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Biodegradability of Selected–Oil Spill Dispersants Commonly Used in Nigeria

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Abstract The study investigates the biodegradation of two oil spill dispersants: 'Eco-Remover' which was obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt and a locally made unapproved 'Rigwash,' obtained from an industrial chemical store at Trans-Amadi, Port Harcourt. The setups were monitored at two weeks interval over 42-days period at room temperature (approximately 30℃) on a static shake-flask system. The physico-chemical parameters of the samples were analyzed using standard chemical methods such as the atomic absorption spectrophotometry, Gas Chromatography (GC-FID) and the titrimetric technique. The result of primary biodegradation rate revealed that the mixture of Eco-Remover, seawater and crude oil (L2) was the highest degraded at 77.1%, followed by Eco-Remover and seawater combination (L1) at 4.39%. K1 (a mixture of seawater and Rigwash) and K2 (a mixture of crude oil, seawater and Rigwash) degraded at 1.22% and 1.19% respectively. The result also indicated that K1 had an adverse effect on crude oil. Analytical Profile Index of bacterial isolates revealed a total of Five genera found growing on the samples. Among them, *Bacillus, Pseudomonas and Kocuria* were dominant and possess the potential to utilize dispersants and/or crude oil as the only carbon sources. The fungal isolates include: *Aspergillus niger* (18.0%), *Cladosporium carionii* (8.20%), *Fusarium oxytoca* (4.92%), *Myxomycete* spp. (13.1%), *Fusarium* spp. (3.28%), *Penicillium* spp. (3.28%), *Phoma* spp. (8.20%), *Pleurotus pulmorarius* (8.20%), *Rhizopus* spp. (3.28%), *Talaromyces flavus* (9.84%), *Trichoderma longibrachiatum* (6.56%), *T. polysporum* (4.92%) and *Verticillium* spp. (8.20%). Analysis of variance at confidence limit $P \leq 0.05$ showed that there was significant difference in the biodegradation of each dispersant or their combination with crude oil. The implication of this study establishes that Eco-Remover is more biodegradable than Rigwash 'dispersant'. However, there is need for further studies on their application in the field to confirm the outcome of this laboratory observations.

Keywords: *pollution, crude oil, dispersants, microorganisms, biodegradation*

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1. Introduction

The aquatic systems, especially the marine and brackish water bodies in the Niger Delta region of Nigeria have experienced lists of the entire spectrum of industrial compounds, mainly approved or unapproved oil spill chemical dispersants which are both refractory and toxic to the environment as well as to humans. The oil spill dispersants use in the sea is permitted by the Nigerian Oil Spill Contingency Plan (NOSCP) as approved by the Department of Petroleum Resources (DPR) *Environmental Guidelines and Standards in the Petroleum Industry in Nigeria,* EGASPIN (2002). The adoption of dispersants among several techniques available to combat oil spills followed a vast array of literatures on the importance of their use [1]. Oil spill dispersants are mixtures of surface active agents (surfactants) in one or more organic solvents, specifically formulated to enhance the dispersion of oil into the seawater column by reducing the interfacial tension between oil and water [2,3]. These agents make oil sink faster and more deeply into the water, and possibly groundwater supplies [4]. Studies have examined the impacts of oil-dispersants mixture on rotifers, which are one of the groups of living organisms that make up the food chain in water sediments.

However, since after the 1967 Torrey Canyon accidental oil spillage in the United Kingdom, where oil dispersant was first used on a large scale as a response method for combating the spills, the use of dispersants have generated serious controversies [2,5,6,7]. This has remained controversial because of the toxicity of dispersed mixtures and their potential negative impacts on the environment. Consequently, aquatic lives below the surface water were potentially exposed to oil and dispersants with different modes of action and exposure routes. In Nigeria, their applications are not recommended onshore and/or in freshwater habitats because of their potential for toxicity, and habitat sensitivities [8,9].

Hassanshahian and Capello [10] have argued that the application of oil spill dispersants on an oil impacted sea

have resulted in the deliberate addition of another toxic substance into the marine environment that is already a potential threat to the biotic and abiotic systems. Contrary to this view, studies have shown that the use of it as countermeasure of pollution is the most frequently employed clean-up method because such liquids can be readily applied to large oil spills, and it is generally assumed to be more cost effective than physical remediation methods [11,12,13]. A study has compared the toxicity of crude oil (a control, without an oil spill dispersant) with crude oil – dispersant mixture and found that a combination of crude oil and dispersant is two times more toxic than the oil alone [14]. A research conducted in late 2012 by *Georgia Tech and Universidad Autonoma de Aguascalientes* revealed the toxicity of oil by up to Fifty-two times after Corexit, a dispersant has been applied during the BP oil spill on Gulf of Mexico [15,16]. A field study of the impact of Dioctyl sodium sulfosuccinate (DOSS), a chemical dispersant on oil has implicated the former to persist in variable quantities in deep-sea coral communities 6 months after the spill on the 2010 Deepwater Horizon (DWH) oil spill and on Gulf of Mexico beaches 26 – 45 months after the spill [17]. Rigwash chemical dispersant has been found to adversely affect the biodegradation of Bonny light crude oil [18]. This, according to the report, may be due to the chemical composition of the cleaning substance which has inhibited the growth and development of microbial degraders.

Evidently, the most quality and highly useful oil spill dispersants used in the petroleum industry are those that have the capacity or ability to solubilize/disperse oil, after which they themselves are readily available to microorganisms for degradation. Microorganisms, especially bacteria have the potential to degrade oil spill dispersants and hydrocarbons. Thus, for an organism with the genetic information for utilizing dispersants as carbon source, the enzyme for degrading chemical dispersant is induced when the chemical reaches the microbial habitat. Bacteria have evolved regulatory systems that ensure the synthesis of enzymes so that the initial attack on these compounds are induced [19].

Nevertheless, the need to investigate the effects of oil spill dispersants used by the petroleum industries in combating oil spills in aquatic systems, before field application, are viewed as panacea to solving most of the problems faced by oil industries. Hence, this study examined the biodegradability of oil spill dispersants (Eco-Remover and locally made 'Rigwash') commonly used in Nigeria by petroleum industries. The objectives were focused on:

- Determination of the biodegradability of some oil spill dispersants used by the Nigerian Petroleum Industries.
- Isolation and identification of bacteria and fungi to genera/species level involved in the biodegradation of oil spill dispersants.
- Investigation of the effects of oil spill dispersants on crude oil.
- Determination of the percentage primary biodegradation and mineralization (ultimate biodegradation) of some oil spill dispersants used in the Nigerian Petroleum Industry.

2. Materials and Methods

The Bonny light crude oil and the Eco-Remover oil spill dispersant were obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Rivers State of Nigeria. And the DPR's unapproved Rigwash oil spill dispersants was purchased from a chemical store in Trans-Amadi, Port Harcourt metropolis. Standard procedures were followed during the collection of samples. The plastic containers used for the collections were sealed and stored at room temperature in the laboratory and used within a period of 30 days. The marine water sample which was aseptically collected from Okrika High Sea through Adedemebiri, Rivers State, was the source of inoculum. The water sample was collected with a 10-liter plastic container by submergence into the water column at a depth of 1m and transported to the laboratory at 25 – 30℃ within 2 hours.

The seawater was the R1, and the mixture of seawater and crude oil was the R2. The combination of seawater and Rigwash oil spill dispersant was K1, and that of seawater, Rigwash dispersant and crude oil was K2. L1 and L2 were mixtures of seawater and Eco-Remover oil spill dispersant; and seawater, Eco-Remover dispersant plus crude oil respectively.

The methodologies adopted for media preparation, preliminary range findings - to determine the non-toxic concentration of the oil spill dispersants to the autochthonous microorganisms of the water sample, biodegradation monitoring, microbiological analyses (including biochemical tests and Analytical Profile Index of isolates), physico-chemical and statistical analyses of samples/data were as described by Nnadozie and Odokuma [18]. The biodegradation tests were carried-out to determine physico-chemical analyses of the samples collected from the static shake-Erlenmeyer biodegradation flasks at day 0, 14, 28 and 42 at approximately 30℃ room temperature. Precisely, for sample R1, 100ml of water sample was poured into the biodegradation flask with no addition of oil or dispersants.

3. Results

3.1. Physico-Chemical Characteristics of the Samples

The results in Figure 1 – Figure 16 showed the physico-chemical analyses of the samples (R1, R2, K1, K2, L1 and L2). The pH for the samples, for periods of 0 to 42 days ranged from 7.39 – 7.49, 9.02 – 9.80, 6.27 – 8.17, 6.42 – 6.56, 7.10 – 8.50 and 6.47 – 8.40 respectively (Figure 1). The acidity (Figure 12) and alkalinity (Figure 13) were between 6.49 – 6.60mg/l & 1.29 – 1.38mg/l, 4.05 – 6.10mg/l & 3.22 – 4.11mg/l, 5.88 – 6.58mg/l & 1.05 – 1.59mg/l, 5.82 – 6.01mg/l & 1.62 – 6.66mg/l, 7.12 – 9.44mg/l & 1.05 – 3.47mg/l, and 4.72 – 6.07mg/l respectively. The results for R1, R2, K1, K2, L1 and L2 for Total Dissolved Solids (TDS) (Figure 4) were 12009 – 12015mg/l, 14001 – 16096mg/l, 11590 – 12030mg/l, 8004 – 10350mg/l, 12910 – 14041mg/l and 3060 – 5012mg/l respectively.

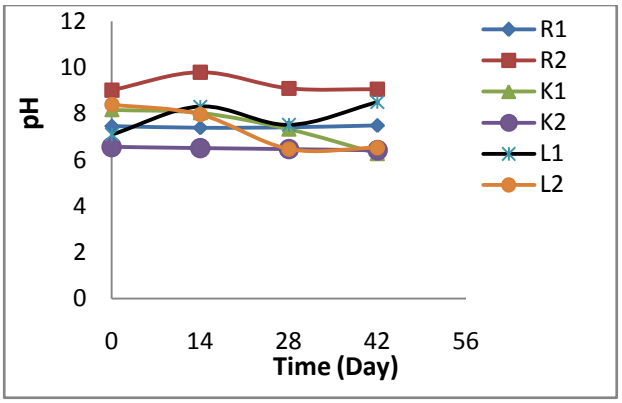

Figure 1. Variation in pH of the Samples during the Degradation

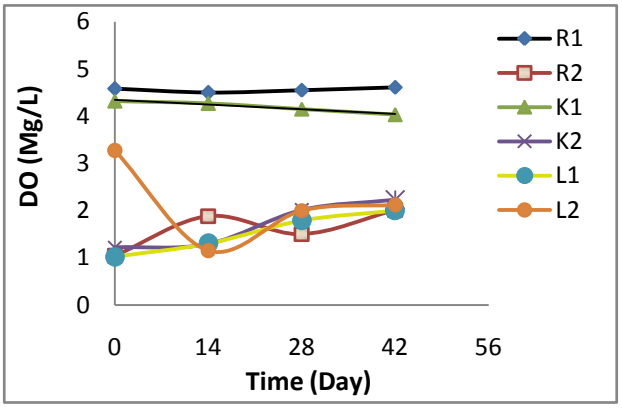

Figure 2. Variation in Dissolved Oxygen Level of the Samples during the Degradation

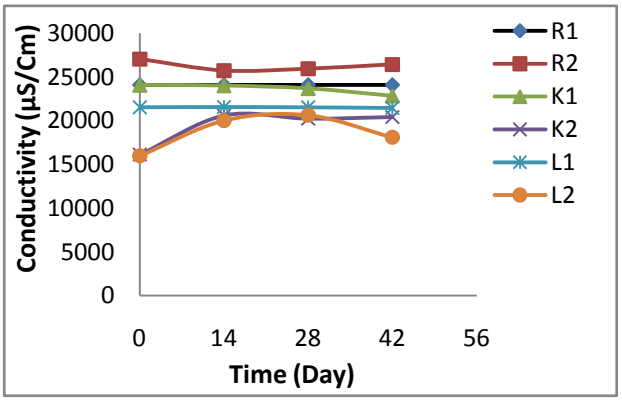

Figure 3. Variation in Conductivity Level of the Samples during the Degradation

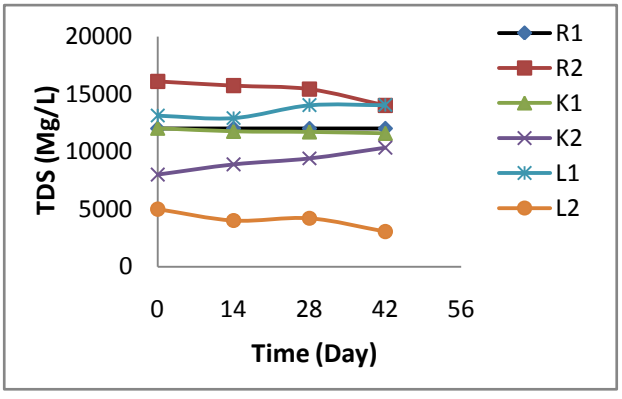

Figure 4. Variation in Total Dissolved Solids Level of the Samples during the Degradation

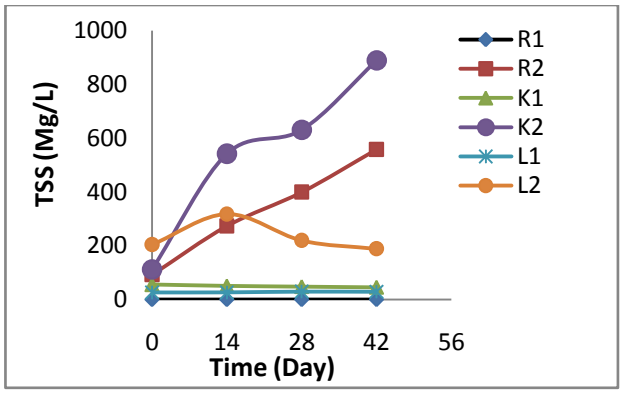

Figure 5. Variation in Total Suspended Solids Level of the Samples during the Degradation

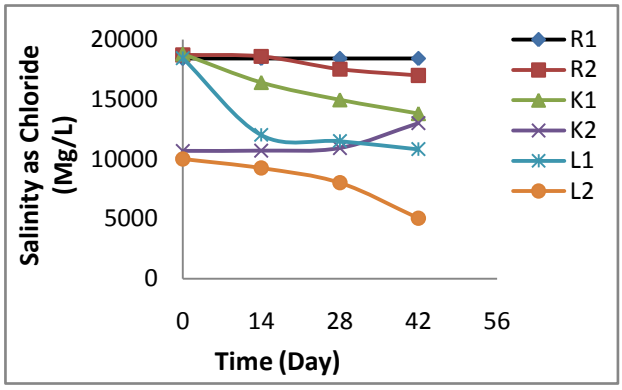

Figure 6. Variation in Salinity Level of the Samples during the Degradation

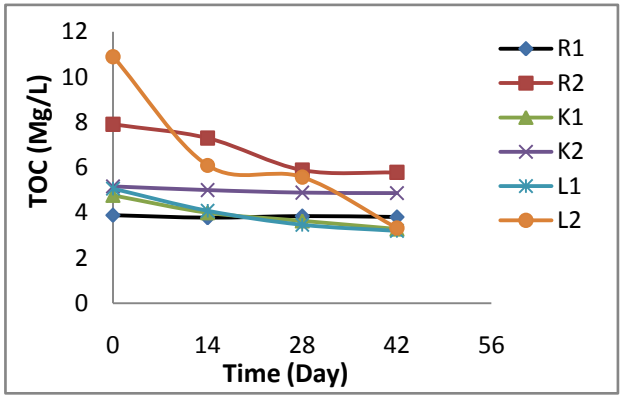

Figure 7. Variation in Total Organic Carbon Level of the Samples during the Degradation

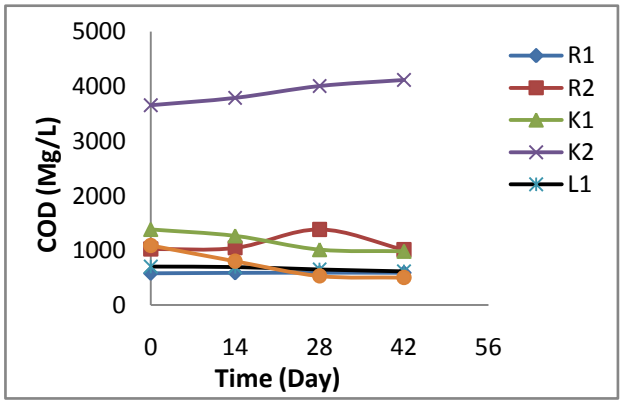

Figure 8. Variation in Chemical Oxygen Demand Level of the Samples during the Degradation

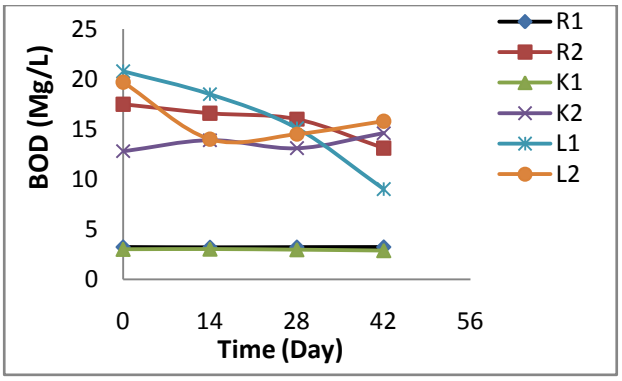

Figure 9. Variation in Biochemical Oxygen Demand Level of the Samples during the Degradation

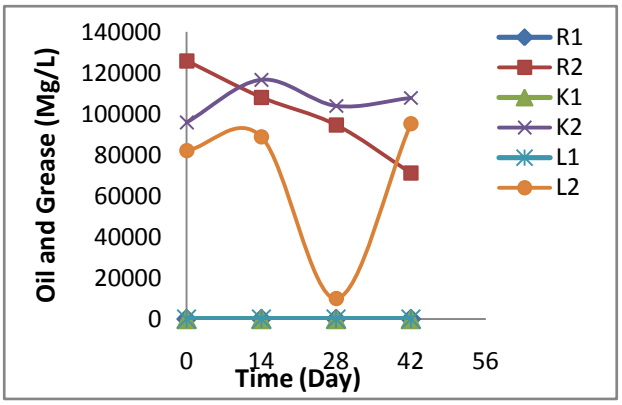

Figure 10. Variation in Oil and Grease Level of the Samples during the Degradation

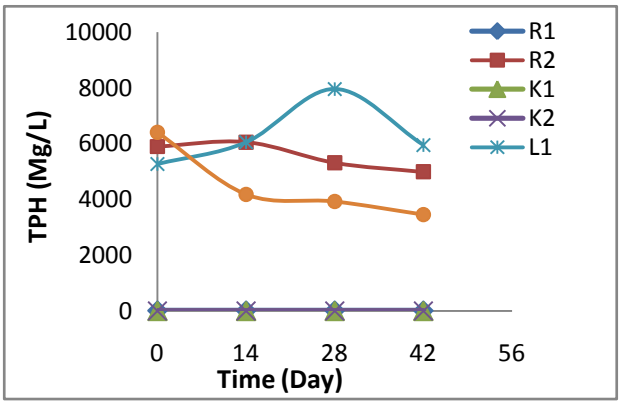

Figure 11. Variation in Total Petroleum Hydrocarbon Level of the Samples during Degradation

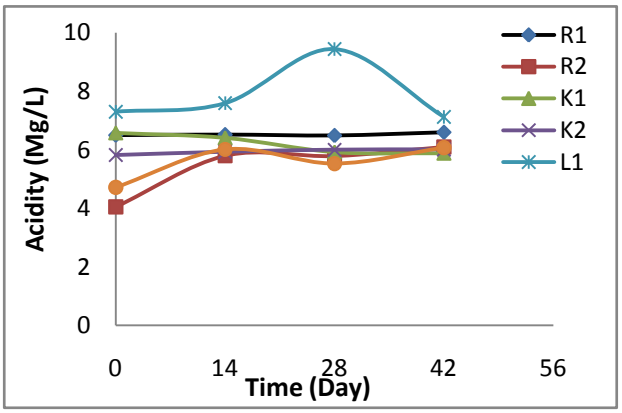

Figure 12. Variation in Acidity Level of the Samples during the Degradation

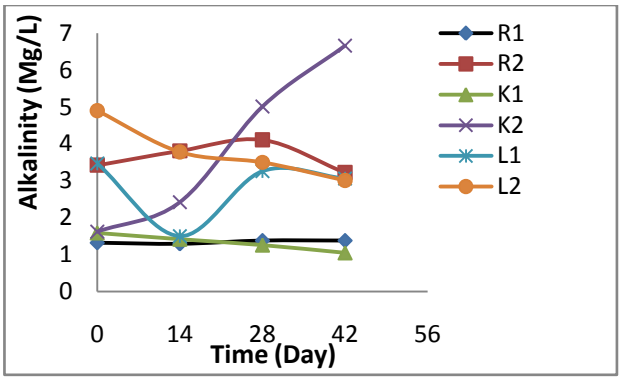

Figure 13. Variation in Alkalinity Level of the Samples during the Degradation

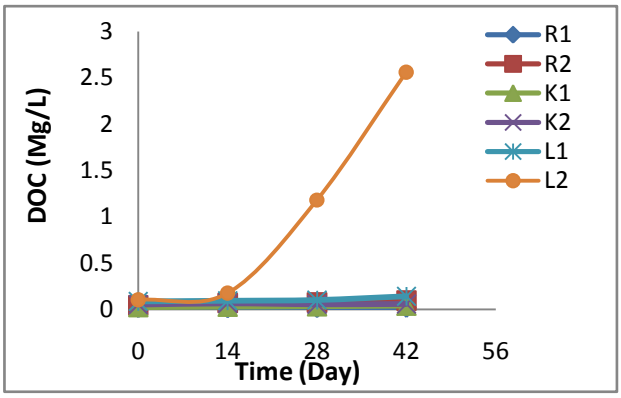

Figure 14. Variation in Dissolved Organic Carbon Level of the Samples during the Degradation

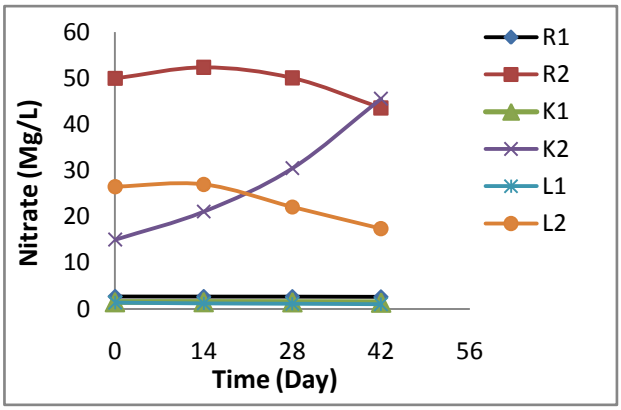

Figure 15. Variation in Nitrate Level of the Samples during the Degradation

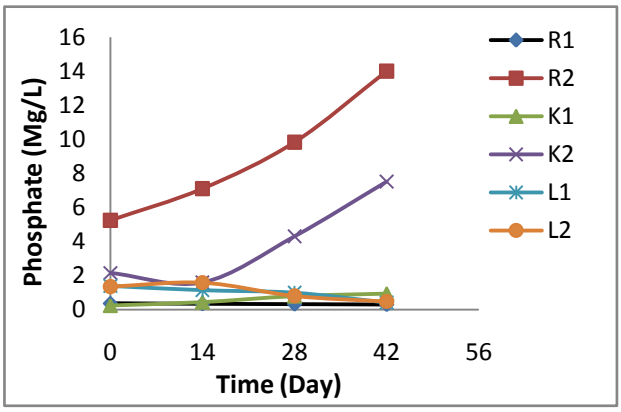

Figure 16. Variation in Phosphate Level of the Samples during the Degradation

The nitrate and phosphate ions are higher in R2 with values ranging from 43.6 – 52.4mg/l and 5.24 -14.0mg/l respectively, and at L1 nitrate was lowest with a range of 1.00 – 1.38mg/l; and at R1 (0.29 –0.36mg/l) and K1 (0.23 – 0.94mg/l) phosphate ions are lower and lowest respectively (Figure 15 – Figure 16). The Potassium (K) and Sodium (Na) contents in R1 sample are within the DPR limit of 200, whereas in sample L1, Na was highest with values ranging from 389mg/l to 518mg/l; and K was highest at K2 (317 – 362mg/l). The Zinc (Zn), Chromium (Cr) ions and Selenium (Se) in the samples are not above regulatory limits of 1.0mg/l, 0.5mg/l and 0.01mg/l respectively. Copper (Cu) and Nickel (Ni) are less than 0.01mg/l in the samples studied. Lead (Pb) ions are less than 0.01mg/l in R2, K1, K2, L1 and L2 samples; while in R1 sample it was between 1.30 and 1.31mg/l. Iron (Fe), Cadmium (Cd) and Sulphur recorded the highest values at L2, K2 and L2 with 0.72 – 2.03mg/l, 0.63 – 1.03mg/l and 0.15 – 0.55mg/l; and lowest at R1 (0.13 – 0.14mg/l), L1(0.01 – 0.03mg/l) and (R1, K1 &L1 – with <0.01mg/l) respectively.

3.2. Microbiological Properties of the Samples and Treatments

3.2.1. Total Heterotrophic Bacterial Count (THB)

The THB was highest in the crude oil-seawater mixture (R2) with 7.5 x 10^2 – 1.2 x 10^6 CFU/ml as shown in Figure 21. The THB in K1 decreased with time from Day 0 (1.0 x 10^4 CFU/ml) to Day 28 (9.4 x 10^2 CFU/ml), and increased to 1.8 x 10^3 CFU/ml at the 42 day compared with L1 where the THB progressively increased from 9.2 x 10^1 CFU/ml to 1.6 x 10^4 CFU/ml over the 42 days experimental period. Unlike the K2, L2 increased with time, having total viable counts ranging from 5.3 x 10^2 CFU/ml (at Day 0) to 5.4 x 10^5 CFU/ml (at Day 42).

The THB results obtained indicates that L1 and L2 are more degradable by bacteria than K1 and K2. The K1 may be toxic to bacterial growth and had adversely impacted on the oil, thus resulting to the recalcitrance of the compound mixture.

3.2.2. Total Heterotrophic Fungal Count (THF)

The THF was highest in K2 at the 28 days of biodegradation period with the total viable count of 7.1 x 10^5 CFU/ml and decreased at Day 42 with 2.7 x 10^4 CFU/ml. The THF counts (Figure 22) for R1, R2, K1, L1 and L2 at the 42 days of sampling were 9.6 x 10^2 CFU/ml, 2.0 x 10^3 CFU/ml, 1.5 x 10^4 CFU/ml, 3.3 x 10^5 CFU/ml and 6.9 x 10^5 CFU/ml respectively.

3.2.3. Dispersants, Oil-Dispersants Mixtures and Hydrocarbon Utilizing Bacterial and Fungal Counts (DUB, DUF, HUB, HUF, DUB-HUB, DUF-HUF)

Figure 23 – Figure 25 showed the counts obtained from R2, K1, K2, L1 and L2 respectively. R2 sample had the highest hydrocarbon utilizing bacteria (HUB) and fungi (HUF) growth at Day 42, and they are 1.1 x 10^4 CFU/ml and 9.6 x 10^3 CFU/ml respectively. K1 and L1 had their highest dispersant utilizing bacteria (DUB) and fungi (DUF) growth on 0 day and 42 day with DUB & DUF counts of 5.2 x 10^2 CFU/ml & 9.2 x 10^1 CFU/ml (Day 0) and 2.4 x 10^3 CFU/ml & 8.9 x 10^2 CFU/ml (Day 42) respectively (Figure 24). The L2 and K2 dispersant-hydrocarbon utilizing bacterial (DUB-HUB) counts were highest with 2.5 x 10^3 CFU/ml and 1.7 x 10^3 CFU/ml on Day 42 and 28 respectively (Figure 25); while that of the fungal counts (DUF-HUF) were 1.7 x 10^6 CFU/ml (on Day 42) and 2.2 x 10^5 CFU/ml (on Day 28) respectively (Figure 25).

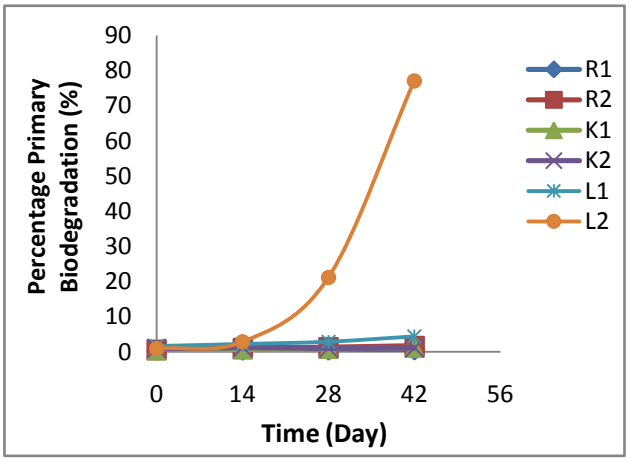

Figure 17. Percentage Primary Biodegradation Rate of the Samples

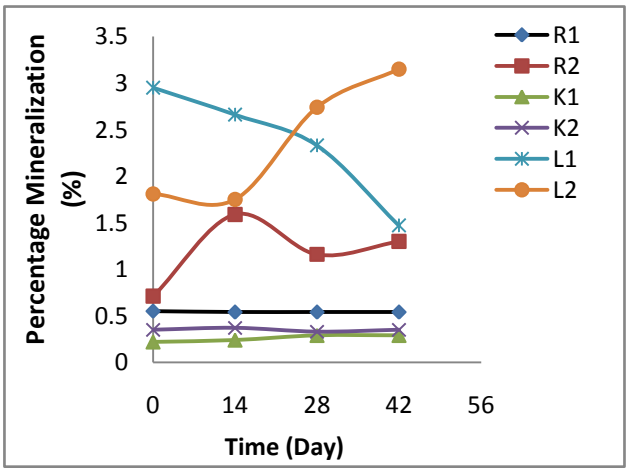

Figure 18. Percentage Mineralization of the Samples

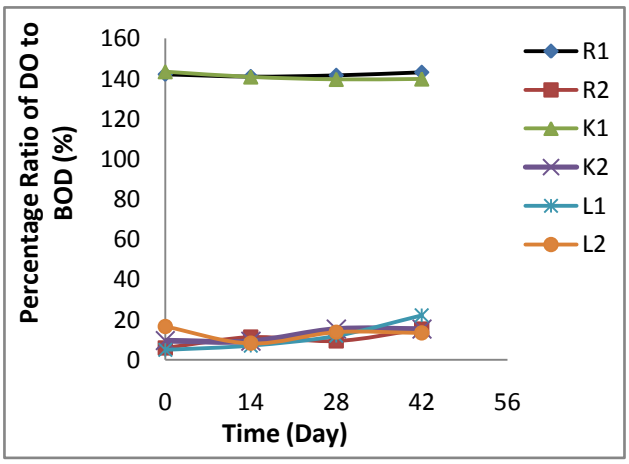

Figure 19. Percentage Ratio of DO to BOD of the Samples

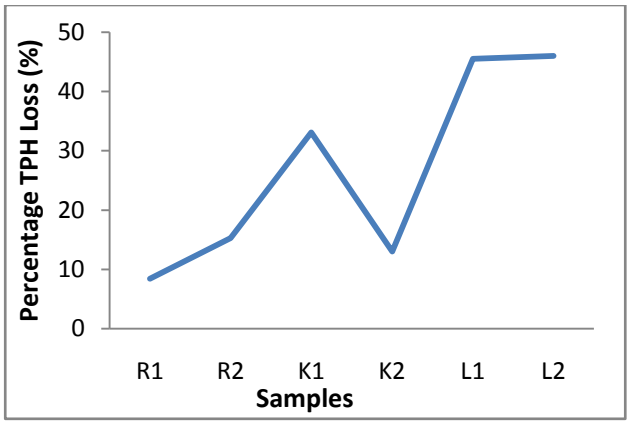

Figure 20. Percentage Total Petroleum Hydrocarbon Loss of Samples

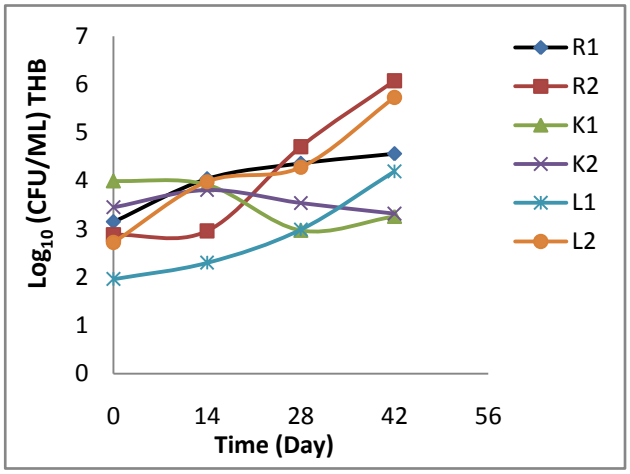

Figure 21. Total Bacterial Counts in the samples

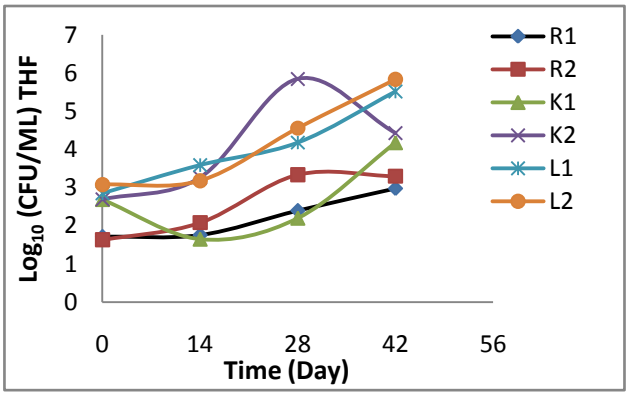

Figure 22. Total Fungal Counts in the samples

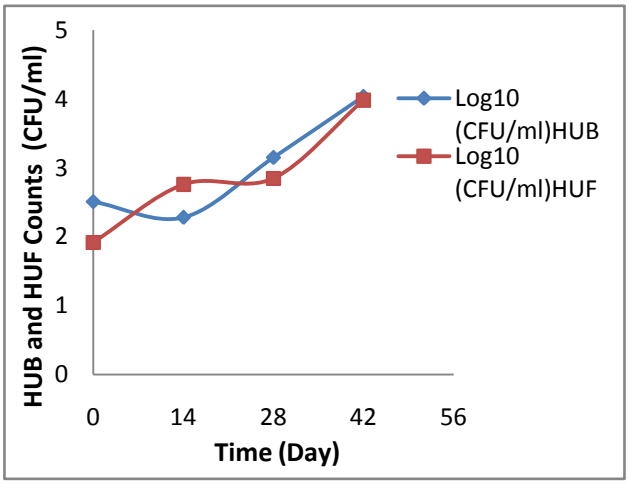

Figure 23. Hydrocarbon Utilizing Bacterial and Fungal Counts for R2 Sample

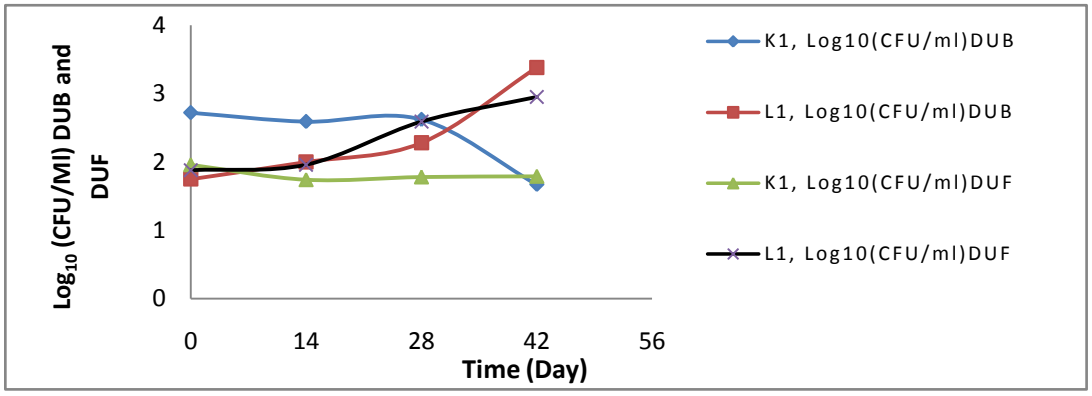

Figure 24. Dispersant Utilizing Bacterial and Fungal Counts for K1 and L1 Samples

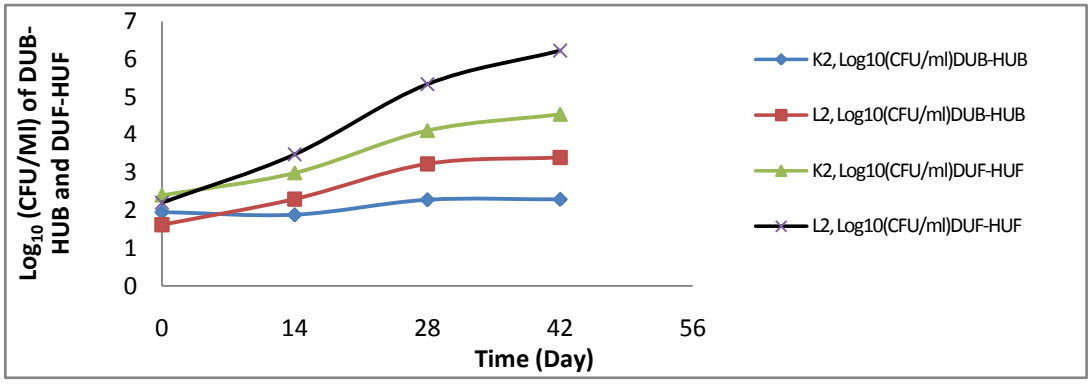

Figure 25. Dispersant-Hydrocarbon Utilizing Bacterial and Fungal Counts for K2 and L2 Samples

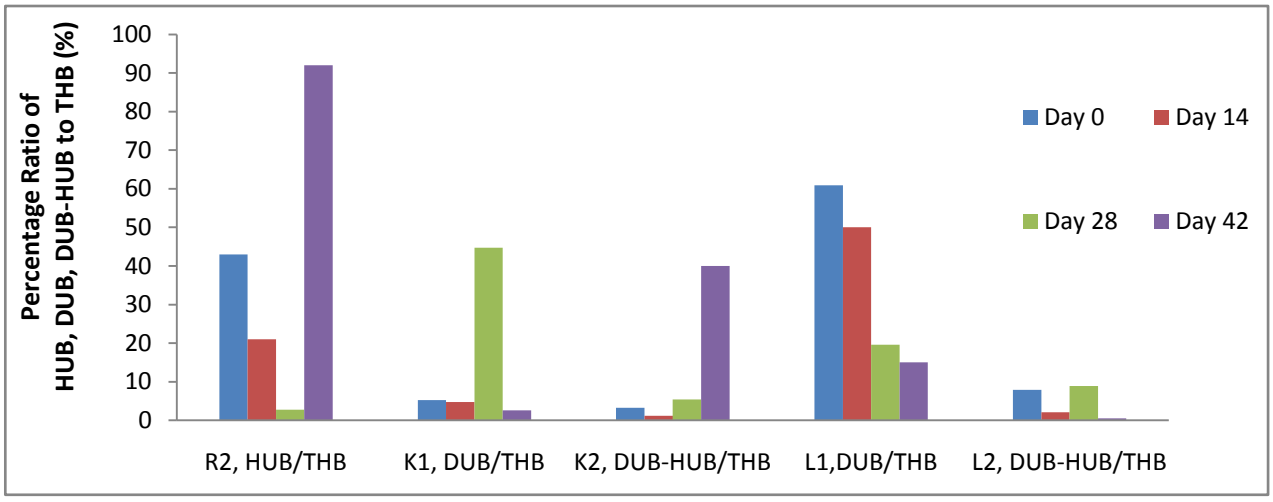

Figure 26. Percentage Ratio of HUB, DUB and DUB-HUB to THB Counts

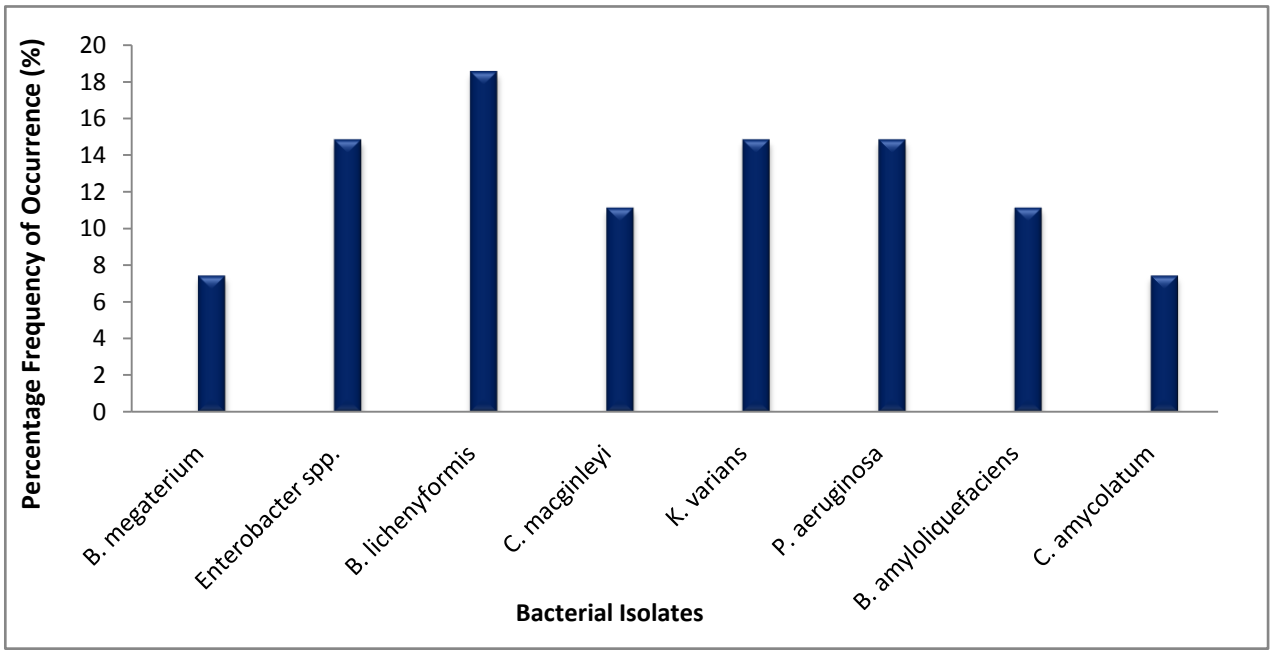

Figure 27. Percentage Occurrence of Bacterial Isolates in Samples

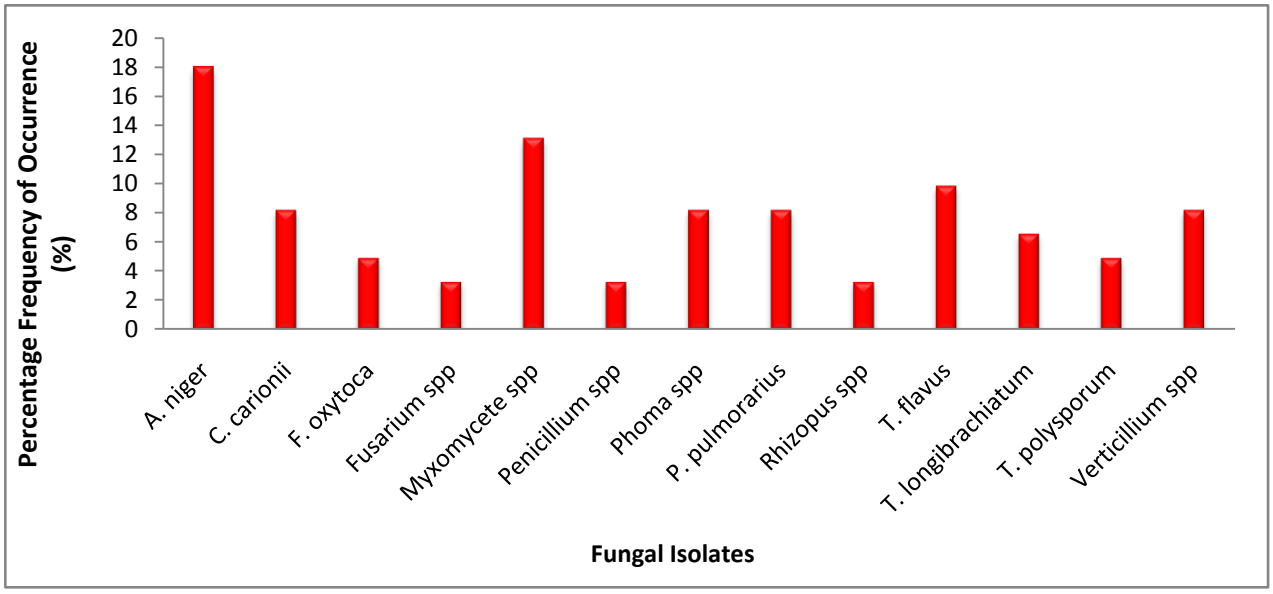

Figure 28. Percentage Occurrence of Fungal Isolates in Samples

Table 1. Analytical Profile Index (API), Morphology and Gram Reaction of the Bacterial Isolates

ISOLATE CODE	GRAM REACTION	MORPHOLOGY	PERCENTAGE SIMILARITY(%)	IDENTITY OF MICROORGANISM
S1	+	Rod	99.5	*Bacillus megaterium*
S2	-	Rod	79.0	*Enterobacter* spp.
S3	+	Rod	79.5	*B. lichenyformis*
S4	+	Rod	79.5	*B. lichenyformis*
S5	+	Rod	97.5	*Corynebacterium macginleyi*
S6	+	Cocci	99.0	*Kocuria varians*
S7	-	Rod	97.8	*Pseudomonas aeruginosa*
S8	+	Rod	60.5	*B. amyloliquefaciens*
S9	+	Rod	65.3	*C. amycolatum*
S10	-	Rod	66.4	*Enterobacter* spp.
S11	-	Rod	90.5	*P. aeruginosa*

3.2.4. Biochemical Characteristics and Gram Reaction of Bacterial Isolates

Analytical Profile Index (API) of bacterial isolates revealed a total of Five (5) genera growing on samples assayed (Table 1). *Bacillus megaterium* (S1) and *Enterobacter* spp. (S2) were isolated from R2 sample. The bacteria *B. lichenyformis* (S3), *Corynebacterium amycolatum* (S9) and *Pseudomonas aeruginosa* (S7) were isolated from L2 sample. S4 (*B. lichenyformis*) was isolated from K1, and S5 (*Corynebacterium macginleyi*) was isolated from both K1 and L2. The other prokaryotes *P. aeruginosa* (S11) and *Enterobacter* spp. (S10) were isolated from L1 sample. *Kocuria varians* (S6) and *B. amyloliquefaciens* (S8) were only isolated from K2 and R1 samples respectively. The percentage similarity of bacterial isolates identified via API ranged between 60.5 to 99.5%. The frequency of occurrence of *B. megaterium*, *Enterobacter* spp., *B. lichenyformis*, *C. macginleyi*, *K. varians*, *P. aeruginosa*, *B. amyloliquefaciens* and C. *amycolatum* were 7.41%, 14.8%, 18.5%, 11.1%, 14.8%, 14.8%, 11.1% and 7.41% respectively.

The result of the biochemical tests identified the microorganisms. The isolate, *P. aeruginosa* was catalase positive and oxidase positive, *K. varians* was lactose negative, trehalose and urease positive. *Enterobacter* spp. was Ortho-Nitrophenyl-βD-Galactopyranosidase (ONPG) positive and urease negative. *Corynebacterium* spp. was pyrazine carboxamide (PYZ) positive and urease negative.

The Gram reaction and morphology characteristics of the bacterial isolates revealed that *Bacillus* spp., *Corynebacterium* spp. and *P. aeruginosa* appeared as rods (Table 1). The S6 was a Gram-positive coccus. The *Enterobacter* spp. appeared as a Gram negative motile rod.

3.2.5. Identification of Fungal Isolates

The colonial and microscopic morphologies of fungal isolates from samples studied showed a total of Eleven (11) fungal genera. The isolates include: *Aspergillus niger* (18.0%), *Cladosporium carionii* (8.20%), *Fusarium oxytoca* (4.92%), *Fusarium* spp. (3.28%), *Myxomycete* spp. (13.1%), *Penicillium* spp. (3.28%), *Phoma* spp. (8.20%), *Pleurotus pulmorarius* (8.20%), *Rhizopus* spp. (3.28%), *Talaromyces flavus* (9.84%), *Trichoderma longibrachiatum* (6.56%), *T. polysporum* (4.92%) and *Verticillium* spp. (8.20%). One (1) un-identified isolate was found in K2 sample. *A. niger* was predominant at R2, K1, K2, L1 and L2; and followed by *Myxomycete* spp. occurring at R1, K2 and L1. The percentage occurrence of bacterial and fungal isolates were shown in Figure 27 – Figure 28.

3.2.6. Percentage HUB, DUB, DUB-HUB to THB of Samples

Figure 26 showed the percentage ratio of HUB to THB for R2 sample which ranged from 2.7 – 92%; whereas the DUB to THB ratio for K1 was 2.6 – 44.7%, and for L1, 15 – 60.9%. The DUB-HUB to THB percentage ratio for K2 was between 1.2 – 40%, and for L2, 0.46 – 8.9% (Figure 26). It was only at L2 after 42 days that the percentage ratio was less than one which indicates that the mixture was not polluted.

3.3. Biodegradation of Test Samples

3.3.1. Percentage Primary Biodegradation of Samples

The percentage primary biodegradation after 28 days of experimentation as showed in Figure 17 has the least rate of biodegradation at R1 with 0.36%, followed by K1 (0.91%), K2 (1.12%), R2 (1.24%), L1 (2.89%) and L2 (21.2%). The result of the primary biodegradation, after 42 days, revealed that L2 was the highest degraded at 77.1%, followed by L1 at 4.39%. K1, K2, R1 and R2 degraded at 1.22%, 1.19%, 0.42% and 1.64% respectively.

3.3.2. Percentage Mineralization of Samples

Figure 18 presented the result of mineralization of the samples. The ratio of BOD to COD of L2, K2, L1 and K1 were 2.74%, 1.16%, 2.33% and 0.29% after 28days. After 42 days, R1 was 0.54%, and R2, K1, K2, L1 and L2 were 1.30%, 0.29%, 0.35, 1.47% and 3.15% respectively.

3.3.3. Percentage Ratio of Dissolved Oxygen to Biochemical Oxygen Demand of Samples

The result on Figure 19 showed the percentage ratio of DO to BOD of the samples between 0 to 42 days interval. L1 and R2 biodegradation setups had the lowest and lower DO/BOD ratios of 4.90% and 5.94% on the 0 Day respectively. The highest percentage was observed at K1 with 143.3% on Day 0 and slightly decreased to 139.9% on the 42 Day.

3.3.4. Percentage TPH Losses

The result on Figure 20 showed that the percentage TPH loss over the 42 days period was highest at L2 followed by L1, K1, R2, K2 and R1 with 46.0%, 45.5%, 33.1%, 15.3%, 13.0% and 8.43% respectively.

3.4. Analysis of Variation and Relations of Data

The results of Analysis of Variance (ANOVA) test at 95% confidence limit ($P \leq 0.05$) for the period of experiment showed that there was significant difference in the Biodegradation of each oil spill dispersant or their combination with Crude oil, as F-calculated and F-critical values were 20.126 and 2.901 respectively. Whereas, using the percentage ratio of BOD to COD, there was no significant difference in the mineralization of each chemical dispersants or their mixture with crude oil as F-calculated value (1.134) was less than F-critical value (3.287).

4. Discussions

The result of this study indicates that many of the bacterial and fungal isolates, when screened for hydrocarbon utilization, showed potential for crude oil and dispersants degradation as the sole source of carbon and energy. Recent studies have showed the growth of *Pseudomonas* spp., *Bacillus* spp., [11,18,20], *Enterobacter* spp. [11] and *Corynebacterium* sp., [18] in the combination of oil spill dispersants with crude oil. The eukaryotes, *Rhizopus* spp., *Penicillium* spp., and *Aspergillus* spp. have been isolated from different mixtures of dispersants and brackish water samples [20]. A total of seven fungal genera, which have also been implicated in this study, have showed tendencies for hydrocarbon and/or dispersants utilization [18]. The ability of the microorganisms to degrade oil spill dispersants and crude oil may be due to co-metabolism, [21], or because of the production of biosurfactants [22,23]. The rod-shaped bacterium, *Pseudomonas aeruginosa* has been linked to the production of rhamnolipids [24], a biosurfactant used in the clean-up of oil. *Enterobacter cloacae* has also shown to secrete exopolysaccharide (EPS) which helps in the reduction of interfacial tension and in addition, shields bacterial cells from direct exposure to toxic substances [25,26]. A consortium of microorganisms known as the biomass can use the dispersant and the crude oil as food [27]. All these and more may have contributed in the degradation of the samples studied.

The hydrocarbon utilizing bacterial and fungal counts for R2 sample were on the increase after 42 days experimentation. This showed that under natural attenuation, oil spills can be degraded by microorganisms. The decline in the counts of THB of K1 from Day 0 to Day 42, THF of K1 from Day 0 to Day 14, DUB of K1 from Day 0 to Day 14 and Day 28 to Day 42, DUF of K1 from Day 0 to Day 14 and DUB-HUB of K2 from Day 0 to Day 14 showed that the oil spill 'dispersant', Rigwash is toxic to the microorganisms. This result is unlike report [7] on four chemical dispersants which none proved to be toxic.

The highest fungal growth observed at K2 sample may be due to its lower pH values ranging from 6.42 to 6.56. Fungi are known to secrete extracellular enzymes during biodegradation, and they grow better under low pH environmental conditions [21]. Results of the pH for L1 and L2 showed some level of compliance with DPR limit whereas the K1 and K2 are not within the regulatory permissible limit. This indicates that there was reduction and/or increment on the volume of hydrocarbon because of the application of a specific dispersant and the activities of bacterial and fungal species in the samples. The chemical constituents of some oil spill dispersants such as the additives and base solvents have been reportedly resulted in the inability of microbial cells to degrade dispersants and/or their mixtures with crude oil [18,28].

The result of the primary biodegradation after 42 days is like the outcome of the 21 days study where L2 was degraded at 18.3%, K2, 1.01% and R2, 1.10% [18]. Although 77.1% of the biodegradation rate of sample L2 was degraded after 42 days, only it was inherently biodegradable under aerobic biodegradation because it degrades greater than 20% of the hydrocarbon mixture after 28 days [29,30]. The primary biodegradation is the alteration in the chemical structure of a substance, brought about by biological action, resulting in the loss of a specific property of that substance. According to OECD [30], it is the structural change (transformation) of an organic chemical by microorganisms resulting in the loss of a specific property. The TPH loss after 42 days of Biodegradation were 8.43%, 15.3%, 33.1%, 13.0%, 45.5% and 46.0% for R1, R2, K1, K2, L1 and L2 respectively. Whereas after 21 days study, it was reported as 37.3% (L2), 4.52% (K2) and 4.07% (R2) [18]. These results implicated the adverse effects of Rigwash on crude oil because under natural attenuation (R2) the TPH loss was increased to 15.3% more than when it was applied (K2 = 13.0%). This result quite supported USEPA observation on Corexit 9527 dispersant, where it was found to increase the toxicity of oil spill after application [31]. The mixture becomes up to 52 times more toxic than oil alone [16].

The percentage mineralization (ultimate biodegradation) of the samples after 42 days ranged from 0.29 to 3.15%. The percentage mineralization of the samples was the level of aerobic degradation obtained when the dispersants and/or crude oil (test compounds) are totally utilized by microbial cells resulting in the production of carbon dioxide, biomass (new microbial cellular constituents), mineral salts and water [18].

The BOD is the amount of oxygen consumed by microorganisms when metabolizing a test compound [32]. The decrease in BOD with time of L1 and R2 indicated that the concentrations of biodegradable organic carbon in the monitoring were decreasing with time and because of a progressive increase in DO. The increase in BOD of other mixtures or samples could have occurred due to increasing microbial activity [33] and decline in DO because the oxygen that is available in the water is being reduced by the microorganisms.

The COD values obtained indicate the amount of oxygen needed to chemically oxidize organic compounds present in the samples. In fact, COD is the amount of oxygen consumed during oxidation of a test compound with hot, acidic dichromate [33]. It provides a measure of the amount of organic compound (oxidizable matter) present in the test material which can be oxidized by a strong chemical oxidizing agent [11,33].

The decrease in nitrate, sulphate and phosphate levels with time indicated that these compounds were being utilized by microorganisms [35]. Nitrogen and phosphorus are limiting nutrients for microbial systems. Their presence or absence influenced the rate of biodegradation.

Salinity was one of the factors that affected the rate of biodegradation. The biodegradation of L1, L2, R2 and K1 increases with decrease in Salinity whereas in R1 and K2, it slightly decreases with increase in salinity. Studies have shown that the biodegradation of oil spill dispersants or crude oil increases with decrease in salinity [8,18,35].

5. Conclusion

The biodegradability of oil spill dispersants, Eco-Remover and Rigwash which are commonly used by petroleum industries in response to oil spill pollution have been evaluated. From the study, Eco-Remover is more biodegradable than Rigwash 'dispersant' within the 42 days' experimental period. The behaviour of the compounds during the study may be due to their synergistic effects. Crude oil-Eco-Remover mixture with the highest number of hydrocarbon-dispersant utilizing bacterial and fungal counts showed the ability to be utilized by microorganisms more than the crude oil-Rigwash combination. However, this study is limited since the percentage rate of microbial cells occurring in the samples are partly dependent on the source of inoculum (seawater). The use of different water samples may present distinct indigenous species of hydrocarbonoclastic microorganisms which have the potentials to use the substrates as the only available carbon and energy. The level of biodegradation achieved showed that there was significant difference in the biodegradation of each oil spill dispersant or their combination with Crude oil.

6. Recommendations

Sequel to the outcome of this study,

(a). There is need for further studies on the application of these dispersants in the field to confirm the outcome of this laboratory observations.

(b). The DPR should ensure that oil industries operating within the Nigerian territory strictly adhere to the rules and regulations guiding the use of oil spill dispersants and adoption of response strategies; oil industries that violated its laws should be brought to book to serve as a deterrent to others.

(c) The un-identified fungal isolate should be subjected to further investigations, preferably, molecular approaches to classify and identify the organism and know whether it's novel.

(d) Other recommendations proposed by Nnadozie and Odokuma [18] should be taken seriously and accorded the necessary attention they deserve.

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