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Assessment of Bacterial Deteriogens of Selected Lubricating Oils Used in Industrial Generators

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Authors' contributions

This work was carried out in collaboration between all authors. Author HOS designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors MD and CJU managed the analyses of the study. Author MD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study investigates microbial deteriogens of lubricating oils used on industrial generators. Total heterotrophic bacterial and total fungal counts were determined using the spread plate method. Bacterial isolates were screened for the utilization of lubricating oil and the rate of biodeterioration was determined by monitoring the optical density and pH of medium containing specific bacterial isolates from lubricating oil. Physico-chemical characteristics of lubricating oil were determined following standard procedures and petroleum hydrocarbon profile was determined using Gas Chromatography. Bacterial counts of used and unused samples ranged from 5.55 log cfu/ml to 7.83 log cfu/ml and from 4.64 log cfu/ml to 4.86 log cfu/ml respectively while fungal counts ranged from 6.60 log cfu/ml to 8.04 log cfu/ml and from 0 log cfu/ml to 7.32 log cfu/ml respectively. Bacterial isolates identified in the study include; *Micrococcus* sp, *Citrobacter* sp., *Bacillus* sp., *Serratia* sp., *Corynebacterium* sp., *Staphylococcus* sp., *Shigella* sp., while the fungal genera isolated include; *Penicillium, Aspergillus, Fusarium, Cryptosporium, Candida and Saccharomyces* species. Screen test for the utilization of used and unused oil samples by bacterial isolates showed that all isolates utilized the lubricating oil. Results further revealed that there was a consistent and significant (P <

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0.05) increase in optical density and a fluctuation in pH during deterioration monitoring. Petroleum hydrocarbon profile analysis after 28 days of study revealed lower concentrations of hydrocarbon components in the used oil samples compared to the unused samples, this confirm the fact that isolated organisms may have utilized the lubricating oil as a source of carbon and energy thereby affecting its quality and performance.

Keywords: Biodeteriogens; optical density; biodeterioration.

1. INTRODUCTION

Biodeterioration is any unwanted defects of inanimate substances caused by the biological activities of living things resulting in a loss in value, while microbial deteriogens are microorganisms that brings about or can cause biodeterioration. The two basic categories of lubricating oil are mineral and synthetic. Mineral oil lubricants are refined from naturally occurring petroleum or crude oil; they are mainly composed of a variety of alkanes and naphthalenes together with small amounts (less than 25%) of aromatic compounds whilst Synthetic lubricants are manufactured from polyalphaolefins, which are hydrocarbon-based polyglycols or ester oils, also consist of methyl silicones. Lubricants contain additives to improve performance, prevent corrosion and reduce oxidation. There may be detergents, dispersants, acid neutralizers, and metal pacifiers to prevent corrosion, making up perhaps 20% of the total oil formulation.

The tendency to decompose some fractions of petroleum can be attributed to a consortium of microorganisms [1]. Scientists over the years have studied the problem associated with the biodeterioration of petroleum products [2]. The issue of biodeterioration of petroleum products is a pressing one that needs urgent attention [3]. This is because Nigeria depends solely on petroleum products to sustain the economy, so whatever that affects this major export will definitely cripple the economy. Indigenous microorganisms play a vital role in the deteriorations of petroleum products in oil channels and tanks [4-6]. Studies and reports on the deterioration of lubricating oil in Nigeria has been very few, car users have little or no knowledge on why oil in motor engines should be constantly changed.

2. MATERIALS AND METHOD

2.1 Collection of Samples

Five brands of Multi grade lubricating oil labeled A, B, C, D and E were used in this study. The used lubricating oil samples were collected from industrial generator (engines) into several universal containers sterilized with 70% ethanol. Samples were collected from generators' engines in various industrial plants in five different companies within Port Harcourt, Rivers state while sealed same brand of unused lubricating oil samples were procured directly from the various producing companies. The samples were then properly labeled and immediately transported to the Microbiology laboratory for immediate analysis.

2.2 Sterilization of Materials

All the glassware and other materials used in the study were autoclave sterilized at 15psi or by dipping into ethanol (95% alcohol) and flaming where necessary.

2.3 Media Preparation

The following media were used to culture the isolates. Nutrient Agar, Potato Dextrose Agar (PDA), Mineral Salt Agar and Normal saline for serial dilution.

2.4 Enumeration of Total Heterotrophic Bacteria

Oil sample was serially diluted up to 10^{-6} using sterile normal saline and plated for total heterotrophic bacteria count (THBC) using 10^{-4} and 10^{-5} concentrations. Aliquots were introduced in to sterile plates of Nutrient Agar and then spread using a flame sterilized hockey stick. The plates were then incubated at room temperature (28+/-2℃) for 24–48 hours.

2.5 Identification of Bacterial Isolates

Gram reaction was used to differentiate between Gram positive and Gram negative bacteria.

2.6 Biochemical Test

The following tests (culture dependent method) were used to identify bacterial isolates: oxidase

test, catalase, citrate, sugar fermentation/ acid production, methyl red and voges proskauer (MRVP), starch hydrolysis, indole and triple sugar iron agar (TSIA).

2.7 Enumeration of Total Saprophytic Fungi

Aliquots from 10^{-4} and 10^{-5} concentrations were introduced in to sterile plates of PDA into which sterile streptomycin (50 mg/ml) had been incorporated to suppress bacterial growth and then spread using a flame sterilized hockey stick. The plates were then incubated at room temperature (28+/-2℃) for 48–72 hours.

2.8 Macroscopic Identification of Isolates

Morphological characteristics such as shape, colour, arrangement of spores, structure of the mycelium, structure of hyphae and arrangement of sporangiophores were all used in identifying the isolates [7].

2.9 Microscopic Identification of Isolates

Isolates were further examined microscopically by staining with a dye cotton blue. An aliquot of cotton blue in lactophenol was placed on a teased fungal isolate on a grease free clean glass slide. The teased sample was covered with clean cover slip avoiding air bubbles, the slide was examined with the microscope (Olympus BX51) using X10 and X40 objective.

2.10 Growth of Isolates Using Used and Unused Oil as Sole Carbon Source

The lubricating oil (A, B, C, D & E) were used at full strength as sole carbon source to determine if the isolates can utilize them for growth in Mineral Salt Agar. The pH and optical density of the medium was monitored for 14 days.

2.11 Determination of Total Petroleum Hydrocarbon

The total petroleum hydrocarbon profile (TPH) of both samples (Used and Unused lubricating oil) was determined using Gas chromatography (Hewlett Packard 5890 series 11) Gas chromatograph equipped with single flame ionization detector (FID) fitted with PerkinElmer Nelson analog digital converter (900 series) and a Compaq deskpro computer. A J and Wscientific DB-1 capillary column of 15 m length and an internal diameter of 0.32 mm wide bore of1micron film thickness were used. A temperature program of 50-305℃ increasing at 3.5℃ per minute for 27.15 min was employed. Hydrogen with a flow rate of 2ml per min was used as a carrier gas while the flow rate of air was 400ml per min. The temperature program used was 50℃ 2 min^{-1}, 35℃ min^{-1}, 270℃ 5 min^{-1}.The extractable petroleum hydrocarbons was identified and quantified by comparison of a sample chromatogram with calibration chromatograms of crude oil.

3. RESULTS

Bacterial isolates obtained from the used and unused lubricating oil are shown in Table 1, while Table 2 shows results fungal isolates. Bacterial isolates obtained from the oil samples include; *Micrococcus* sp., *Citrobacter* sp., *Bacillus* sp., *Serratia* sp., *Corynebacterium* sp., *Staphylococcus* sp. and *Shigella* sp. *Shigella* sp were however not detected in the unused samples. Fungal isolates were *Aspergillus* sp, *Fusarium* sp, *Penicillium* sp, *Cryptococcus* sp, *Candida* sp. and *Saccharomyces* sp., *Fusarium* sp., *Cryptosporium* sp. and *Candida* sp. were not detected in the unused lubricating oil samples. The results for percentage occurrence rates of bacteria in used and unused oil samples show *Micrococcus* sp. as the most predominant bacterial isolate and *Shigella* sp. as the least. *Cryptosporium* was the most predominant for fungi, with *Candida* and *Saccharomyces* species the least.

Results for total culturable heterotrophic bacterial count of used and unused lubricating oil are shown in Fig. 1. Bacterial counts of used lubricating oil were higher in all used samples compared to the unused samples. Bacterial counts of used and unused lubricating oil samples ranged 5.55 log cfu/ml to 7.83 log cfu/ml and from 4.64 log cfu/ml to 4.86 log cfu/ml respectively. Results of the total fungal count of used and unused lubricating oil are shown in Fig. 2. Fungal counts of used lubricating oil were generally higher in all used samples compared to the unused samples. Fungal counts of used and unused samples ranged from 6.60 log cfu/ml to 8.04 log cfu/ml and from 0 log cfu/ml to 7.32 log cfu/ml respectively.

Table 1. Bacterial isolates obtained from used and unused lubricating oil

Isolate	Used	Unused	Percentage occurrence
Shigella sp.	+	-	4.68
Bacillus sp.	+	+	17.18
Micrococcus sp.	+	+	29.68
Citrobacter sp.	+	+	25.00
Serratia sp.	+	+	7.81
Corynebacterium sp.	+	+	10.94
Staphylococcus sp.	+	+	4.71

+ = present; - = absent

Table 2. Fungal isolates obtained from used and unused lubricating oil

Isolate	Used	Unused	Percentage occurrence
Penicillium sp.	+	+	7.31
Aspergillus sp.	+	+	26.83
Fusarium sp.	+	-	17.07
Cryptosporium sp.	+	-	41.46
Candida sp.	+	-	2.43
Saccharomyces sp.	+	+	2.43

+ = present; - = absent

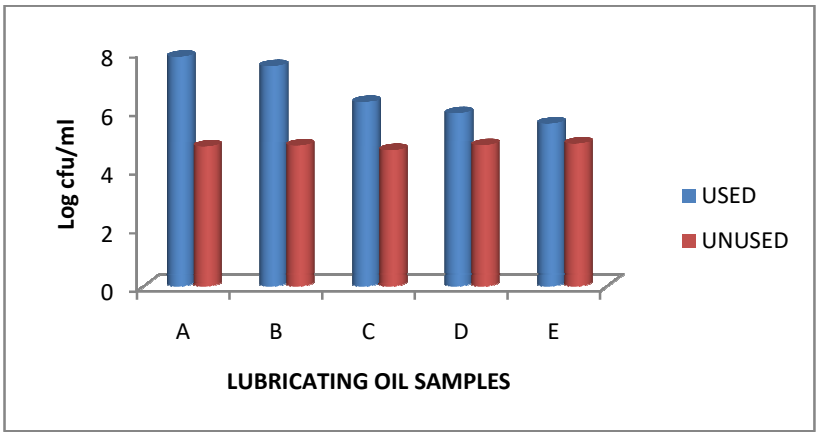

Fig. 1. Changes in the total culturable heterotrophic bacterial counts of lubricating oil samples

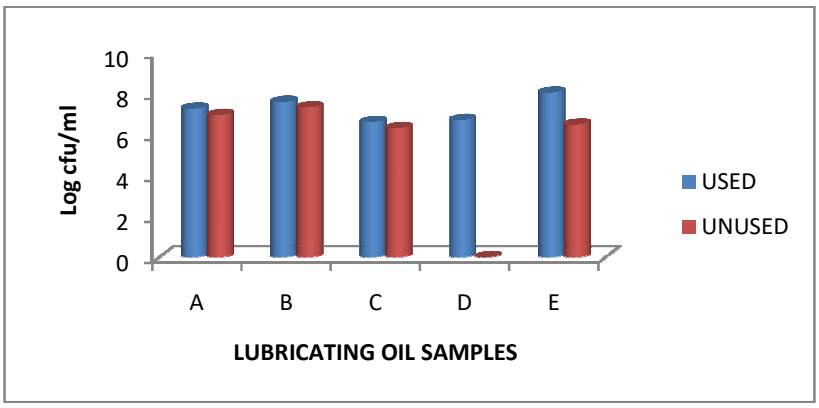

Fig. 2. Changes in the total fungal counts of lubricating oil samples

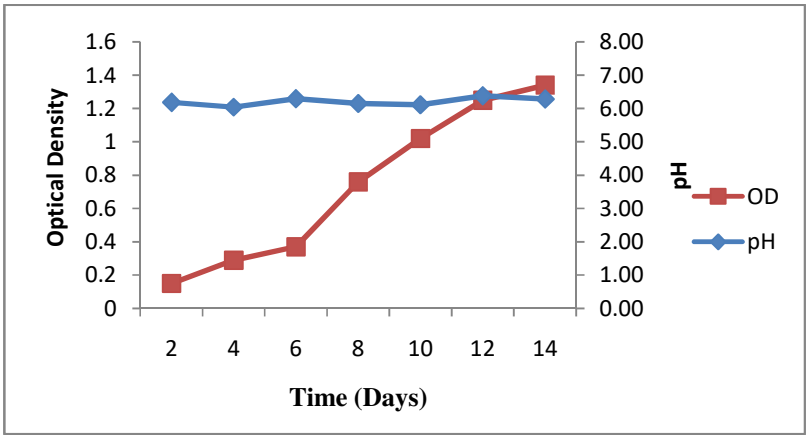

Fig. 3. Changes in the growth rate of *Micrococcus* sp. in medium containing used lubricating oil as sole source of carbon and energy

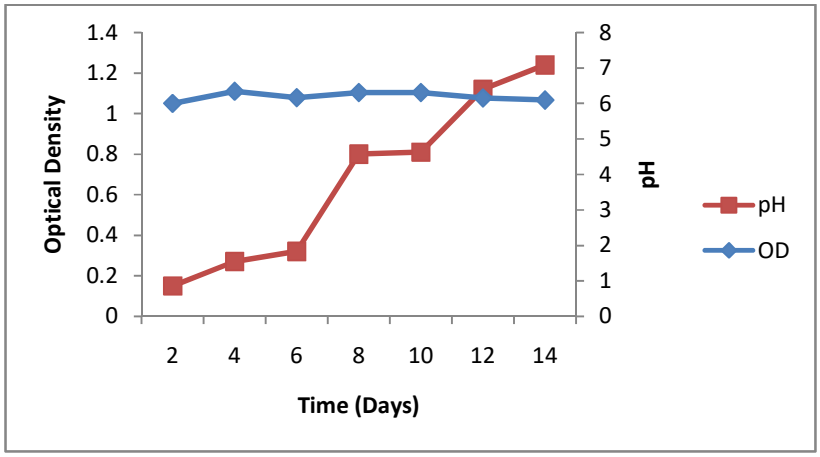

Fig. 4. Changes in the growth rate of *Citrobacter* sp. in medium containing used lubricating oil as sole source of carbon and energy

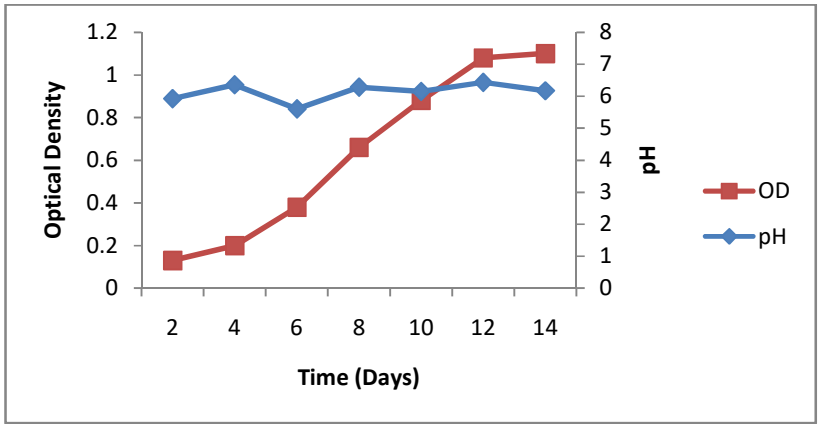

Fig. 5. Changes in the growth rate of *Serratia* sp. in medium containing used lubricating oil as sole source of carbon and energy

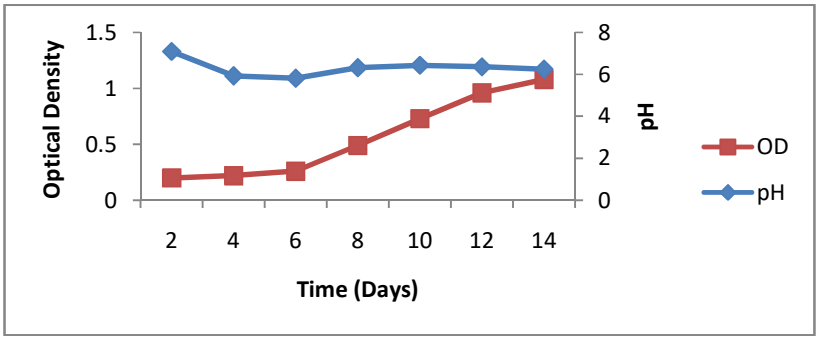

Fig. 6. Changes in the growth rate of *Corynebacterium* sp. in medium containing used lubricating oil as sole source of carbon and energy

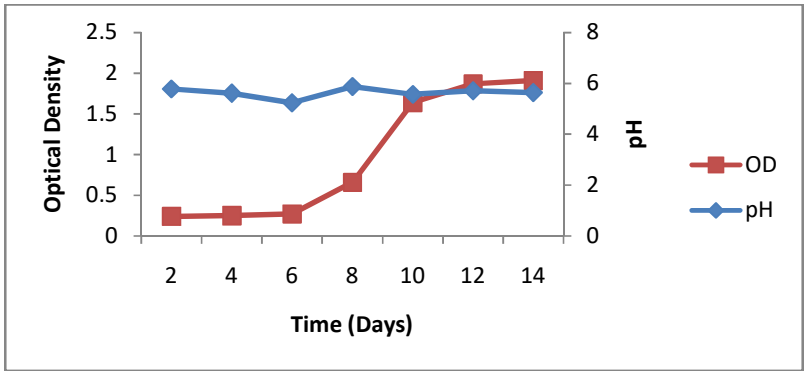

Fig. 7. Changes in the growth rate of *Micrococcus* sp. in medium containing unused lubricating oil as sole source of carbon and energy

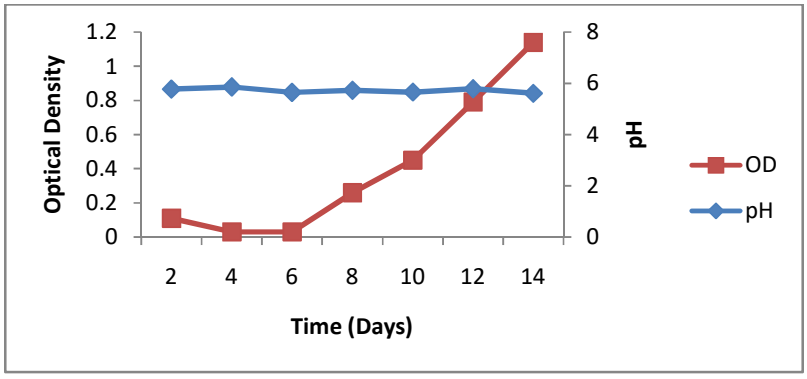

Fig. 8. Changes in the growth rate of *Citrobacter* sp. in medium containing unused lubricating oil as sole source of carbon and energy

The screen test results for the utilization of lubricating oil (used and unused) by bacteria are presented in Table 3. The organisms subjected to lubricating oil utilization test were *Micrococcus* sp., *Citrobacter* sp., *Bacillus* sp., *Serratia* sp., *Corynebacterium* sp., *Staphylococcus* sp. and *Shigella* sp. All isolates showed higher growth in medium containing used oil compared to medium containing unused oil. The growth profile of selected isolates (based on their utilization potentials) in mineral salt medium containing lubricating oil that served as energy and carbon source as shown in Figs. 3–10. Results indicate that the growth (optical density) of *Micrococcus* sp. in used and unused lubricating oil increased significantly ($P < 0.05$) from 0.15 to 1.34 and

from 0.24 to 1.91 respectively while the pH profile fluctuated insignificantly between pH 6.04 to pH 6.29 and from pH 5.24 to pH 5.88 respectively. Growth (OD) of *Citrobacter* sp. in medium containing used and unused engine oil increased from 0.15 to 1.24 and from 0.11 to 1.14 respectively during the 14-day period of incubation while the pH fluctuated between 6.00 to 6.34 and from 5.61 to 5.85 respectively. Growth (OD) of *Serratia* in media containing used and unused lubricating oil increased significantly from 0.13 to 1.10 and from 0.08 to 1.22 respectively while the pH fluctuated significantly between 5.60 to 6.34 and from 5.59 to 6.24 respectively during the 14-day period of incubation. Growth of *Corynebacterium* sp. in medium containing used and unused lubricating oil increased significantly from 0.20 to 1.08 but the growth of *Corynebacterium* sp. decreased significantly in day 2 from 0.29 to 0.05 OD and showed a subsequent increase from the third day to the final day with OD from 0.07 to 1.07. The pH was revealed to fluctuate insignificantly between 7.09 to 6.24 and from 5.03 to 5.78 in medium containing lubricating oil (used and unused).

Table 3. Screen test for the utilization of used and unused lubricating oil samples by the bacterial isolates

Isolates	Used oil	Unused oil
Shigella sp.	++	+
Bacillus sp.	++	+
Micrococcus sp.	+++	+
Citrobacter sp.	+++	+
Serratia sp.	+++	+
Corynebacterium sp.	+	+
Staphylococcus sp.	+	+

+ = Less growth; ++ = Moderate growth; +++ = Abundant growth

Table 4. Percentage of reduction in total petroleum hydrocarbon profile

Lubricant sample	% TPH (Unused)	% TPH (Used)
A	29.32	60.40
B	28.13	62.08
C	29.33	60.35
D	29.31	64.73
E	39.14	59.30

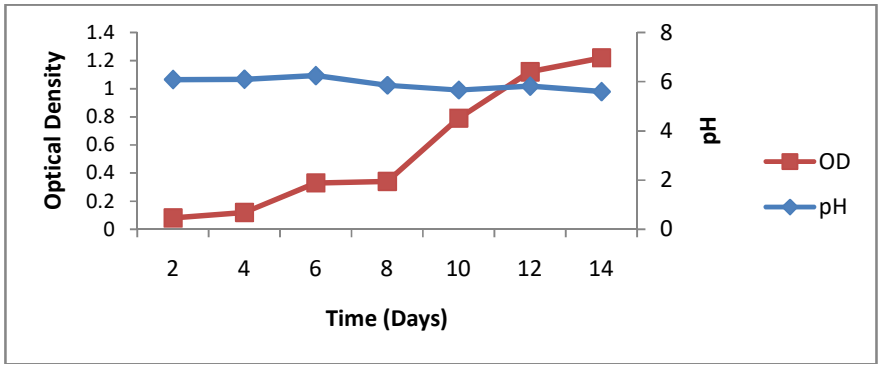

Fig. 9. Changes in the growth rate of *Serratia* sp. in medium containing unused lubricating oil as sole source of carbon and energy

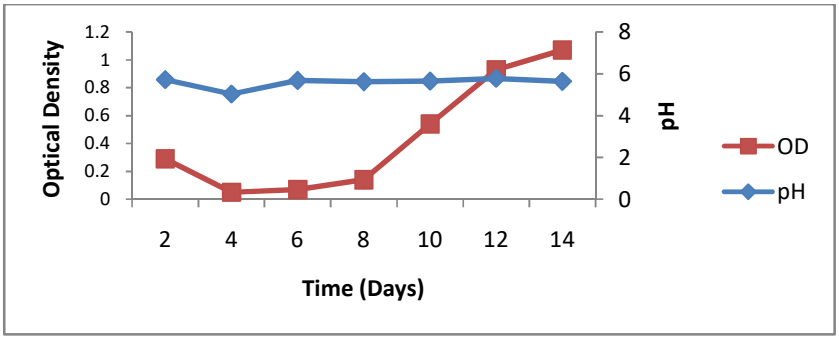

Fig. 10. Changes in the growth rate of *Corynebacterium* sp. in medium containing unused lubricating oil as sole source of carbon and energy

Table 4 shows percentage of reduction in total petroleum hydrocarbon profile in used and unused lubricating oil. Results showed that used oil was more susceptible to microbial degradation than unused oil.

4. DISCUSSION

The study investigated the biodeterioration of used and unused lubricating oils samples. Results of the research indicate that bacterial and fungal counts were higher in used oil samples compared to unused oil samples. Bacterial counts of used and unused lubricating oil samples were very high. The high bacterial and fungal counts obtained from the used lubricating oil samples can be as a result of the fact that the lubricating oil samples had been exposed to the environment making it prone to contamination by indigenous microorganisms.

Bacterial isolates obtained from the study include; *Micrococcus* sp., *Citrobacter* sp. *Bacillus* sp., *Serratia* sp., *Corynebacterium* sp, *Staphylococcus* sp. and *Shigella* sp*., Shigella* sp. was however not detected in the unused oil samples. This further buttressed the point earlier explained that living organisms (microbes) must have entered into the used oil from the external environment. In a similar study carried out by Okpokwasili and Okorie [8] on microbe-enhanced degradability especially the ones isolated from lubricating oil, *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Acinobacter* sp., *Serratia* sp, *Norcadia* sp., *Edwardsiella* sp. and *Corynebacterium* sp were identified. In another study on biominerability of microorganisms in brake fluid, *Pseudomonas* sp., *Micrococcus* sp. and *Serratia* sp were isolated from spent and new lubricants [9].

From the research carried out, *Micrococcus* sp were found to be higher in number, followed by *Citrobacter* sp., *Bacillus* sp., *Corynebacterium* sp., *Serratia* sp.,, *Staphylococcus* sp*.,* and *Shigella* sp., in that order. Several studies have identified *Micrococcus* and *Bacillus* species as hydrocarbon utilizers [8,9].

The predominance of Gram positive bacteria in lubricating oil have been mentioned by Maduka and Okpokwasili [9] and their abundance can be as a result of the fact that this group of bacteria are spore formers which allow them to survive adverse conditions. Furthermore, the survival and dominance of Gram positive bacteria in lubricating oils of industrial generators whose temperatures are elevated during operation is possible because the formation of spore ensures their survival when the environmental temperature is elevated beyond the organisms' temperature requirement.

Fungal isolates obtained from the lubricating oil in the present study include: *Penicillium, Aspergillus, Fusarium, Cryptosporium, Candida and Saccharomyces* species. However, *Fusarium, Cryptosporium* and *Candida* species were not detected in the unused lubricating oil samples. In a similar study carried out by Sanyaolu et al. [10], *Aspergillus* species and *Trichorderma* sp. were isolated from used lubricating oil. Among the mould isolates obtained from the used samples, *Aspergillus* was the highest occurring mold, followed by *Fusarium Penicillium*, while *Cryptococcus* was the predominant yeast followed by *Candida* and *Saccharomyces*. The presence of these fungal organisms in used oil implies that they are capable of utilizing the substrate as their carbon source and hence capable of negatively affecting its quality and performance. In the study of Okerentugba and Ezeronye [11], it was shown that *Aspergillus* and *Penicillium* species are capable of degrading hydrocarbons. Due to the ability of fungi to produce extracellular enzymes, they are capable of utilizing hydrocarbon as a substrate for growth and survival [12].

Finding feasibility in utilization of lubricants by microbial groups revealed all isolates utilized these oily materials. The isolates showed different degrees of turbidity with the highest turbidity showed in the used sample for some of the tested strains. This result is in concordance with the study of Okpokwasili and Okorie [8] who reported that the highest turbidity was shown in the medium containing used lubricating oil. This result suggests that these organisms are fast hydrocarbon degrader and they possess the enzyme systems to break down complex organic carbon. Okpokwasili and Okorie [8] however explained that some constituent of the used oil may have been affected somehow providing easily metabolizeable substrates for the organisms.

The consistent and significant increase in the growth (OD) of all isolates in medium containing used and unused lubricating oil is an indication that the organisms are utilizers of petroleum hydrocarbon and possible degraders. Rise in biomass quality by the groups [13] (Okpokwasili and Nweke, 2006). However, growth of isolates

was observed to be higher in media amended with used lubricating oil compared to medium containing unused lubricating oil for some of the tested strains. The used lubricating oil may have undergone degradation while exposed, to allow for the availability of precursor molecules which are easily utilizeable and metabolizeable by microorganisms. The easily metabolizeable substrates would support a higher growth compared to the complex organic hydrocarbon present in the unused lubricating oil which can only be degraded or broken down by a specialized physiological group of organisms that possess the specific enzyme system to do so. This finding agreed with the study of Nweke and Okpokwasili [14] and further supported by the study of Okpokwasili and Okorie [8]. Okpokwasisli and Okorie [8] reported that used lubricating oil supported a higher growth compared to the unused lubricating oil. They further stated that enzymes needed for the breakdown of complex hydrocarbon are inducible and the synthesis of these enzymes is stepwise. Therefore, it will require an extended duration for the breakdown of the unused lubricating oil where the factors are optimal.

Preservatives added to lubricating oil to guarantee an extended shelf-life could also have inhibited the growth of the organisms in unused oil while these components may have been oxidized in used oil during service or exposure thereby allowing for proper growth without inhibition or elimination.

The fluctuations in pH (though statistically insignificant) in medium containing both used and unused lubricating oil does not agree with previous studies [8,9]. However, this may be because of the production of acidic and alkaline metabolites by the organisms. The pH values of medium containing used lubricating oil were all within neutral. This further supports the results of the optical density analysis which revealed higher growth in the medium containing used lubricating oil. The optimal pH requirements of the isolates used in the analysis were all in the neutral range.

Petroleum hydrocarbon profile of unused lubricating oil samples analyzed did not difer significantly. The decrease in petroleum hydrocarbon components in the used oil samples was more compared to the unused samples, and this can be attributed to microbial contamination/ activity and oxidation leading to carbon loss through volatilization. In a similar study carried out by Shaieb et al. [15], the concentrations of petroleum hydrocarbon components in a crude oil medium containing known isolates decreased to 18.86%, 26.16% and 39.04% for bacteria isolated from crude oil samples. In another study carried out by Uba and Ifeanyi [16], the concentrations of petroleum hydrocarbon components in a crude oil medium containing known isolates was shown to quantitatively decrease from 12,405 mg/L to 122 mg/L for *Bacillus* sp and from 12,405 mg/L to 712 mg/L for *Trichosporon* sp. The result for petroleum hydrocarbon content between the used and unused lubricating oil samples showed a significant difference ($p < 0.005$).

5. CONCLUSION

Lubricating oils are prone to deterioration when they are exposed to microbial activities, especially when they are in-use as shown from the study. Therefore microbial growths are proxies for deterioration in lubricating oils. Once they ensue they utilize the lubricating oil, degrade its quality and retard the performance of the industrial generators' engines where they are used. Hence, it is impossible to prevent penetration of microorganisms into lubricating oil when good storage techniques are not employed. The introduction of preservatives, additives and biocides in the lubricating oil could deter microorganisms from infesting the oil thereby increasing its shelf life and preventing the engine parts from touching each other. Preventive measures of microbiological contamination for lubricating oil should include a monitoring system of storage conditions, strict storage and maintenance rules.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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