001721176	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.003442351	
t Critical two-tail	4.30265273	

GAS CHROMATOGRAPHIC DETERMINATION OF THE EXTENT OF BIODETERIORATION OF SOME POLYAROMATIC HYDROCARBON (PAH) CONSTITUENTS OF PMS BY ASPERGILLUS FLAVUS

The GC determined biodeterioration rate of the PAH constituents of the PMS by *Aspergillus flavus* was also analyzed at days 0 and 21. At day 0, the concentration of the naphthalene was 3.12313mg/L, while at day 21; the value had reduced to 0.976152mg/L. The value for the control was 5.67124mg/L (figure 1).

Also, for acenaphthylene, the biodeterioration rate caused by *Aspergillus flavus* as determined by the GC determined biodeterioration rate of by *Aspergillus flavus* in PMS was 0.870552mg/L, 0.102997 mg/L and 1.49312mg/L for Day 0, day 21 and control respectively (figure 2).

Lastly, the GC determined biodeterioration rate of fluorene by Aspergillus *flavus* in PMS at day 0 and 21 was nil or probably it was not obvious, but the concentration of the control was 0.00021941mg/L (figure 3).

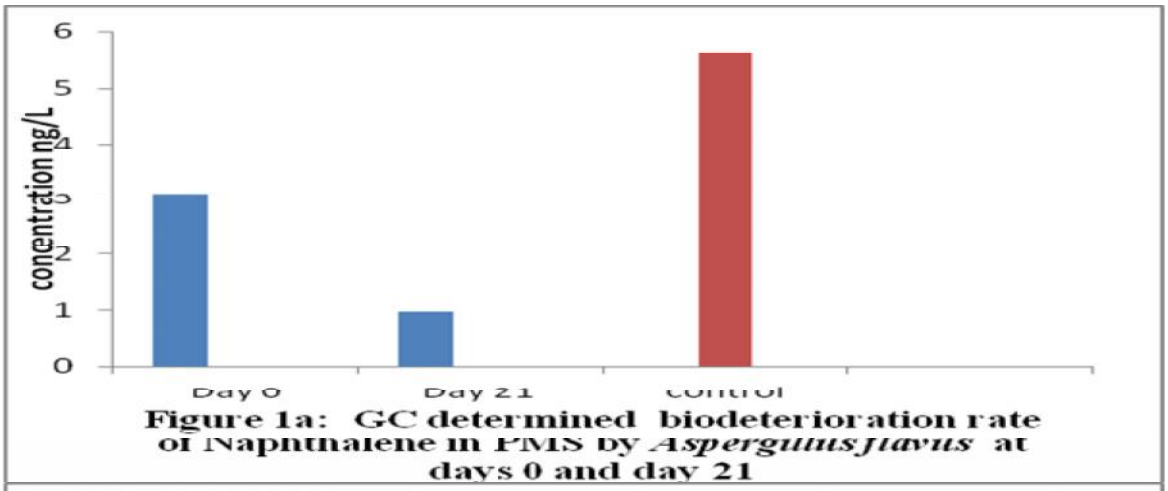

Figure 1a: GC determined biodeterioration rate of Naphthalene in PMS by *Aspergillus flavus* at days 0 and day 21

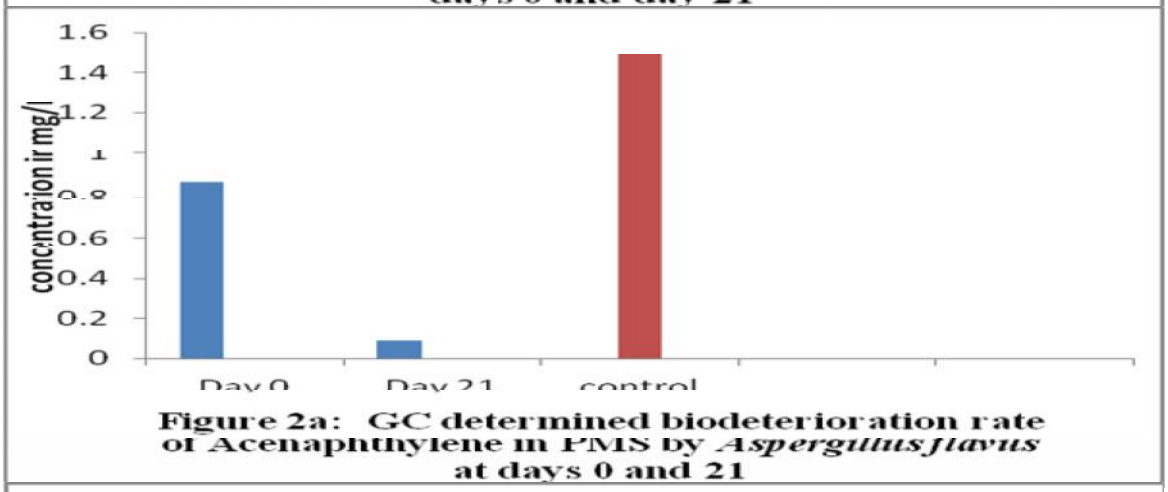

Figure 2a: GC determined biodeterioration rate of Acenaphthylene in PMS by *Aspergillus flavus* at days 0 and 21

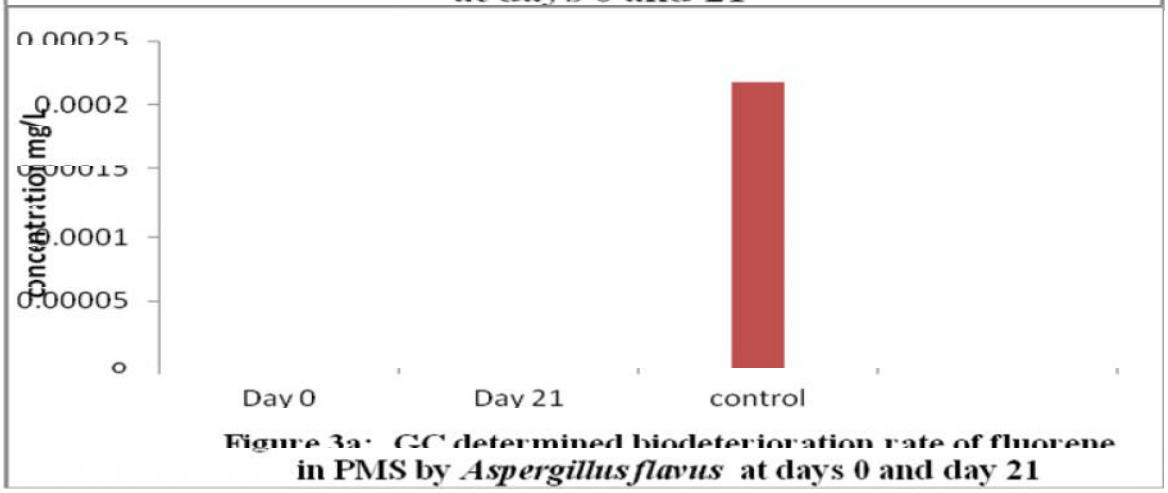

Figure 3a: GC determined biodeterioration rate of fluorene in PMS by *Aspergillus flavus* at days 0 and day 21

DISCUSSION AND CONCLUSION

Okerentugba and Ezeronye (2003) demonstrated that *Penicillium spp., Aspergillus spp. and Rhizopus spp.* are capable of degrading hydrocarbons especially when single cultures are used. This position is in agreement with the results obtained in this study as *Aspergillus niger, Aspergillus flavus and Trichoderma spp* were all found to be capable of utilizing and reducing hydrocarbon compounds in PMS to about 75% after day 21 using Gas chromatographic technique. Santos *et al*., (2008) had previously reported the ability of *Aspergillus sp*. to biodegrade PMS (gasoline) at different rates. The result obtained from this study also showed that the rate of hydrocarbon utilization among the fungi isolated varies. This position is hinged on the differences in the growth rate of each of the fungus under consideration. Oboh *et al*., (2006) have reported the abilities of bacterial species such as *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Citrobacter* and fungi such as *Aspergillus sp*., *Penicillum, Rhizopus* and *Rhodotorula* species to grow on crude petroleum as the sole carbon and energy source when screened for hydrocarbon utilization. At some point in this study, it was observed that there was growth and extension of the hyphae forming mycelium in the medium. In their report, Uzoamaka *et al*., (2009) reported that some eight isolates of fungi that showed potentials for hydrocarbon biodegradation include *A. versicolor*, *A. niger*, *A.flavus*, *Syncephalastrum* spp., *Trichoderma* spp., *Neurospora sitophila*, *Rhizopus arrhizus* and *Mucor* spp, thus including the three fungal isolates that were encountered in this study.. Atlas and Bartha (1972) observed that both water in oil and oil in water emulsions are formed following oil spillage. The two phase liquid medium where the bulk of the carbon and energy source are found is water insoluble and that all other minerals nutrients are dissolved in the water phase. Microbial growth is known to typically occur at the interface of the two liquids. Thus, the ability of microorganisms to lower the interfacial tension will increase the accessibility of the hydrocarbon substrate. Similar observations were made in this study, as the oil-water interface was clearly observed in the test tubes. Shaw (1995) found that organisms break down hydrocarbons and use the energy to synthesize cellular components. After being completely broken down, the reaction releases Carbon (IV) oxide, water and energy used to create cellular biomass (Keeler, 1996).

However, it must be noted that there was nutrient present in the minimal salt broth, though more of it could have been present in the oil which stimulated the growth of each fungus. In view of this therefore, the additional nutrients present in the minimal salt solution helped in overcoming nutrient limitation to microbial growth and also helped in creating a favorable environment for the rapid development of the fungi.

In this study, degradation of the PMS has been shown to occur by an attack on the aromatic fractions of this oil. The petrol (PMS) used in this experiment contained 3.12313mg/l naphthalene and 0.870552mg/l acenaphthylene at day 0. By day 21 however, the concentration of naphthalene and acenaphthylene had reduced to 0.976152 and 0.102997mg/l respectively. This can be adduced to the usage of these fractions contained in the PMS by each of the fungus for their metabolic processes, which includes their growth in the minimal salt solution as evidenced by the progressive increase in their optical densities as seen from the data obtained from the spectophotometric analysis.

In conclusion, the results obtained from this study shows that deterioration of oil and fuels or its ‘‘ageing’’ due to microbial activity is real. It must be appreciated that the protection of oils and fuels against this undesirable phenomenon is regarded as a serious economic and environmental issue in some developed economies (Wilhelms *et al*., 2001; Watanabe *et al*., 2002; Roling *et al*., 2003). Unfortunately however, a number of engine inefficiencies and the uneconomical consumption of fuels by a number of engines in Nigeria is seldom (if at all) adduced to the deteriorative action of microorganisms on these fuels in storage. The result here shows that the fungus (*A. flavus*) isolated from the PMS was able to deteriorate and reduce the quantity of some of the hydrocarbons found in it. Similar phenomenon has been previously reported to have caused great operational problems, as occasioned in accidents to aircrafts (Chesneau, 2000), and it is suspected to be responsible for a great deal of an inefficient combustion of these fuels in engines, thereby making these engines to be less efficient, as well as resulting in a higher consumption of fuels in these engines.

REFERENCES

Adekunle, A.A. and Adebambo, O.A. (2007) The hydrocarbon utilization of crude petroleum and Petroleum products by fungi isolated from *Detarium senegalense* (Gmelin) seeds. *Journal of American Science* **3(1):** 69-76.

Alexander, M. (1994) Biodegradation and Bioremediation. Academic Press, New York. 692pp.

Allsopp, D., Seal, K.J., and Gaylarde, C.C., (2004) Introduction to biodeterioration, 2nd Ed. Cambridge University Press, UK

Bartha, R., and Atlas, R.M. (1997) Biodegradation of Oil in seawater, Writing Factor and Artificial Stimulation in: The Microbial degradation of Oil Pollutants (D.G. Ahern and S.P. Meyers (Eds). Centre for Wetland Resources, Louissiana pp.148.

Bonch-Osmolovskaya, E.A., Miroshnichenko, M.L., Lebedinsky, A.V., Chernyh, N.A., Nazina, T.N., Ivoilov, V.S., Belyaev, S.S., Boulygina, E.S., Perov, A.N., Mirzabekov, A.D., Hippe, H., Stackebrandt, E., L’Haridon, S., and Jeanthon, C. (2003) Radioisotopic, culturebased, and oligonucleotide microchip analyses of thermophilic microbial communities in a continental high-temperature petroleum reservoir. *Applied Environmental Microbiology* **69**(**10**): 6143–6151

Chesneau, H.L., (2000) The silent fuel killers (stability and microbiologicals). In: Proceedings of 2000 International Joint Power Generation Conference, Miami Beach, Florida, July 23–26, pp 1–8

Efremenko, E.N., Azizov, R.E., Raeva, A.A., Abbasov, V.M., and Varfolomeyev, S.D. (2005) An approach to the express control of oil spill bioremediation by bioluminescent method of intracellular ATP determination. *International Biodeterioration Biodegradation* **56**: 94–100

Ferrari, MD., Neirotti, E., and Albornoz, C. (1998) Occurrence of heterotrophic bacteria and fungi in an aviation fuel handling system and its relationship with fuel fouling. *Rev Argent Microbiol* **30**(**3**): 105–114

Frundzhan, V.G., Babunava, V.S., and Ugarova, N.N. (2002) bioluminescent detection of microbe contamination of crude chopped beef. *Moscow State Bulletin*. Line 2. Chemistry **43**(**6**): 389–392

García de Oteyza, T., Grimalt, J.O., Llirós, M., Esteve, I. (2006) Microcosm experiments of oil degradation by microbial mats. *Sci Total Environ* **357**:12-24

Hueck, H.J. (1965) The biodeterioration of materials as part of hylobiology . *Mater.Org*., **1**(**1**):5-34

Muthukumar, N., Rajasekar, A., Ponmariappan, S., Mohanan, S., Maruthamuthu, S., Muralidharan, S., Subramanian, P., Palaniswamy, N., and Raghavan, M. (2003) Microbiologically influenced corrosion in petroleum product pipelines- a review. *Indian Journal Exp Biol* **41**(**9**): 1012–1022

Nwachukwu, S.C.U. (2000) Enhanced rehabilitation of tropical aquatic environment polluted with crude petroleum using *Candida utilis. Journal of Environmental Biology* **21**(**3**): 241 – 250

Odier, E. (1976) Microbial growth and fuel tanks hazards. *Annual Microbiologia* **127B**(**2**): 213–225.

Olliver, B., and Magot, M. (2005) Petroleum microbiology. ASM Press, USA

Roling, W.F., Head, I.M., and Larter, S.R. (2003) The microbiology of hydrocarbon degradation in subsurface petroleum reservoirs: perspectives and prospects. *Res Microbiol* **154**(**5**):321–328

Watanabe, K., Kodama, Y.u., and Kaku, N. (2002) Diversity and abundance of bacteria in an underground oil-storage cavity. *BMC Microbiol* **2**:20-23

Wilhelms, A., Larter, S.R., Head, I., Farrimond, P., di-Primio, R., and Zwach, C. (2001) Biodegradation of oil in uplifted basins prevented by deep-burial sterilization. *Nature* **411(6841)**: 1034–1037

Yang, S.S., Chen, C.Y., Sung, Y., and Lin, Y.T. (1992) Effect of moisture content on the microbial activity in JP-5 fuel oil. *Chinese Journal of Microbiology and Immunology* **25**(**4**):223–231

Zhigletsova, S.K., Rodin, V.B., Kobelev, V.S., Akimova, N.A., Aleksandrova, N.V., Rasulova, G.E., Mironova, R.I., Noskova, V.P., and Kholodenko, V.P. (2000) Increase in the ecological danger upon the use of biocides for fighting corrosion induced by microorganisms. *Prikl Biokhim Mikrobiol* **36**(**6**):694–700