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Biodeterioration of Premium Motor Spirit and Automotive Gas Oil by Bacterial and Fungal Deteriogens

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Abstract: The study of Biodeterioration of Premium Motor Spirit and Automotive Gas Oil by bacterial and fungal deteriogens was conducted to ascertain the level of spoilage on petroleum samples obtained from African Petroleum and Oando Filling Stations, Port Harcourt. The Spread plate technique was employed for the enumeration of the microorganisms. The samples were cultured on petri-dishes containing nutrient media and potato dextrose agar and were incubated at temperature of 28–31°C for 48 hours and 72 hours for bacterial and fungal growth respectively. The bacterial and fungal isolates were further tested for hydrocarbon utilization potential with a modified mineral salt agar at room temperature for 48 hours and 168 hours respectively. Results showed a high range of total viable counts of Bacterial and Fungal species in samples collected from the stations. *Bacillus* spp., *Pseudomonas* spp., *Aspergillus* spp., *Penicillium* spp. were isolated from Diesel samples; while *Pseudomonas* spp., *Bacillus* spp., yeast spp. and *Aspergillus* spp. were isolated from Petrol. The Petrol and Diesel distributed by the African Petroleum to customers is of a higher quality than that of Oando Filling Station. There was abundance of microbial growth at Diesel substrates and was least supported by Petrol. This may be as a result of heavy contamination of the samples when in the storage tanks underground. The lead, anti-knock additive (tetra-ethyl lead) in Petrol may be the cause of growth inhibition. It is therefore recommended that adequate control-spoilage measures should be taken in order to prevent bioattack of the petroleum products.

Keywords: Biodeterioration, Petrol, Diesel, Fungal Spp., Bacterial Spp

1. Introduction

Crude oil is a complex biodegradable substance containing a large variety of hydrocarbons such as, “straight, branched and cyclic aliphatics, aromatic and heterocyclic compounds [1, 2].” Petroleum like all fossil fuels primarily consists of a complex mixture of molecules called hydrocarbons with minor impurities such as nitrogen, oxygen and sulphur [3]. The Premium Motor Spirit (PMS) also known as petrol, and the Automotive Gas Oil (AGO) also referred to as diesel are used all over the world for powering light and heavy vehicles, machineries, electricity generating sets, etc. However, these have made the petroleum refining industry one of the largest manufacturing industries in the world [4]; and filling stations one of the most commonly found business stands, where petrol and diesel are sold in Nigeria.

PMS and AGO can be readily deteriorated by algae, protozoans, and mainly fungal and bacterial species. The activities of sulfur reducing bacteria (SRB) and *Aspergillus* species on petroleum and its products cannot be overemphasized. The ability to actively decompose specified fractions of petroleum oil is expressed by many microorganisms [5]. According to Okpokwasili and James [6], the microbial spoilage of crude oil and petroleum products has obvious economic implications, as well as serious environmental problems [7]. The related financial burden and the threat to health and safety of the operators are of importance. The negative effects of spoilt fuels on motor engines have gained momentum over recent times. Colonization of the petrol and diesel engines metal surfaces by microbial deteriogens have resulted in biofilm/microbial

mat formation and biocorrosion of the metal surfaces. Plugging of power generating sets and vehicle engines fuel flow lines and filters is also a major challenge facing the global oil industry and end users.

2. Problem Statement

Biodeterioration is any undesirable change in the quality of a material caused by the vital activities of organisms [1], [3], [8], [9], [10]. The biodeterioration of PMS and AGO are a biochemical assimilatory process (i.e. the organism uses the petroleum products as a source of food and energy). Subsequently, the utilization of these products by microbial deteriogens spurs the release of metabolites or products which may disfigure or alter the physical and chemical properties of the fuels (Chemical dissimilatory process). 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 [11].

However, several problems associated with the biodeterioration of PMS and AGO have been of immense interest to experts and scientists for a long period of time. Fuel biodeterioration has been well documented for more than a century [4]. There have been reported incidences of fuel going "off specification" perhaps due to sulphide production, colour deterioration or haziness. Other cases included situations where the physical presence of the organisms caused filter blocking and slime formation. There were instances relating the effects of microorganisms inducing emulsion formation, water uptake, release of microbial products into the fuel phase and finally corrosion caused by microorganisms in storage vessels [12]. The presence of water at the bottom of storage tanks and in oil pipelines is a primary requirement for microbial growth in fuels [4], [7], [13]. Bacteria and fungi proliferate and are most metabolically active at interfaces within fuel systems [14]. The ability of microorganisms to grow both in a water phase as well as on interphase of water and hydrocarbon worsen the physical and chemical properties of oils and fuels [7].

The problem of oil spoilage is not limited to PMS and AGO alone, the microbiological contamination of aviation fuel is also a major concern as the deterioration of kerosene and rocket fuels often lead to accidents [15], [16], [17]. Obviously, the problem of hydrocarbon material biofouling is an urgent issue at the present time [18], [19], [20]. However, despite the application of biocides, additives such as stabilizers and octane enhancers (mainly tetra-ethyl lead), anti-freezing and anti-corrosion agents which are undertaken to solve the problem associated with oil and petroleum products spoilage [17], [21], a long-term storage of oils and oil products in industrial tanks for strategic purposes lead to their deterioration [22]. In Nigeria however, as posited by Sanyaolu *et al.* [3], 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.

3. Aims

The aims of this research are:

- To isolate and identify the fungal and bacterial species that are suspected to be involved in the biodeterioration of petrol and diesel from two locations at Alakahia-Choba Axis, Port Harcourt.
- To evaluate the quality of PMS and AGO that are being distributed by African Petroleum (AP) and Oando Filling Stations at Alakahia-Choba Axis, Port Harcourt.

4. Materials and Methods

- Sample location

Petrol and diesel samples were collected from AP and Oando Filling Stations, all located at Alakahia-Choba axis, East-West Road, near University of Port Harcourt, Choba, Rivers State of Nigeria. The two Filling Stations are 5 and 7.2 kilometers away from the Choba River respectively.

- Collection of samples

A total of eight sterile containers were used for the collection of petrol and diesel samples: four sterile containers for each station–of which two was used after 3 weeks of the first sample was collected. The containers were made sterile by first washing it with distilled water and detergent and thereafter rinsed with sterile distilled water, after which they were soaked in absolute ethanol for 5 minutes.

- Sample preparation

(a) Sterilization of petrol and diesel

The PMS and AGO were sterilized using the membrane filtration technique. The Membrane filter (Millipore 47mm) used was first sterilized by wrapping it in an aluminum foil and placed in an autoclave at 121°C for 15 minutes. It was allowed to cool. Then the PMS/AGO 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 aseptic condition in a UV room.

(b) Isolation and identification of total heterotrophic fungi and bacteria

The 0.1ml of PMS/AGO was evenly distributed with the use of a sterile dropper on 8 sterile Petri dishes per week with previously prepared sterile potato dextrose agar and nutrient agar respectively. Serial dilution, 10^{-1} to 10^{-3} was made and afterwards, the plates were incubated at a temperature of 28–31°C for 48hours and 72hours to enable bacteria and fungi growth respectively.

For pure cultures of the fungal and bacterial isolates, developing fungal and bacterial cultures were aseptically sub-cultured into fresh Potato Dextrose Agar (PDA) plates and nutrient agar (NA) respectively. The PDA plates are incubated until the fungus begins to sporulate, while the NA plates are incubated at temperature of 28–31°C for 48hours. A part of the pure cultures were then aseptically transferred

into sterile agar slants. The bottles were then incubated till full growth of the fungus and bacteria were 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 sterile slide. To identify the bacteria, Gram staining and biochemical tests were carried out on the isolates.

(c) Test for hydrocarbon utilization potentials of the microorganisms

To test the fungal and bacterial isolates for their ability to utilize various hydrocarbons as sole sources of carbon and energy for growth, each isolate was streak-inoculated onto a modified mineral salt agar (MSA) medium as described by Okpokwasili and James [6] which was inverted over the dish cover containing a filter paper soaked with the samples under study. For the samples, because of their volatility, the filter paper was resoaked at intervals to maintain a continuous supply of the carbon source. Plates were incubated at room temperatures for 48hours and 168hours for hydrocarbon utilizing bacterial and fungal counts respectively.

5. Results

The result of the total viable bacterial counts of the petrol samples obtained from AP and Oando Filling Stations ranged between 3.6 x 10^1CFU/ml–4.9 x 10^2CFU/ml and 1.0 x 10^1CFU/ml–2.5 x 10^4CFU/ml respectively; while for the diesel samples obtained from the same stations are 4.5 x 10^1CFU/ml–1.0 x 10^3CFU/ml and 6.5 x 10^2CFU/ml–1.3 x 10^5CFU/ml respectively.

The fungal counts obtained from AP and Oando Filling Stations are shown in Figure 1–2 (Petrol: 1.2 x 10^1CFU/ml–3.4 x 10^1CFU/ml and 1.5 x 10^1CFU/ml–5.6 x 10^2CFU/ml; Diesel: 1.6 x 10^1CFU/ml–5.2 x 10^2CFU/ml and 5.8 x 10^3CFU/ml–8.1 x 10^4CFU/ml) respectively.

The results of the hydrocarbon utilizing bacterial and fungal counts were shown in Table 1. The graphical representations of petrol and diesel utilization by bacterial and fungal isolates are shown in Figure. 3–4.

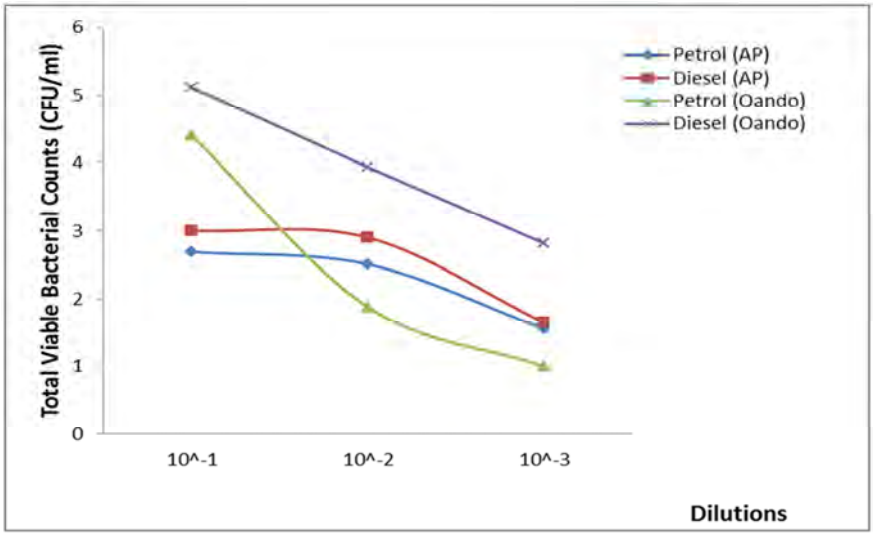

Figure 1. *The Total Heterotrophic Bacterial Counts of Petrol and Diesel Samples.*

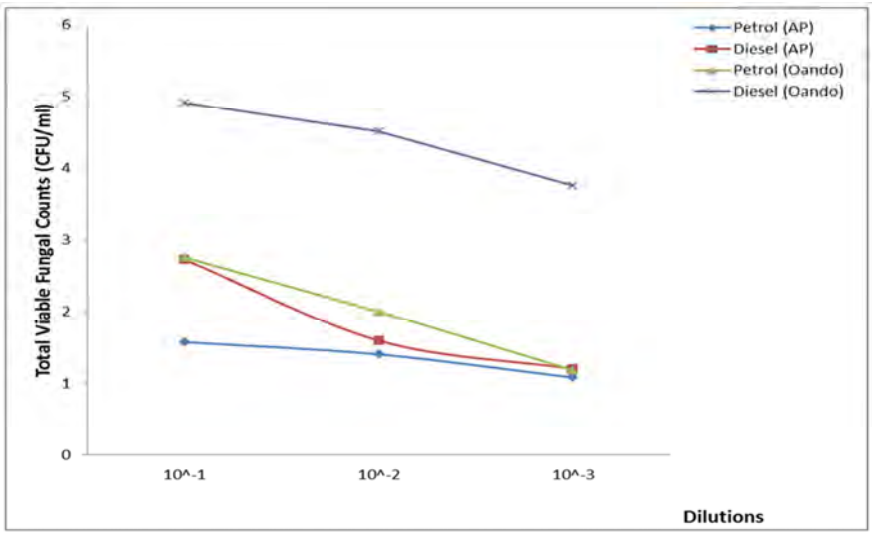

Figure 2. *The Total Heterotrophic Fungal Counts of Petrol and Diesel Samples.*

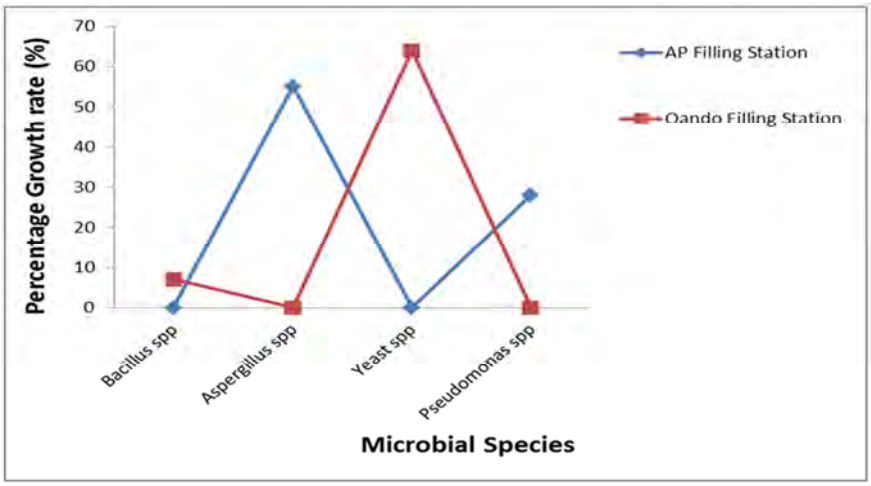

Figure 3. *Graphical Representation of Petrol Utilization by Fungal and Bacterial Isolates.*

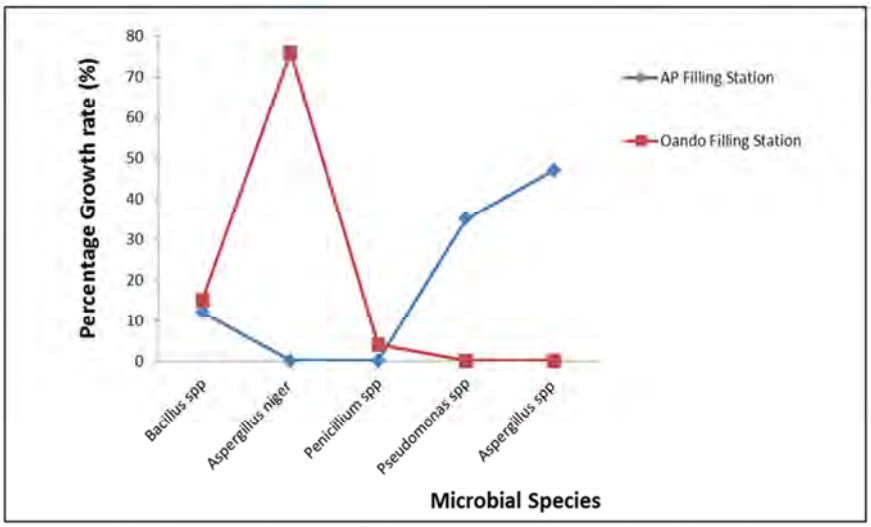

Figure 4. *Graphical Representation of Diesel Utilization by Fungal and Bacterial Isolate.*

Table 1. *Hydrocarbon Utilizing Bacterial and Fungal Counts of Petrol and Diesel Samples.*

Filling Station	Petrol		Diesel	
	HUB (CFU/ml)	HUF (CFU/ml)	HUB (CFU/ml)	HUF (CFU/ml)
AP	8.4×10^1	1.7×10^2	1.3×10^2	6.1×10^2
OANDO	5.4×10^1	3.2×10^2	2.4×10^3	3.8×10^4

The bacterial and fungal species isolated from cultured plates of the diesel samples obtained from Oando Filling Stations are *Bacillus* spp., *Aspergillus niger*, and *Penicillium* spp.; while from the petrol samples of the same station are yeast spp. and *Bacillus* spp. The isolates from diesel samples collected from AP Filling Station are *Pseudomonas* spp., *Bacillus* spp., and *Aspergillus* spp.; while that of petrol are *Aspergillus* spp and *Pseudomonas* spp. Growth of the isolates was most abundant with diesel as sole source of carbon and energy, especially from cultured plates of the sample obtained from Oando Filling Station, while it was least supported by petrol sample.

6. Discussion

The high growth rate of microorganism on diesel culture plates may be as a result of heavy contamination of the sample when in the storage tanks underground. The assimilatory biodeterioration of petrol by microbial deteriogens may be attributed to its simple chemical composition. The diesel which ordinarily should have supported growth less because of its complexity (having chain lengths C_{15}–C_{21}) more than petrol (with chain lengths C_5–C_9) is more readily absorbed by microorganisms as carbon source than petrol. The lead, anti-knock additive (tetra-ethyl lead) in petrol is probably the cause of the inhibitory [6], [23].

The petrol and diesel distributed by the Oando to customers is of a lower quality than that of AP Filling Station. This is because of the recorded increase in the frequency of bacterial and fungal cells occurrence in the samples obtained from Oando Filling Station.

The microorganisms isolated from the samples may be responsible for the biodeterioration of petrol and diesel which have been found to deteriorate car engines and block fuel-pump filters, cause bioufouling, and biocorrosion of metals, pipes and fuel storage tanks; and form slime and emulsions. Sanyaolu *et al.* [3] have isolated *Aspergillus niger, Aspergillus flavus, Trichoderma* sp and *Aspergillus terreus* from PMS/AGO samples collected from Oando Filling Station and MRS Service Station in Lagos State of Nigeria. This included the fungal species we isolated from the culture plates of PMS and AGO samples in Port Harcourt.

The works of Uzoamaka *et al.* [24] and Oboh *et al.* [25] have revealed that some fungal and bacterial species including the ones we isolated from PMS and AGO showed potentials for hydrocarbon biodeterioration. The microorganisms reported by these scholars are: *A. versicolor*, *A. niger*, *A. flavus*, *Syncephalastrum* spp., *Trichoderma* spp., *Neurospora sitophila*, *Rhizopus arrhizus, Mucor* spp, *Pseudomonas* spp., *Bacillus* spp., *Alcaligenes* and *Citrobacter* spp. Fungi such as *Aspergillus* spp., *Penicillum, Rhizopus* and *Rhodotorula sp.* possess the ability to grow and utilize crude petroleum as the sole carbon and energy source [3].

7. Conclusion

The results of this study revealed that bacterial and fungal species with petroleum biodeterioration potentials abound in petrol and diesel. To store them longer without adequate control-spoilage measures, being put in place, will result in their bioattack and subsequently may lead to the breakdown of motor engines. The petrol and diesel distributed by the AP to customers is of a higher quality than that of Oando Filling Station. Therefore, we recommend that adequate measure should be taken in order to reduce to the barest minimum the sources of microbial contamination on PMS and AGO in storage tanks of filling stations. Storage tanks undergrounds should have durable special coatings to prevent water from entering them; this is because water has been observed by many researchers to be an important prerequisite for development of microbial cells in fuels and oil and their subsequent deterioration.

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