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Comparison of *In vitro* Study of *Aspergillus niger* and *Pseudomonas aeruginosa* Biodegradation of Spent Lubricating Oil

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Authors' contributions

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

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ABSTRACT

Comparison of biodegradation of spent lubricating oil by *Aspergillus niger* and *Pseudomonas aeruginosa* was studied for 16 days. pH, turbidity, nitrate, phosphorus and Gas Chromatographic - Mass Spectrophometry (GC-MS) analyses of the media were carried out. The results showed lower pH and phosphorus levels in *A. niger* medium compared to *Pseudomonas aeruginosa* medium and the control while nitrate was higher in *Pseudomonas aeruginosa* compared to *A. niger*. The GC-MS revealed that more compounds were degraded by *A. niger* than *Pseudomonas aeruginosa* after 16 days. Azulene and benzene were not degraded by both organisms. This study suggests that *A. niger* grows and metabolize compounds in spent lubricating oil better than *Pseudomonas aeruginosa* and that neither of the two organisms had the competent enzymes to degrade azulene and benzene in two weeks.

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Keywords: Azulene; biodegradation; benzene; spent lubricating oil; Aspergillus niger; Pseudomonas aeruginosa.

1. INTRODUCTION

Bacteria, fungi, yeasts and algae play a paramount role in bioremediation of hydrocarbons in soil [1]. These organisms have been repeatedly isolated from hydrocarbon polluted soils. According to Nkweenleng et al. [1], *Pseudomonas, Acetobacter, Chromobacterium, Candida* species are frequently isolated from hydrocarbon enriched soil. Other researchers reported *Bacillus, Actinomyces, Flavobacterium, Fusarium, Aspergillus and Rhodotorula* species [2,3].

Aspergillus species especially *A. niger* and *A. flavus* have been reported as fungi that degrade hydrocarbons. Kuiper et al. [4], Okereke et al. [5], Nkweenleng et al. [1] and Chikere et al. [6] reported the presence of *A. niger, A. flavus and A. fumigatus* in oil spill soil and their crude oil degradative abilities. Okwute and Ijah [7] also reported the presence of *Aspergillus niger* in palm oil mill effluent (POME) polluted soil undergoing biodegradation.

According to Stephen et al. [8], *Aspergillus* spp can initiate the degradation of normal alkanes by sub-terminal oxidation, hence their relative abundance in soil polluted by hydrocarbons.

Spent lubricating oil refers to any oil refined from crude oil or any synthetic oil that has been used and as a result of such use, is contaminated by physical or chemical impurities [9]. Spent lubricating oil pollution is responsible for several environmental problems, risk to human health and plants [10]. Spent lubricating oil creates unsatisfactory conditions for plant growth ranging from heavy metal toxicity to insufficiency in aeration of the soil. Hence, there is the need to degrade it in soil and also determine the compounds that could be degraded or not. The choice of *Aspergillus niger and Pseudomonas aeruginosa* stem from their widely reported activity in biodegradation studies. This study was therefore undertaken to compare their individual abilities in degrading spent lubricating oil under same conditions and the various compounds degraded.

2. MATERIALS AND METHODS

2.1 Collection of Isolate and Lubricating Oil

Aspergillus niger and *Pseudomonas aeruginosa* were collected from stock cultures from the Department of Microbiology, Kogi State University Anyigba while spent lubricating oil was collected from the mechanic workshop opposite First City Monument Bank, Anyigba, Nigeria. Both organisms were inoculated into peptone broth for 24 hours. Mineral Salt Medium 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 $MgSO_4.7H_2O$ was prepared in 1000 ml of distilled water. The initial pH of the mineral salt medium was 5.7. 10 ml of Mineral Salt Medium was dispensed into twenty test tubes each. 2ml of spent lubricating oil was added into each test tube and the solution sterilized by autoclaving. 2ml of overnight broth culture (peptone broth) of *Aspergillus niger* and *Pseudomonas aeruginosa* were seeded into seven test tubes separately while the remaining six without *Aspergillus niger* and *Pseudomonas aeruginosa* served as the control. The test tubes were incubated at ambient temperature for 16 days without shaking. Degradation of the spent lubricating oil was monitored at 4 days interval for 16 days. Growth pattern of the organisms were determined by measuring the turbidity using turbidity meter (WGZ-113 Shanghi, China). pH was determined at ambient temperature using glass electrode pH and conductivity meter (Hannia, Italy). Phosphorus was determined using the method described by Murphy and Riley [11. Nitrogen was determined by the micro Kjedahl method as described by Ibitoye [12].

2.2 Gas Chromatography-mass Spectrophometry

The Gas Chromatographic –Mass Spectrophotometric analysis was carried out at day 0 (for control) and days 7 and 14 (for spent lubricating oil inoculated with *Aspergillus niger* and *Pseudomonas aeruginosa*). The mineral salt medium containing spent lubricating oil and the organisms were decanted into a 50 ml beaker using Whatmann filter paper. The oil on the filter papers was recovered by rinsing with 25ml of carbon trichloromethane (chloroform) in another

50ml beaker. The oil was placed in a water bath for 20 minutes to evaporate the solvent. The oil was then analysed using gas-liquid chromatography-mass spectrophotometer (GCMS Qp 2010 plus, Shimadzu, Japan). The oil was diluted with n-hexane to a volume of 10ml from which one micro litre was injected into the GC-MS. Injector and detector chamber temperatures were set at 250°C and 380°C respectively. The oven temperature was initially set at 60°C for 4 minutes, ramped at 10°C per minutes to 210°C for 3 minutes. It was further held for 2 minutes and ramped at 20°C per minutes to 280°C.

2.3 Data Analysis

Descriptive statistics and analysis of variance (ANOVA) was performed using procedure of SPSS version 16 (2007). Experimental precision achieved was reported at $p \leq 0.05$ level.

3. RESULTS

Fig. 1 shows the change in pH of the spent lubricating oil undergoing biodegradation. The pH ranged from 5.5 to 7.8. The pH was lower in *Aspergillus niger* inoculated medium compared to *Pseudomonas aeruginosa* medium between days 4 and 12. The pH in both *Aspergillus niger* and *Pseudomonas aeruginosa* medium decreased from day 12 till the last day of the experiment. There was significant difference ($p<0.05$) in the pH between control, *Aspergillus niger* and *Pseudomonas aeruginosa* medium.

Fig. 2 shows the turbidity produced by the organisms in the spent lubricating oil medium during the course of the experiment. Turbidity increased in all treatments from day 0. *Pseudomonas aeruginosa* had higher turbidity compared to *Aspergillus niger* and the control at day 8. However, an appreciable increase was observed in *Aspergillus niger* medium from day 12 till day 16. There was no significant difference ($p<0.05$) in the turbidity between control, *Aspergillus niger* and *Pseudomonas aeruginosa* medium.

Fig. 3 shows the change in nitrate concentration of the spent lubricating oil undergoing biodegradation. The nitrate concentration declined after day 0 in all treatments. However, the decline was greater in *Pseudomonas aeruginosa* medium compared to *Aspergillus niger* and control. There were significant differences ($p<0.05$) in the nitrate concentrations between control, *Aspergillus niger* and *Pseudomonas aeruginosa* medium.

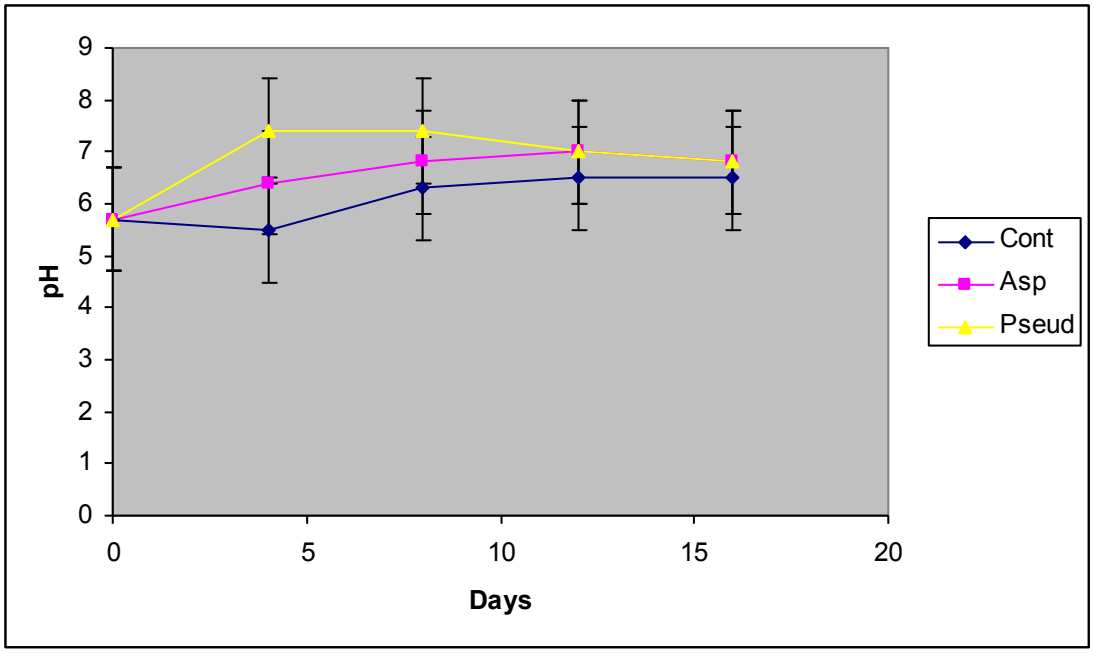

Fig. 1. pH of spent lubricating oil undergoing biodegradation
Cont: Control, Asp: Aspergillus niger, Pseud: Pseudomonas aeruginosa

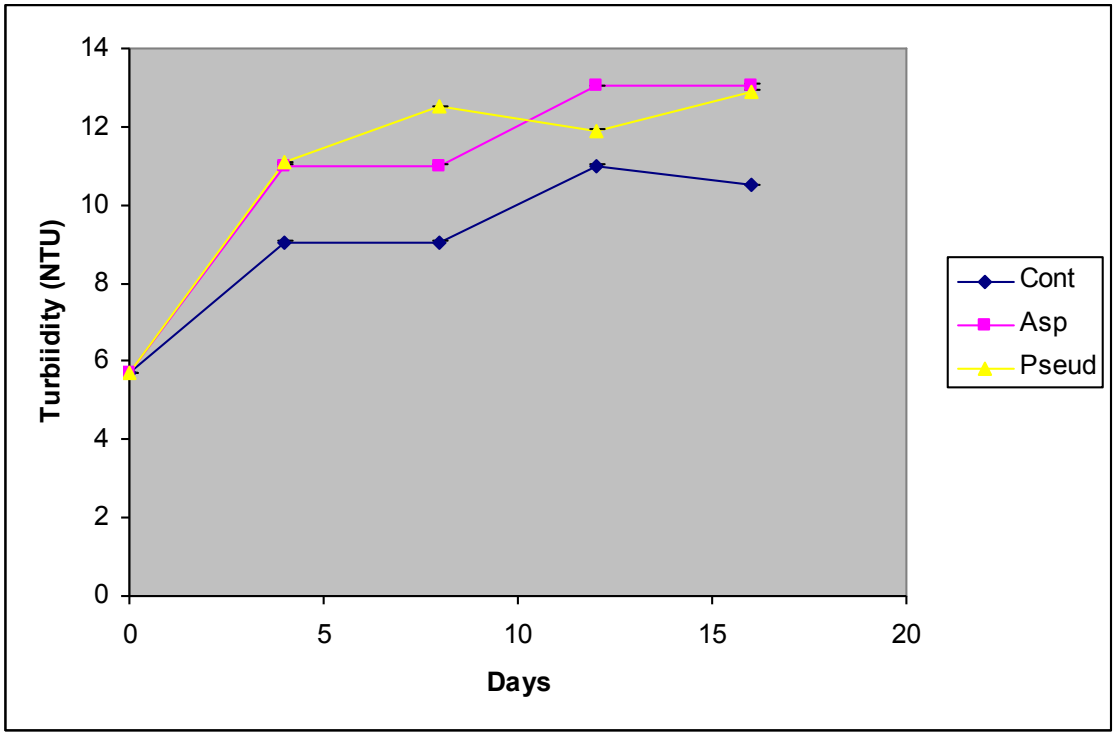

Fig. 2. Turbidity of spent lubricating oil undergoing biodegradation
Cont: Control, Asp: Aspergillus niger, Pseud: Pseudomonas aeruginosa

Fig. 4 shows the phosphorus content of the spent lubricating oil undergoing biodegradation. The phosphorus contents decreased in the inoculated samples from day 0 till the end of the experiment. Phosphorus was lower in samples inoculated with *Aspergillus niger* and *Pseudomonas aeruginosa* than the control. However, there was a gradual decline until day 16 of the experiment in both *Aspergillus niger* and *Pseudomonas aeruginosa* samples. There were no significant differences ($p<0.05$) in the phosphorus contents between control, *Aspergillus niger* and *Pseudomonas aeruginosa* medium.

Fig. 5 shows the gas chromatographic tracing of the uninoculated spent lubricating oil (control). The compounds present were methylbenzene, ethylbenzene, o-xylene, propylbenzene, octane, pentadecane and hexane. The chromatogram showed that spent lubricating oil had more aromatic and cycloalkanes than straight chain alkanes.

Fig. 6 shows the chromatogram of the spent lubricating oil inoculated with *Aspergillus niger* after one week (7 days). The branched aromatic compounds were degraded after one week. There was a reduction in the peak heights after one week. New compounds such as azulene (bicyclo (5,3,0) decapentene) may have been introduced as a result of the utilization of the spent oil by *Aspergillus niger*.

The chromatogram of the inoculated spent oil by *Aspergillus niger* after 14 days is shown in Fig. 7. The peak heights and compounds were further reduced compared to Figs. 5 and 6. Organic acids were more in Fig. 8 than 7. The compounds remaining in the spent lubricating oil after 14 days of degradation were benzene, azulene, dodecane, tetradecane, heptadecane, hexadecanoic acid, 9-octadecanoic acid and octadecanoic acid.

Fig. 8 shows the chromatogram of the spent lubricating oil inoculated with *Pseudomonas aeruginosa* after one week (7 days). The branched aromatic compounds were degraded after one week resulting in multiple benzene and straight chain alkanes. There was a reduction in the peak heights after one week. Azulene (bicyclo (5,3,0) decapentene) was also introduced as a result of the utilization of the spent oil by *Pseudomonas aeruginosa*.

The chromatogram of the inoculated spent oil by *Pseudomonas aeruginosa* after 14 days is shown in Fig. 9. The peak heights and compounds were higher and more in number compared to those in Fig. 7. Organic acids were also less than those in produced by *Aspergillus niger* after two weeks. The compounds remaining in the spent lubricating oil after 14 days were benzene, azulene, undecane, tetradecane, heptadecane, 7-octadecanoic acid and octadecane and henicosane.

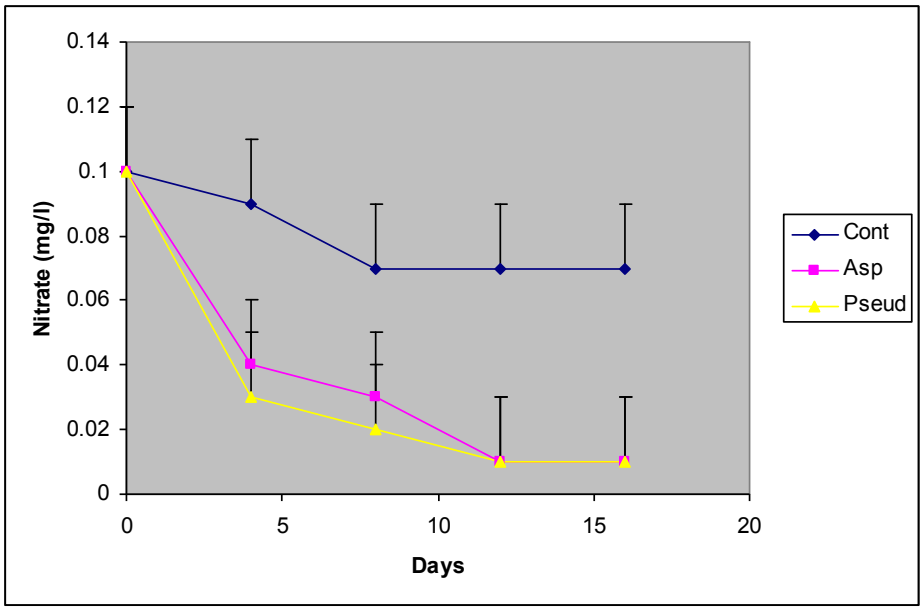

Fig. 3. Nitrate concentration of spent lubricating oil undergoing biodegradation
Cont: Control, Asp: Aspergillus niger, Pseud: Pseudomonas aeruginosa

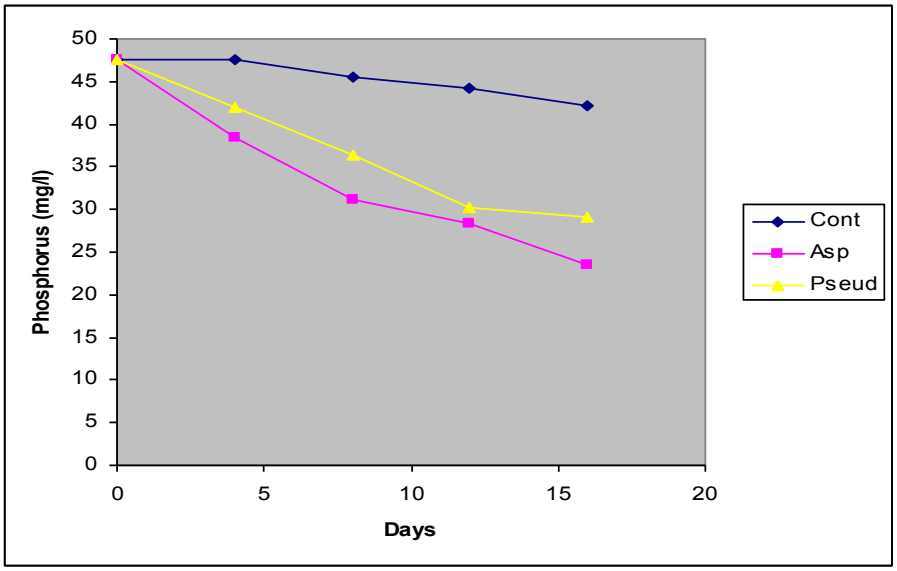

Fig. 4. Phosphorus content of spent lubricating oil undergoing biodegradation
Cont: Control, Asp: Aspergillus niger, Pseud: Pseudomonas aeruginosa

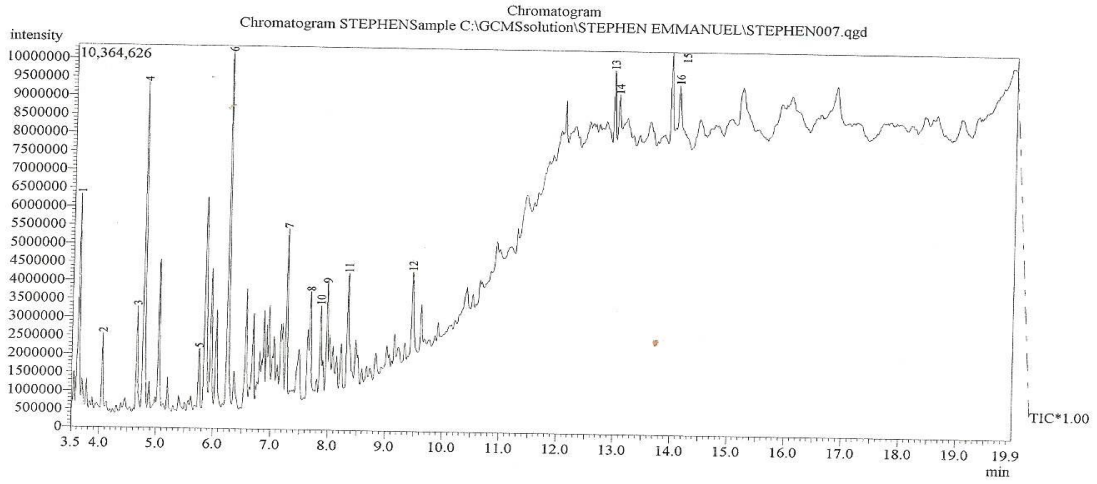

Fig. 5. Gas Chromatographic analysis of Spent Lubricating Oil, SLO

Peak sequences: 1: methylbenzene 2: octane 3: ethylbenzene 4: o-xylene 5: propylbenzene 6: ethylbenzene 7: benzene 8: 1-bromomethyl-4-isopropylbenzene 9: methyl-p-ethyltoluene 10: 1-phenyl-1-butene 11: cyclopentacycloheptene 12: 1,6-methanol 13: pentadecane 14: pentadecane 15: Hexadecane 16: hexadecane

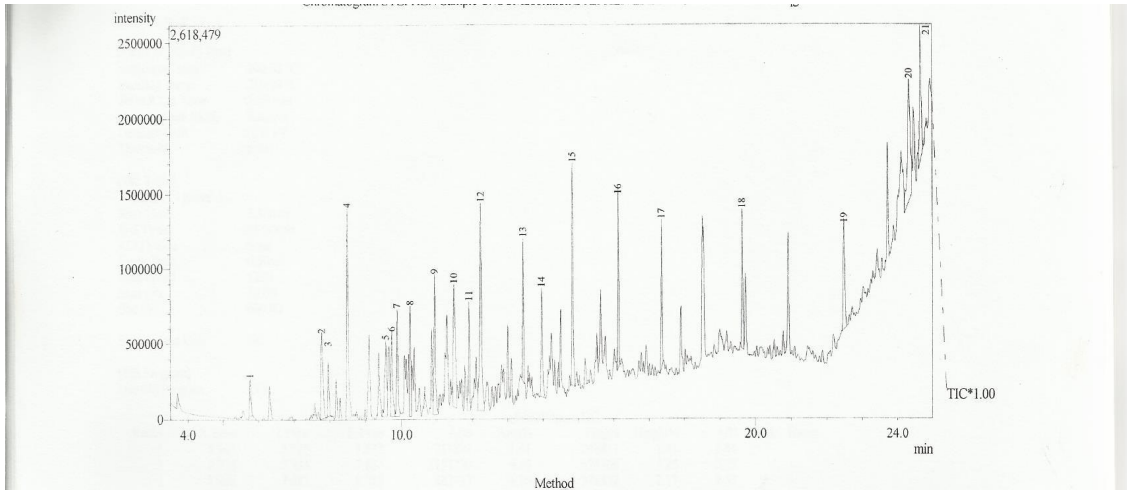

Fig. 6. Gas chromatographic analysis of spent lubricating oil degraded by *Aspergillus niger* after one week

Peaks: 1: benzene 2: benzene 3: benzene 4: benzene 5: benzene 6: benzene 7: 4,7- methanoindene 8: 1,3-cyclohexadiene 9: benzene 10: 3-phenyl-1-butene 11: undecane 12: azulene 13: undecane 14: napthalene 15: tetradecane 16: pentadecane 17:hexadecane 18:heptadecane 19: heptadecane 20: tetracosanoic acid 21: tricontane

4. DISCUSSION

The range of pH (5.3-7.8) observed in both *A.niger* and *Pseudomonas aeruginosa* media compared to the control has been reported to favour biodegradation of hydrocarbon [13]. The range in pH observed in this study were similar to those reported earlier by Stephen et al. [8]. The lower pH observed in *A.niger* medium is an indication of higher number of organic acids produced by the metabolic activity of *A. niger* compared to *Pseudomonas aeruginosa* [14]. This can be seen in the resulting chromatogram (Fig. 7).

The turbidity produced during biodegradation was higher in *Pseudomonas aeruginosa* medium up till the 12th day compared to the control and *A.*

niger medium. This implied that *Pseudomonas aeruginosa* was able to utilize the spent lubricating oil better than *A. niger* at the early stage of the study. The higher turbidity observed in *A. niger* at the end of the experiment indicates that metabolic activity of the fungus increases with time. This result is in line with an earlier report by Stephen et al. (8) who observed higher metabolic activity in an *A. niger* inoculated medium compared to the control.

Lower nitrate concentration was observed in *Pseudomonas aeruginosa* medium compared to *A. niger* and the control throughout the study. The lower nitrate concentration in *Pseudomonas aeruginosa* is an indication that *Pseudomonas aeruginosa* utilizes nitrate better than *A. niger* [14].

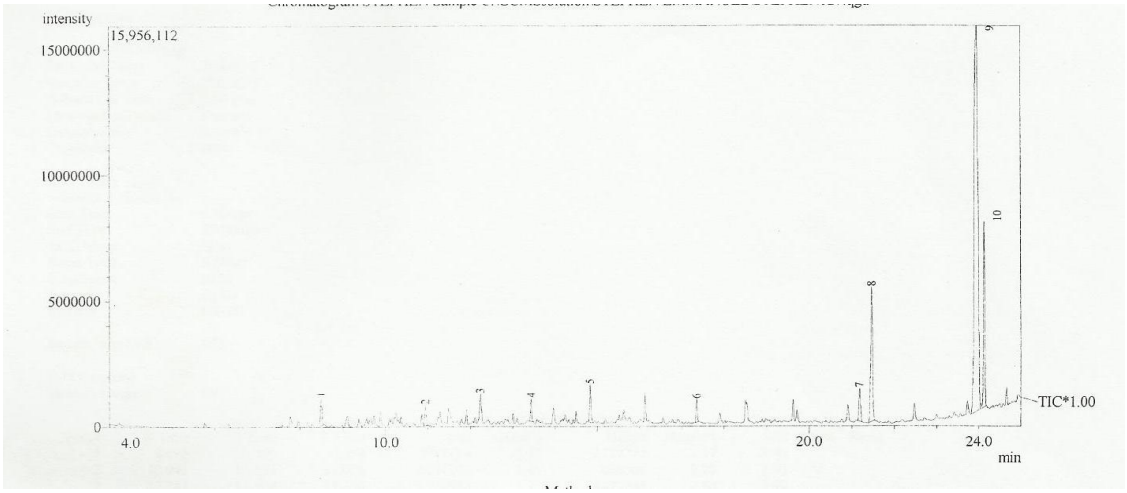

Fig. 7. Gas chromatographic analysis of spent lubricating oil degraded by *Aspergillus niger* after two weeks

Peaks: 1: benzene 2: benzene 3: azulene 4: dodecane 5: tetradecane 6: heptadecane 7: hexadecanoic acid 8: hexadecanoic acid 9: 9- octadecanoic acid 10: octadecanoic acid.

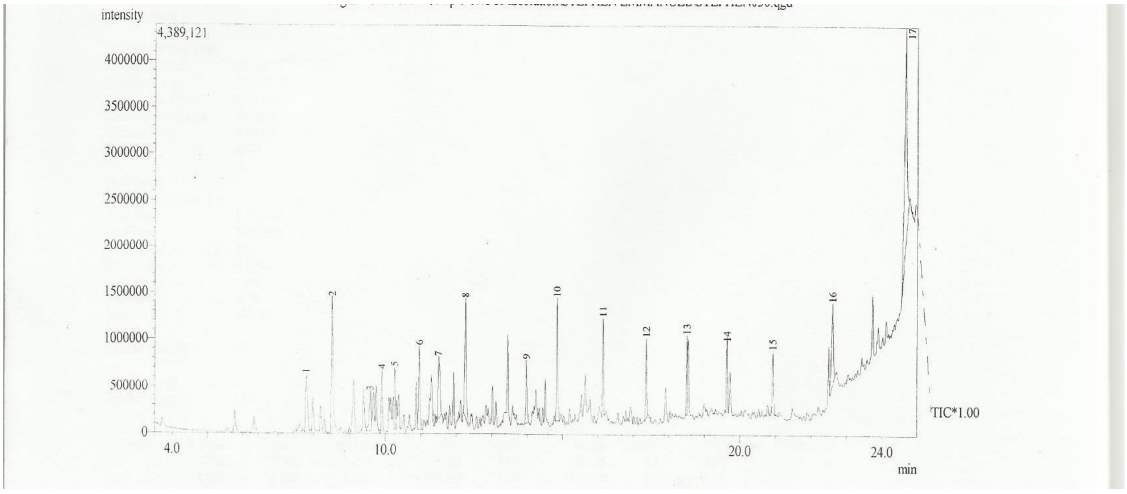

Fig. 8. Gas chromatographic analysis of spent lubricating oil degraded by *Pseudomonas aeruginosa* after one week

Peaks: 1: benzene 2: benzene 3: benzene 4: 4,7-methano-1-indene 5: benzene 6: benzene 7: benzene 8: azulene 9: 1,4-methanonaphthalene 10: tetradecane 11: nonane 12: dodecane 13: heptadecane 14: hexadecane 15: heptadecane 16: hexadecanoic acid 17:6-octadecanoic acid

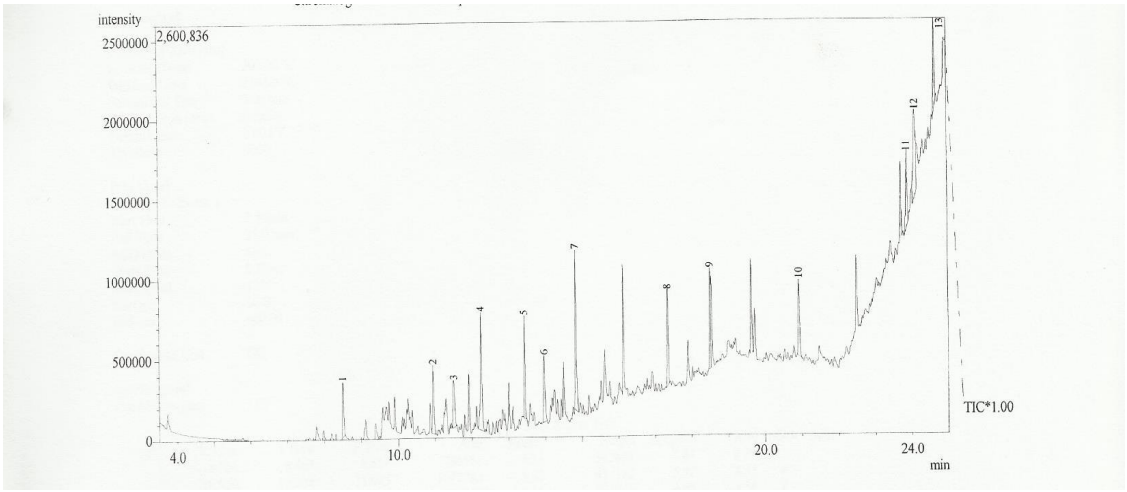

Fig. 9. Gas chromatographic analysis of spent lubricating oil degraded by *Pseudomonas aeruginosa* after two weeks

Peaks: 1: benzene 2: benzene 3: benzene 4: azulene 5: undecane 6: 1, 4-methanonapthalene 7: tetradecane 8: heptadecane 9: undecane 10: octane 11: 7-ocatdeccanoic acid 12: octadecane 13: henicosane

The phosphorus content was lower in *A. niger* medium than *Pseudomonas aeruginosa*. Phosphorus like nitrate is required by organisms during biodegradation. This result is an indication that phosphorus was better utilized by *A.niger* than *Pseudomonas aeruginosa* in the course of biodegradation of spent lubricating oil.

Some of the compounds present in the spent lubricating oil (control) may have been due to prolonged usage. The branched aromatic compounds may have resulted from chemical contamination during usage [9]. Stephen [15] observed more branched aromatic compounds in spent lubricating oil compared to unused lubricating oil and attributed it to impurities from engines.

The GC-MS of the spent lubricating oil degraded by *A.niger* after 7 days revealed the presence of more benzene than *Pseudomonas aeruginosa* medium and the control. This may be due to the cleaving of the methyl, ethyl, propyl from benzene present in the control by the organisms. However, *Pseudomonas aeruginosa* medium had fewer benzene compounds and carboxylic acids compared to *A. niger* medium. This is an indication that the rate of degradation and disappearance of some of the compounds were higher in *Pseudomonas aeruginosa* than *A. niger* medium. The presence of benzene in both media shows that benzene cannot be degraded by both *A. niger* and *Pseudomonas aeruginosa*. This is in agreement with Atlas and Bragg [16]. These researchers reported that some aromatic hydrocarbons cannot be degraded by some organisms.

The chromatographic analysis of the samples after 14 days revealed less peaks and compounds in both *A. niger* and *Pseudomonas aeruginosa* media compared to the chromatograms of both organisms at day 7. This may be attributed to increased biodegradation [17]. Benzene and azulene could not be degraded after 14 days by both organisms. The increased number of carboxylic acids in both medium (*A. niger* and *Pseudomonas aeruginosa*) is an indication of biodegradation of the alkanes found in the spent lubricating oil (control) and after 7 days of biodegradation [18]. The higher number of carboxylic acids in *A. niger* medium compared *Pseudomonas aeruginosa* also indicates that *A. niger* degrades spent lubricating oil better than *Pseudomonas aeruginosa*.

5. CONCLUSION

This study revealed that *A. niger* grew and utilized spent lubricating oil better than *Pseudomonas aeruginosa*. pH and phosphorus concentration were lower in *A. niger* medium than *Pseudomonas aeruginosa*. Some of the branched aromatic compounds such as ethylbenzene, methylbenzene, propylbenzene and straight chain alkanes such as hexadecane, heptadecane and octane were degraded into benzene, hexadecanoic acid and octadecanoic

acid. This study also revealed that benzene and azulene present in the spent lubricating oil could not be degraded further by *A. niger* and *Pseudomonas aeruginosa* after two weeks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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