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Evaluation of Percentage Degradation of Crude Oil in Contaminated Soil by Isolated Consortia

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The Biodegradation of spilled crude oil in contaminated soil could reach insignificant quantity and harmless compounds formed through the application of effective and viable hydrocarbon degraders. In this study, 15 micro-organisms were isolated, sub-cultured, purified and used for the bio-augmentation process of a crude oil contaminated soil. The isolated consortium used the crude oil as their sole carbon source and in the process lead to the bio-degradation of the oil contaminant. The quantity of residual oil obtained following bio-augmentation with each isolate was determined gravimetrically. The best hydrocarbon degraders identified include: *Staphylococcus epidermis, Pseudomonas* Spp, *Baccilus* Spp, *Klebsiella* Spp., *Micrococcu* Spp. These findings from this study have environmental implication as it could help in the selection and application of bacterial species that can be used for effective bio-degradation of crude oil in contaminated soil in the Niger Delta region of Nigeria through bio-augmentation process.

Keywords: Soil pollution; bio-degradation; bio-augmentation; crude oil; hydrocarbon degraders, percentage degradation.

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1. INTRODUCTION

One of the causes of environmental pollution across Nigeria today is oil spillage which may originate from the oil wells, refineries, industries, filling stations, loading and pumping stations, petroleum products depots, during transportation and at auto-mechanic workshops [1]. All forms of oil spillage contribute to soil and water contamination. Petroleum hydrocarbon such as crude oil, engine oil, diesel fuel, creosote products, gasoline, spent motor oil, etc could contaminate the environment [2]. The chemical composition of crude oil include fractions of aliphatic, aromatics, asphaltenes and resins. Most of these compounds have recalcitrant property, bioaccumulation potential, toxic, mutagenic, carcinogenic property and resistance to biodegradation [2].

Petroleum-contaminated soil can be remedied either by using physical, chemical or biological methods. Biological methods are regarded as the best because they are cost effective, environmentally friendly, conserve soil texture and characteristics, have simplicity of operation and design, and relatively high treatment efficiency [1,3,4]. Biological methods (bioremediation) make use of three processes: bio-degradation, bio-accumulation and bio-sorption. With these processes, the diverse abilities of microorganisms are exploited to convert the complex chemical components of crude oil to harmless products [5].

Biodegradation is a process whereby bacteria or other organisms use hydrocarbon contaminants as sole carbon and energy sources and in the process; they break down the organic contaminants into other substances, eventually producing simple compounds, carbon dioxide and water [6]. Approaches to biodegradation include: the use of natural attenuation, bioreactor, bio-piling, composting, bio-sparging, bio-venting, land-farming, phyto-remediation, bio-augmentation and bio-stimulation.

The bio-augmentation approach involves the addition of highly concentrated and specialized populations of specific microbes into a contaminated matrix, to enhance the rate of biodegradation of the contaminat in the affected environment. Bio-augmentation is useful when the micro-organisms necessary to degrade the contaminants are completely absent or occur at a very low population to be effective and efficient in the degradation of the contaminant [7]. According to Sharma [8], it is commonly used in the treatment of municipal wastewater so as to restart activated sludge bioreactors and also used to increase the biological activity added Ubani [7]. However, it can be used in the soil environment, as Abdulsalam et al. [1] compared bio-stimulation and bio-augmentation for remediation of soil contaminated used motor oil.

The limitations of bio-augmentation as studied by Ubani [7] include: poor survival of the introduced strains of micro-organisms; non-biodegradability of targeted contaminants; uneven flow of liquid or gas containing the microbes as a result of heterogeneity of the soil or media containing contamination which leads to uneven bio-degradation and the general slow process of bio-augmentation if compared to land-farming and composting.

Most micro-organisms have been studied by researchers and were found to degrade petroleum contaminated samples. Some of the isolated and identified bacterial species include: *Pseudomonas* sp., *Streptococcus* sp., *Escherichia coli, Staphylococcus* sp., *Klebsiella* sp., *Bacillus* sp., *Mycobacterium* sp., *Enterobacter aerogenes*, *Salmonella* sp., and *Micrococcus* sp [1,9-13]. Yakubu [14] identified *Proteus* among other organisms. The biochemical test of isolated bacteria of Abbassi & Shquirat [15] showed that *stenotrophomonas multophilia* degrades petroleum. Gram negative bacteria isolated and identified by Morelli, et al. [16] *were Alcaligenes*, *Agrobacterium*, *Acinetobacter* and *Comamonas* while the gram positive bacteria they identified were principally *coryneforms. More so,* Rhodococcus sp and *Pseudomonas sp were reported* [17]. Saadoun et al. [18] reported S*treptomyces* sp; *Penicillium sp and Aspergillus* sp. as fungi; *and Pseudomonas* sp, *Corynebacterirum, Enterobacter cloaca, Actinetobacter as bacteria.* The results of most studies revealed that *Pseudomonas* sp., *Streptococcus* sp., and *Bacillus* spp. (*subtilis and cereus*), are the most versatile species of bacteria that could utilized all the petroleum products in the soil environment [11,16,17,19].

In the Niger Delta region of Nigeria, crude oil exploration, accidental spillage, pipeline vandalization, and illegal drilling had resulted to the pollution of agricultural land and water. Therefore, efficient and effective hydrocarbon degraders need to be identified and incorporated into the polluted environment to effect the degradation of the crude oil so as to reclaim the

agricultural land for food production. This paper is aimed at evaluating effective crude oil degraders via bio-augmentation by monitoring their percentage oil degradation.

2. MATERIALS AND METHODS

Contaminated soil were sampled from two mechanic workshops in Gwagwalada Area Council of Abuja while uncontaminated soil was gotten from a farmland, void of any form of contamination, within SHESTCO environment at Sheda-Kwali, Abuja.

The serial dilution was performed using Joel & Amajuoyi [20] method. All the media and laboratory apparatus used were autoclaved before use for 15 min at 121 Atmospheric Pressure using the autoclave. About 1 g of the contaminated soil sample was added to 9 ml of sterile distilled water previously dispersed in sterile test tubes. This was agitated to make loose the microbes from the soil particles. Suspension of each soil sample was allowed to settle down and then serial ten-fold dilutions were performed up to 10^{-6}.

Isolation was carried out using the method of Makut & Ishaya [11]. Aliquots of 0.1 ml from 10^{-4} to 10^{-6} dilution were aspirated using syringes and inoculated on the surface of an already prepared nutrient agar plates and modified Beneth's media plates. These were evenly spread over the surface of the agar using L-shaped spreader, which was flamed after been dipped into 100% ethanol. The plates were labeled accordingly, inoculations were performed in duplicates and plates were incubated at 35ºC for 24 h in an incubator. The plates were then observed at the end of the incubation. Thereafter, the isolates were sub-cultured, purified accordingly and preserved on agar slant at 4ºC.

The identification of the bacterial isolates was based on their cultural, morphological and biochemical characteristics as described by Bergey's manual of determinative bacteriology [21].

Modified method of Alvarez et al. [22] was used for the bio-augmentation of the artificially contaminated soil as about 50 g sterilized soil samples were weighed into 250 ml conical flask and spiked with 4 ml of crude oil. This was allowed to stand for 5 day to allow for the volatilization.

The isolates were resuscitated from the agar slant culture by streak inoculating the micro-organisms on freshly prepared nutrient agar. Subsequently, inoculums of 18-24 h were introduced into 10 ml sterile nutrient broth contained in bottles. After 24 h growth, the 10 ml cultures were introduced individually to the sterile contaminated soil in conical flask and labeled accordingly and in duplicates. The conical flasks were capped with cotton wool and wrapped with a foil paper to avoid contamination. The modified method of McBirney et al. [23] and Kim et al. [24] were used to prepare the standard curve and obtain the exact concentration of the isolates spiked into the sterile contaminated soil prior to bio-augmentation with the isolates. These were incubated for 24 days after which the percentage oil degradation was determined using Borah and Yaday method [25].

The residual oil was cold extracted using dichloromethane [26-27]. The aluminum boat used for the extraction were previously labeled accordingly and weighed before extraction and after evaporation of the solvent used for extraction. The dry weight of the oil was determined and the weight loss was calculated as residual oil, according to Marquez-Rocha et al. [28] and Chang [29].

Gain in weight of boat (g) = (weight of boat and residual oil after evaporation of solvents) – (weight of empty weighing boat)

Residual oil (g) =

$$\frac{\text{gain in weight of the weighing boat (g)}}{\text{weight of the wet soil (g)}}$$

Thereafter, the percentage oil degradation was determined using Borah and Yadav method [25].

Oil degradation (%) =

$$\frac{\text{weight of oil in control} - \text{weight of oil in stimulated microcosms}}{\text{weight of oil in control}} \times 100$$

3. RESULTS AND DISCUSSION

The soil physicochemical properties are as shown on Table 1. The pH of the soil determines the availability of nutrients to both plants and micro-organisms. The micro-organisms that will survive in any soil depend largely on the pH of that very soil, since certain micro-organisms would survive under a particular pH range. The pH value of the soil sample used for the bio-augmentation was slightly alkaline in nature. The

pH in water was 8.11 while that measured in KCl was 8.00. There was no significant difference in the values of the pH obtained. Therefore, using either of the electrolytes is considered appropriate. Orji et al. [30] reported a pH of 7.41, very slightly alkaline lower than that in this present soil study. In other literatures, the authors [31-33] reported similar slightly alkaline pH values as found in this study while others reported acidic pH values [34-36]. Frankenberger and Arshad [37] and Sharma [32] explained that biodegradation of hydrocarbon in contaminated soil is enhanced when the pH of the soil is neutral or slightly alkaline.

Table 1. Physicochemical properties of the soil used for bio-augmentation

Soil parameters	Values.
pH (water)	8.11
pH(KCl)	8.00
Organic Carbon (%)	0.10
Organic Matter (%)	0.17
Nitrogen content (%)	2.07
Electro-conductivity	646
Moisture content (%)	3.04

The total organic carbon of Orji et al. [30] and Sharma et al. [32] were higher than that reported in the present study, indicating low carbon content of the soil. The nitrogen content of the present soil is higher than that of Agamuthu et al. [35], showing that their contaminated soil is deficient in nitrogen which is a through representation of soil contaminated with hydrocarbon oil. While the soil used in this study was artificially contaminated for only 5 days prior to bio-augmentation and as such the effect of the crude oil contamination has not yet affected the nitrogen content of the soil under study.

The mean residual oil obtained from microcosms spiked with similar isolate was obtained and used to compare the performance of the isolates with respect to the reduction of the residual crude oil. The residual oil obtained following bio-augmentation of crude oil in artificially contaminated soil after 24 days is as shown in Fig. 1.

The mean value of the least extracted residual oil was 0.03 g/g from isolate La followed by isolate Bb and thirdly by isolate E. meanwhile, the highest mean residual oil was 0.08 g/g extracted from isolate G, seconded by isolate C and thirdly by isolate B. The extraction of much residual oil from the microcosms of some isolates implies that these isolates were not able reduce the quantity of crude oil that was artificially introduced into the soil. Therefore, the least performing organism with the poorest ability to bio-augment and utilized the crude oil contaminant as the sole carbon source was isolate G, followed by isolate C and B. The range of the extracted residual oil following 24 days bio-augmentation was 0.03 to 0.08 g/g. The residual oil extracted from the control experiment was 0.12 g/g.

From the ANOVA analysis result of the extracted residual oil, the residual oil of 0.08 g/g extracted from isolate C was significant higher than 0.03 g/g extracted from each of the following isolates: La ($p = .030$), E ($p = .048$) and Bb ($p = .046$). Whereas, the quantities of residual oil

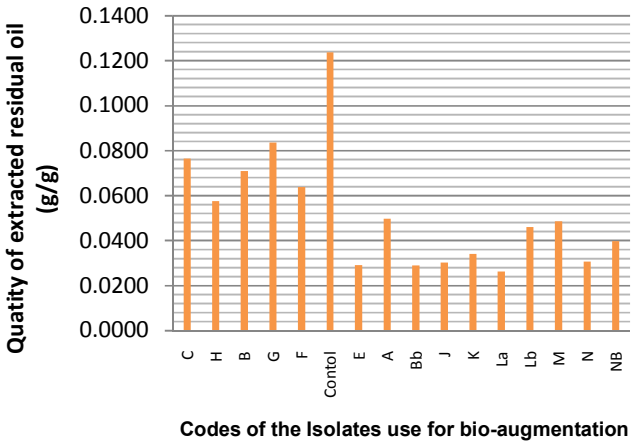

Fig.1. Residual oil (g/g) obtained after 24 days bio-augmentation of crude oil artificially contaminated soil

of 0.030 g/g extracted following bio-augmentation with isolate J was significantly lower than the residual oil of 0.08 g/g, extracted from G (*P* = .081) and 0.10 from the Control (*P* = .002). Meanwhile, isolate K reduced the oil contamination to 0.03 g/g, which was significantly lower than 0.08 g/g, extracted from G (*P* = .034) and 0.10 g/g from the Control (*P* = .003). Nevertheless, isolate La yielded a residual oil of 0.03 g/g that was significantly lower than that, 0.08, 0.08 and 0.10 g/g extracted from C (*P* = .030), G (*P* = .010), Control (*P* = .001). While a residual oil of 0.08 g/g extracted following bio-augmentation with isolate G was significantly higher than 0.03 g/g extracted from J (*P* = .018), K (*P* = .034), La (*P* = .010), N (*P* = .020), E (*P* = .016) and Bb (*P* = .015). The quantity of 0.10 g/g of residual extracted from the control microcosms was significantly higher than the residual oil of 0.03g/g extracted from each of Bb (*P* = .001), E (*P* = .001), J (*P* = .002), K (*P* = .003), La (*P* = .001) and N (*P* = .002); 0.04 g/g of Nb (*P* = .007); 0.05 g/g extracted from each of Lb (*P* = .018), M (*P* = .027), A (.032).

Though isolate G had the poorest performance, measured by the quantity of residual oil following extraction after bio-augmentation, there was no significant difference between its performance and that of the control experiment microcosms, had only sterilized distilled water.

The percentage oil degradation of the crude oil in the contaminated soil following bio-augmentation is shown on Fig. 2.

Among the top six highly performed isolates as shown in Fig. 2, isolate La had the highest percentage oil degradation and therefore, the most effective and efficient in bio-augmenting the inherent micro-organisms in the contaminated soil, that were deliberately destroyed during autoclaving of the soil. This was followed by isolate Bb and thirdly by isolate E. The poorly performed isolate of the six isolates was isolate K followed by isolate N.

Therefore, the order of performance of the isolates is: La > Bb > E > J > N > K > Nb > Lb > M > A > H > F > B > C > G > Control.

The bio-chemical test showed that three of the isolates with codes B, E and N are Cocci while while isolate with codes J, K and L are Rods.

Also the best six degraders, B, E, J, K, La and N were identified as *Staphylococcus epidermis, Pseudomonas spp, Baccilus spp, Klebsiella spp, Klebsiella spp, Micrococcus Spp respectively.* The isolate with the highest percentage oil degradation of 78.80% was *Klebsiella spp*, a Rod, whose performance was followed by all the three Cocci: *Micrococcus Spp, Baccilus spp and*

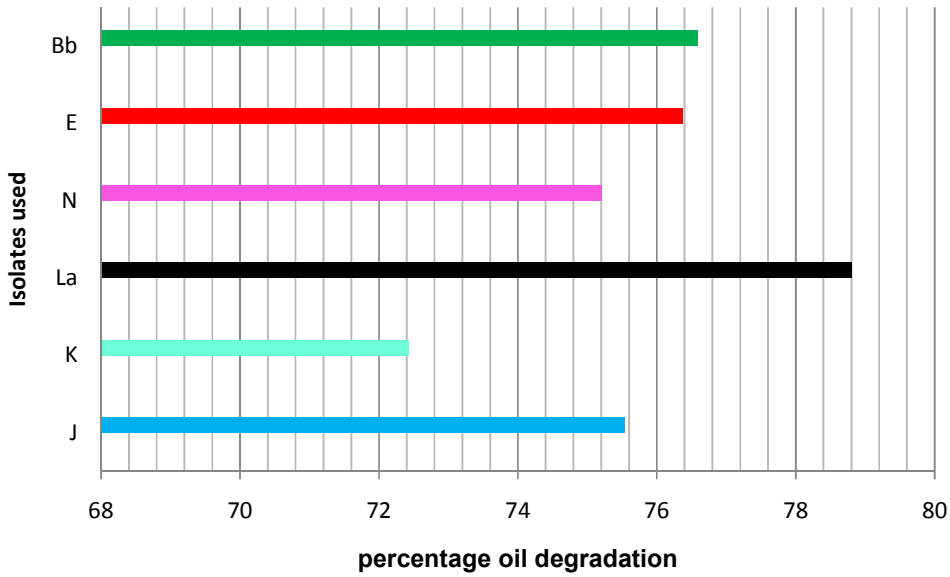

Fig. 2. Percentage oil degradation crude oil in the artificially contaminated soil following bio-augmentation

Pseudomonas spp. having 76.57, 76.37 and 75.20% oil degradation abilities. According to Makut and Ishaya [11], the *Pseudomonas spp. and Baccilus spp* they isolated had highest percentage oil degradation abilities while *their Klebsiella spp*. was among other bacteria with the lowest percentage oil degradation abilities, which was contrary to what was observed in this study. Though one *Klebsiella spp.* in this study had the highest percentage oil degradation ability of 78.80%, another *Klebsiella spp.* had the least performance of 72.42%. The report of Ijah et al. [12] revealed that Baccilus spp SOB-06 had the highest degrading ability followed by *Micrococcus* and *Pseudomonas* spp respectively, though another *Bacsilus* spp SOB-01 had a much lower degrading ability.

The ANOVA analysis of this study showed that there were no significant differences in the performances of these six highly performed crude oil degraders. Therefore, despite the fact that they all had different percentage oil degrading abilities, statistically, their performances are the same.

Some authors used Cocci to degrade organic compounds: *Pseudomonas putida* was used by Massa et al. [38] and Filonov et al. [39] to degrade 4-chlorobensoic and Naphthalene respectively; *Pseudomonas fluorescens* was used by Monti *et a*l [40], Boldt et al. [41] and Brazil et al. [42] to degrade 2, 4-Dinitrotoluene , Biphenyl, polychlorinated biphenyl respectively. While Sharma et al. [32] used *Pseudomonas aeruginosa* for the degradation of a diesel contaminated soil. *Bento* et al. [43] *used mostly rods: Bacillus cereus, Bacillus sphaericus, Bacillus fusiformis, and Bacillus pumilu; for their bio-augmentation process.* In the bio-augmentation process of Wright and Weaver [44], they combine inoculants with either nutrients or dispersing agent to enhance the degradation of the hydrocarbon contamination in the soil through bio-augmentation. Abdulsalam et al. [1] used both rod and Cocci: *Bacillus subtilis* and *Micrococcus leteus,* for degradation of spent motor oil in a closed aerobic fixed bed bioreactor.

The average percentage oil degradation, 75.82%, of these six isolates was higher than the percentage oil degrading ability of the isolates of Bento et al. [43]. Sharma et al. [31] studied bio-augmentation with nutrient and *Pseudomonas aeruginosa* on the contaminated soil after 30 days and reported percentage oil degradation of 66%, a value lower than that from this study. Zawierucha and Malina, [45] reported that bio-augmented microcosms degraded enzo[a] pyrene and anthracene, with a percentage degradation of 70 and 72% respectively, while the values for the control microcosms were much lower, they added. Their observation on the control microcosms was similar to that observed in this study. Other authors that reported percentage oil degradation lower than that from this study and include: Wright and Weaver [43] with average of 62% of total petroleum hydrocarbon (TPH) contaminant after 33 days following bio-augmentation under continuously-flooded conditions where as, Abdulsalam et al. [1] stated that their bio-augmentation showed 66% removal of the spent oil in the bio-reactor they used.

5. CONCLUSION

The study showed that *Staphylococcus Epidermis, Pseudomonas Spp, Baccilus Spp, Klebsiella Spp, Micrococcus Spp*., can all be used for the biodegradation of crude oil in contaminated soil. The crude oil removal efficiency can reach up to 78.80% using a particular *klesbsiella spp* with much ability in degrading the oil contaminant more than the other bacteria.

The information from this study can help in the selection of bacterial species that can be used for the bio-degradation of crude oil contaminated soil in the Niger Delta region of Nigeria through the process of bio-augmentation.

For further investigation, the bacterial species identified need to be characterized using molecular method. The compounds formed after the crude oil degradation via bio-augmentation, should be identified using GC/MS.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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