



## Impact of Rigwash Oil Spill ‘Dispersants’ on the Biodegradation of Crude Oil in Nigeria

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### Authors' contributions

This work was carried out in collaboration between both authors. Author PCN designed the study, performed the statistical analysis, wrote the protocol and the manuscript, managed the analyses of the study and the literature searches under the strict supervision of author LOO. Both authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** To ascertain the impact of Rigwash oil spill ‘dispersant’ on the biodegradation of crude oil.

**Study Design:** The study employs experimental design and statistical analysis of data and interpretation.

**Place and Duration of Study:** The water sample for the study was collected from Okrika High Sea via Adedemebiri, Rivers State of Nigeria at a depth of 1 m. It was transported to the laboratory at 25 – 30°C within 2 hours. The ‘dispersant’ and the oil were obtained from an industrial chemical store at Trans-Amadi, Port Harcourt and from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Rivers State of Nigeria respectively. Experimental analyses were done at the Departments of Microbiology, and Biochemistry, University of Port Harcourt, Rivers State, and at the Federal Institute of Industrial Research, Oshodi (FIRO), Lagos State. The biodegradation setups were monitored at a weekly interval over 21-days period at room temperature (approximately 30°C) on a static shake-flask system.

**Methodology:** Standard techniques such as the spread plate method, titrimetric technique, atomic

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absorption spectrophotometry and Gas Chromatography (GC-FID) were adopted during the study. The preliminary range findings involves plating out in duplicates on nutrient agar an 0.1 ml aliquot of a mixture of 100 ml seawater and each of the different concentrations of 'dispersants' (0, 0.01,0.1,1.0, 2.0, 5.0,10, 20, 50, 100,1000) ml (v/v) respectively. The cultures were incubated at 35°C for 48 hours. Analysis of variance at confidence limit  $P \leq 0.05$  was adopted.

**Results:** The result of the study revealed that Rigwash oil spill 'dispersant' may have negative impact on the biodegradation of Bonny light crude oil over time. The effect may portend great danger to the health, safety and environment of aquatic floras and faunas of marine systems. The level of biodegradation achieved for the crude oil-dispersant mixture (K2) and crude oil alone (R2) were 1.01% and 1.10% respectively. Analysis of variance at confidence limit  $P \leq 0.05$  showed that there was no significant difference in the biodegradation of crude oil or its combination with Rigwash oil spill 'dispersant'.

**Conclusion:** Rigwash oil spill 'dispersant' may have negative impact on the biodegradation of Bonny light crude oil. However, this may be injurious to the health, safety and environment of living organisms.

*Keywords: Rigwash 'dispersant'; oil spill; biodegradation; crude oil and marine systems.*

## 1. INTRODUCTION

In recent times, between 2005 to 2016, the activities of multinational oil companies, oil pipeline vandals and militants such as the Niger Delta Avengers (NDA), the Movement for the Emancipation of Niger Delta (MEND) and other splinter militant groups in the Niger Delta region of Nigeria have seriously affected health, environment as well as the economic fortunes of Nigeria. In Nigeria, there are over seventy companies involved in petroleum and exploration activities. Most notable are Shell Petroleum Development Company (SPDC), Nigerian Agip Oil Company (NAOC), ExxonMobil, Chevron, Schlumberger, Total, Addax Petroleum, Petrobas, African Petroleum Oil Field Services, Conoil, South Atlantic Petroleum (SAPETRO), Nexen Inc. and SEPLAT Petroleum. Consequently, as the number of oil industries increases, so the increase in the rate of oil spill pollution. Given the dynamic nature of oil pollution and the extent of contamination in the Niger Delta, several remediation strategies have been adopted as a countermeasure to combat oil spill pollution [1]. Among these remediation methods include the use of oil spill dispersants [2,3]. Dispersants are blends of surface active agents (surfactants) in solvents which are designed to enhance natural dispersion by reducing the surface tension at the oil and water interfaces [4].

Studies have shown the negative impacts of oil spill dispersants on human health and other biota in the environment. Scientific reports have shown increasing number of cases of toxicity on marine life [5,6]. The use of oil spill dispersants for dealing with oil pollution in sea of the coast of the

Niger Delta of Nigeria has been approved by the Department of Petroleum Resources (DPR) *Environmental Guidelines and Standards in the Petroleum Industry in Nigeria* (2002). Reports showing the biodegradability of these dispersants are few [7,8].

The study on the impact of Rigwash oil spill 'dispersants' on the biodegradation of crude oil in Nigeria will help bring to the fore-knowledge about the effects of oil spill dispersants used by some oil industries during oil spill control and abatement. It will reveal the microbial population capable of degrading crude oil and/or its mixture with dispersants. This is very important because many environmentalists in the Niger Delta of Nigeria believe that the consequences of the impact of crude oil and/or oil spill dispersants on the Bodo Community and Ogoniland (2009), on the Ikara – Ikpoba Oka (2014) which is caused by ruptured oil pipelines owned by the Nigerian Petroleum Development Company (NPDC), on some of the Lagos rivers which is caused by the vandalization of the Trans-Forcados and the Escravos-Lagos pipelines by militants (2015), and on some of the riverine communities in Delta State of Nigeria which is caused by the bursting of the Chevron Gas pipeline by NDA (2016) would be addressed if these degraders are known and utilized in the field.

## 2. MATERIALS AND METHODS

### 2.1 Sources of Samples

#### 2.1.1 Crude oil samples

The Bonny light crude oil samples used for this study were collected from the Nigerian National

Petroleum Corporation (NNPC), Rivers State of Nigeria. The plastic containers used for the collection were first rinsed out with a little quantity of the sample before the final collection. The plastic vessel was sealed and stored at room temperature in the laboratory and used within a period of 30 days.

### **2.1.2 Marine water sample**

In this study, the seawater sample served as the source of inoculum. It was collected aseptically from Okrika High Sea via Adedembiri, Rivers State. The sample was collected by submerging a 10-liter plastic can into the water at a depth of 1m and transported to the laboratory at 25 – 30°C within 2 hours. Where analysis could not be conducted immediately, the fresh seawater samples were stored at 4°C for at most 24 hours before commencement.

### **2.1.3 Dispersants**

The Department of Petroleum Resources (DPR) unapproved chemical, Rigwash used by some oil industries in Nigeria as 'oil spill dispersant' was locally purchased from a chemical store at Trans Amadi, Port Harcourt, Rivers State of Nigeria. The DPR's approved dispersant Eco-Remover (control) was obtained from NNPC, Port Harcourt.

## **2.2 Media Preparation**

The nutrient media and mineral salt media were used [9]. The Potato Dextrose Agar (PDA) for the culture of fungi, particularly the filamentous molds, is prepared by dissolving 62 g of PDA powder in 1000 ml of distilled water. It was sterilized by autoclaving at 121°C for 15 minutes, cooled to 47°C and poured into sterile petri-dishes.

## **2.3 Preliminary Range Finding**

The spread plate method was adopted to determine the non-toxic concentration of the chemical 'dispersants' to the indigenous microbial flora of the seawater. An 0.1ml aliquot of a mixture of 100ml seawater and each of the different concentrations of 'dispersants' (0, 0.01,0.1,1.0, 2.0, 5.0,10, 20, 50, 100,1000) ml (v/v) were respectively plated out in duplicates on nutrient agar. The cultures were incubated at 35°C for 48 hours.

## **2.4 Biodegradation Monitoring**

The static shake flask method was used for the biodegradation tests. The setups were monitored at a weekly interval over 21-days period at room temperature (approximately 30°C). A total of Three (3) biodegradation flasks were setup for the experiment. Two of the setups contained each 0.1 ml non-toxic concentration of either the Rigwash or Eco-Remover, with 98.1 ml of water sample and 9 ml of Bonny light crude oil (Sample K2 and L2), while the other flask contained 9 ml of crude oil in 91 ml of seawater (Sample R2). The biodegradation test of the samples was determined by conducting physico-chemical analyses on the test samples collected from the biodegradation flasks at day 0, 7, 14 and 21.

## **2.5 Microbiological Analysis**

### **2.5.1 Isolation and enumeration of total heterotrophic bacteria (THB)**

The THB for each biodegradation setup was enumerated by spread plate method [7]. The dilutions were respectively transferred onto well dried sterile nutrient agar plates (in duplicates) and incubated at 35°C for 48 hours. After incubation, the bacterial colonies that grew on the plates were counted and sub-cultured onto fresh nutrient agar plates using the streak-plate technique [10]. Discrete colonies on the plates were then transferred into nutrient agar slants, properly labeled and stored as stock cultures for preservation and identification [11].

### **2.5.2 Isolation and enumeration of total heterotrophic fungi (THF)**

The THF population in the biodegradation setups were enumerated and isolated by inoculating 0.1 ml aliquot of the 10<sup>-1</sup> to 10<sup>-6</sup> dilutions mixtures onto well-dried potato dextrose agar (PDA) containing lactic acid to inhibit bacterial growth [10]. Pure cultures of the fungal isolates were enumerated and transferred onto PDA slants as stock cultures for preservation and identification [7].

### **2.5.3 Enumeration of hydrocarbon utilizing bacteria (HUB)**

Aliquots (0.1 ml) of the 10<sup>-1</sup> to 10<sup>-6</sup> dilutions of R2 biodegradation test were plated onto modified mineral salt media in duplicates [9]. The vapour-phase transfer method was adopted. The filter papers were saturated with Bonny light crude oil

after the agar plates have been inoculated, and they were aseptically placed inside the plates cover and inverted. Subsequently, the petri-dishes were cultured for seven days at room temperature. The colonies were counted and recorded for the calculation of colony forming units (CFU/ml).

#### **2.5.4 Enumeration of hydrocarbon utilizing fungi (HUF)**

Aliquots (0.1 ml) of each 10 – fold serially dilutions of sample from R2 Erlenmeyer flask were transferred into duplicate plates of mineral salt agar (MSA) supplemented with streptomycin (50 mg/ml) to suppress bacterial growth. Culturable HUF were enumerated by vapour-phase transfer method at 30°C for fourteen days after aseptically placing Bonny light oil-soaked Whatman filter paper into the lids of each inoculated agar plates. Colonies on MSA plates were further purified by subsequent culture on PDA and final subculture on nutrient agar plates. The purified fungal isolates were identified based on their morphological characteristics [12].

#### **2.5.5 Isolation and enumeration of HUB-DUB from mixtures**

Aliquots (0.1 ml) of the 10<sup>-1</sup> to 10<sup>-6</sup> dilutions from the oil: Dispersant K2 and L2 biodegradation setups were plated onto modified mineral salt media. After which some other described enumeration methods [10,13] were followed.

#### **2.5.6 Isolation and enumeration of HUF-DUF from mixtures**

The method described above for enumeration of HUF was employed except that in this case, 0.1 ml aliquots of the 10<sup>-1</sup> to 10<sup>-6</sup> dilutions from the K2 and L2 biodegradation setup flasks were plated onto modified mineral salt media containing the antibiotics.

#### **2.5.7 Bacterial and fungal isolates identification**

The criteria for the identification of discrete bacterial isolates were per Bergey's Manual of Determinative Bacteriology [14]. The morphological and biochemical tests used for bacterial identification include; Gram staining, motility, catalase, oxidase, citrate utilization, hydrogen sulphide production, indole production, methyl red and voges-proskauer tests.

To identify the fungi, small portion of the fungal colony isolated was picked and teased with a sterile inoculating wire loop into 2-3 drops of lactophenol cotton blue solution on a clean slide. The cover slip was placed after teasing the colony out; and the preparation was observed under microscope using x10 and x40 objective lenses. The absence or presence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata and other microscopic characteristics as well as culture characteristics revealed the identity of the fungal isolates [15].

Culturable bacterial isolates were further characterized using Analytical Profile Index (API) in the identification of microorganisms via API system [16,17]. The API 20E V4.0, API 50 CH V3.0, API 20 NE V4.0 and API CORYNE V2.0 strips stored at 2 – 8°C were used in the identification of bacteria.

### **2.6 Physico-chemical Analysis**

The physico-chemical parameters such as conductivity and pH were measured using a HANNA Conductivity/TDS meter (HI 9835) and a digital Oakton pH meter (model PCD 650) respectively. The experimental analyses of the samples followed standard chemical techniques such as the Gas Chromatography (GC-FID), atomic absorption spectrophotometry and the titrimetric methods at different intervals of 0, 7, 14 and 21 days at room temperature. The percentage mineralization was determined using the ratio of Biochemical Oxygen Demand (BOD) to Chemical Oxygen Demand (COD) [18], and the primary biodegradation rate was estimated using the percentage ratio of Dissolved Organic Carbon (DOC) to Total Organic Carbon (TOC) [19].

### **2.7 Statistical Analysis**

The significant variations of parameters were determined using the Analysis of Variance (ANOVA) at 95% confidence limit ( $P \leq 0.05$ ). The analysis was carried out using Microsoft Excel 2010 software.

## **3. RESULTS AND DISCUSSION**

The results of the percentage primary and ultimate biodegradation after 21 days of study for L2 were 18.3% and 2.32% respectively. For the K2 mixture, the results were 1.01% and 0.35% respectively; while for the R2 sample, they were

1.10% and 1.46% respectively (Figs. 1 – 2). The percentage TPH loss (Fig. 3) for L2, K2 and R2 were 37.3%, 4.52% and 4.07% respectively. The total bacterial count and hydrocarbon utilizing bacterial count for R2, within the experimental period (0 – 21 days), were between  $7.5 \times 10^2$  CFU/ml –  $1.0 \times 10^3$  CFU/ml and  $1.9 \times 10^2$  CFU/ml –  $7.5 \times 10^2$  CFU/ml respectively. While for the L2 mixture, the total bacterial count and the hydrocarbon-dispersants utilizing bacterial count were between  $5.3 \times 10^2$  CFU/ml –  $1.1 \times 10^4$  CFU/ml and  $4.2 \times 10^1$  CFU/ml –  $1.2 \times 10^3$  CFU/ml respectively; and for the K2,  $2.2 \times 10^3$  CFU/ml –  $6.4 \times 10^3$  CFU/ml and  $7.5 \times 10^1$  CFU/ml –  $9.8 \times 10^1$  CFU/ml respectively. The result of the total fungal count for R2 ranged between  $4.3 \times 10^1$  CFU/ml and  $2.5 \times 10^2$  CFU/ml, while the hydrocarbon utilizing fungal count ranged between  $8.3 \times 10^1$  CFU/ml and  $6.4 \times 10^2$  CFU/ml. The total fungal count and the hydrocarbon-dispersants utilizing fungal count for L2 were  $1.2 \times 10^2$  CFU/ml –  $6.7 \times 10^3$  CFU/ml and  $1.6 \times 10^2$  CFU/ml –  $3.6 \times 10^4$  CFU/ml respectively. And for the K2 oil-dispersants mixture, the total fungal count and the hydrocarbon-dispersants utilizing fungal count were  $4.9 \times 10^2$  CFU/ml –  $1.3 \times 10^4$  CFU/ml and  $2.5 \times 10^2$  CFU/ml –  $3.3 \times 10^3$  CFU/ml respectively.

A total of Seven (7) bacterial isolates identified by Analytical Profile Index were isolated. *Enterobacter* sp. and *Bacillus* sp. showed increasing abilities to degrade R2. *Pseudomonas aeruginosa* and *Corynebacterium striatum* possess biodegradation potential for K2 sample, and *Pseudomonas* sp., *Bacillus licheniformis* and *Corynebacterium* sp. were isolated from L2 mixture. The fungal isolates identified from R2 were *Trichoderma* sp., *Aspergillus* sp. and *Fusarium* sp. K2 were *Pleurotus* sp., *Talaromyces* sp. and *Myxomycetes* sp. The fungi isolated from L2 combination were *Penicillium* sp., *Aspergillus* sp. and *Talaromyces* sp.

The result of the physico-chemical analysis (Table 1) at R2 and K2 generally does not show some level of compliance with DPR's limits, except that some heavy elements such as Chromium, Selenium and Zinc are within the regulatory limits in the test samples studied. The pH for the control (L2) is within DPR's limit (Table 2). The pH for R2 increases with time and the values are above regulatory limit. On the application of 'Rigwash' to crude oil mixture (i.e., K2), the pH value decreased to 6.49 at day 21 while the combination of Eco-Remover with crude oil mixture (i.e. L2) resulted in pH decrease

from 8.40 (at Day 0) to 6.80 (at Day 21). The Dissolved Oxygen (DO) for R2 increases with time at Day 0 – 14 and decreased to 1.65 at day 21. For the L2 DO, there is a noticeable increase at Day 7 and at Day 21. The DO for K2 relatively increased with time during the 21 days' interval. The salinity has direct effect on the biodegradation of the samples as R2 decreases with time at 0 – 14 days and increased at day 21. The Chemical Oxygen Demand (COD) level is higher in K2 than in R2 and L2; while the Biochemical Oxygen Demand (BOD) level is higher in L2 and R2 than in K2. Unlike K2 and R2, the Total Petroleum Hydrocarbon (TPH) level of L2, during the study, progressively decreases with time. As the salt in the water reacts with crude oil and Eco-Remover (L2), there is an appreciable decrease in the salinity level with time. And for K2, salinity increases progressively with time during the 0 – 21 days' period. The result of the statistical Analysis of Variance (ANOVA) at confidence limit  $P \leq 0.05$  showed that there was no significant difference in the Biodegradation of Crude oil or its combination with Rigwash oil spill 'dispersant'.

The results obtained from this study have shown that some hydrocarbonoclastic microorganisms have potentials for crude oil and dispersants degradation. The bacterial genera *Bacillus* spp., *Pseudomonas* spp. and *Enterobacter* spp. that were isolated from the mixture of oil spill dispersants and crude oil have also been implicated by several researchers [20]. The ability of the microorganisms to degrade the K2 and L2 may be because of co-metabolism, a form of microbial interaction involving simultaneous degradation of two compounds [12], or due to the production of biosurfactants by microorganisms [21,22]. The increase in the counts of THB of R2 from day 0 to 21 revealed that the crude oil may not be toxic to the microorganisms and thus may show that the organisms have potential to utilize the test compound. The higher hydrocarbon-dispersant utilizing bacterial and fungal counts observed at L2, than that of the K2, may imply that there are availability of other sources of nutrients such as the limiting elements or compounds, nitrogen and phosphorus for microbial growth other than the hydrocarbon/dispersant [12]. The toxic nature of the 'dispersant' can be attributable to the base solvents and other additives [3]. The result of the primary biodegradation resulted in the loss of more of the specific property of R2 than K2. This indicated that R2 is more utilized as the only carbon source than K2. Although the TPH loss

**Table 1. Physico-chemical analysis of bonny light crude oil and “Rigwash” oil spill dispersant**

Parameters	R2				K2				DPR limit
	0	7	14	21	0	7	14	21	
pH	9.02	9.36	9.80	9.61	6.56	6.60	6.51	6.49	6.5 – 8.5
Acidity, mg/l	4.05	4.18	5.80	5.72	5.82	5.87	5.93	5.96	-
Alkalinity, mg/l	3.42	3.50	3.81	4.00	1.62	1.93	2.42	4.11	-
Conductivity, $\mu$ S/cm	27030	26250	25710	25519	16120	18360	20590	20310	900
Total dissolved Solids, mg/l	16096	15802	15725	15631	8004	8120	8890	9001	2000
Total suspended solids, mg/l	92.5	186	274	312	112	202	542	550	< 50
Salinity as chloride, mg/l	18722	18689	18600	17714	10680	10691	10701	10880	2000
Dissolved oxygen, mg/l	1.04	1.20	1.88	1.65	1.22	1.24	1.29	1.85	4.0 – 5.0
Total organic carbon, mg/l	7.91	7.52	7.30	6.38	5.16	5.11	5.00	4.93	-
Chemical oxygen demand, mg/l	1023	1036	1044	1118	3655	3690	3789	3955	125
Biological oxygen demand, mg/l	17.5	17.1	16.6	16.3	12.8	12.5	13.9	13.7	125
Dissolved organic carbon, mg/l	0.042	0.049	0.068	0.070	0.051	0.055	0.065	0.050	-
Oil and grease, mg/l	125960	120104	108152	91650	95871	100150	116703	112400	10
Total petroleum hydrocarbons, mg/l	5890	5902	6049	5650	5262	5712	6043	5500	40
Nitrate, mg/l	50.0	50.9	52.4	51.5	15.0	19.3	21.1	25.0	20
Phosphate, mg/l	5.24	6.00	7.10	7.55	2.15	2.08	1.59	3.14	-
Sodium, mg/l	366	360	356	281	401	370	373	377	200
Potassium, mg/l	291	287	285	232	317	330	347	353	200
Zinc, mg/l	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	1.0
Copper, mg/l	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	No limit
Chromium, mg/l	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.5
Iron, mg/l	2.01	1.83	1.52	1.77	0.63	0.81	0.99	1.00	No limit
Lead, mg/l	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	No limit
Nickel, mg/l	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Cadmium, mg/l	0.09	0.07	0.05	0.06	0.01	0.01	0.03	0.05	Unobjectionable
Selenium, mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.01
Sulphur, %	0.25	0.23	0.20	0.17	0.17	0.18	0.20	0.29	0.2

Key: R2 = Mixture of Seawater and Crude oil; K2 = Mixture of Seawater, Crude oil and Rigwash 'Dispersant'

after 21 days of Biodegradation is greater in K2 (4.52%) than R2 (4.07%) by 0.45%, there is no significant difference in the value.

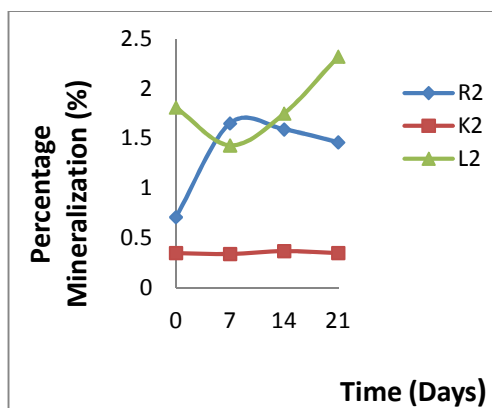
The percentage mineralization of the samples after 21 days' biodegradation ranged from 0.34 to 1.65%. Mineralization (ultimate biodegradation) is the level of aerobic degradation achieved when the test compound is totally utilized by microorganisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass). The higher COD levels mean higher

amount of oxidizable organic material in the samples or crude oil-dispersant mixture studied; by implication, the higher the COD the higher the amount of pollution in the test samples, thus this may lead to the reduction of DO levels in the system. A decline in DO level can lead to anoxic condition, which is deleterious to aquatic lives. The higher BOD level showed the increased amount of oxygen consumed which indicates enhanced microbial activity [20]. The more TPH level decreases with time, the faster will the degradation of petroleum fractions occur.

**Table 2. Physico-chemical analysis of bonny light crude oil and eco-remover oil spill dispersant**

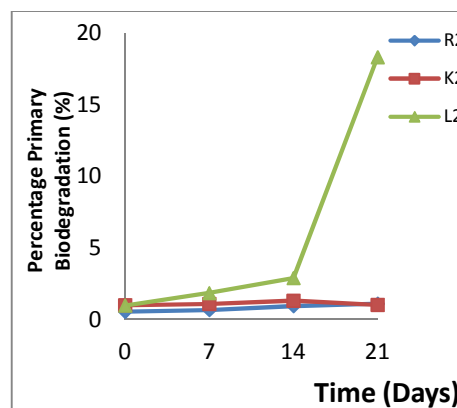
Parameters	L2				DPR Limit
	0	7	14	21	
pH	8.40	8.21	7.97	6.80	6.5 – 8.5
Acidity, mg/l	4.72	5.34	6.01	5.79	-
Alkalinity, mg/l	4.90	4.41	3.78	3.61	-
Conductivity, $\mu$ S/cm	15970	17501	20005	20213	900
Total dissolved solids, mg/l	5012	4800	4020	4117	2000
Total suspended solids, mg/l	205	265	318	300	< 50
Salinity as chloride, mg/l	10010	9710	9250	8311	2000
Dissolved oxygen, mg/l	3.27	4.00	1.15	1.19	4.0 – 5.0
Total organic carbon, mg/l	10.7	8.43	6.09	5.45	-
Chemical oxygen demand, mg/l	1090	941	800	612	125
Biological oxygen demand, mg/l	19.7	13.5	14.0	14.2	125
Dissolved organic carbon, mg/l	0.104	0.155	0.176	1.00	-
Oil and grease, mg/l	82120	80710	88900	92300	10
Total petroleum hydrocarbons, mg/l	6400	6011	4180	4014	40
Nitrate, mg/l	26.5	25.1	27.0	25.0	20
Phosphate, mg/l	1.35	1.43	1.58	1.24	-
Sodium, mg/l	516	473	409	361	200
Potassium, mg/l	235	233	228	222	200
Zinc, mg/l	0.03	0.02	0.02	0.01	1.0
Copper, mg/l	<0.01	<0.01	<0.01	<0.01	No limit
Chromium, mg/l	<0.01	<0.01	<0.01	<0.01	0.5
Iron, mg/l	0.72	0.55	1.25	1.43	No limit
Lead, mg/l	<0.01	<0.01	<0.01	<0.01	No limit
Nickel, mg/l	<0.01	<0.01	<0.01	<0.01	-
Cadmium, mg/l	0.07	0.07	0.09	0.07	Unobjectionable
Selenium, mg/l	<0.001	<0.001	<0.001	<0.001	0.01
Sulphur, %	0.55	0.35	0.32	0.28	0.2

Key: L2 = Mixture of Seawater, Crude oil and Eco-Remover Dispersant



**Fig. 1. Percentage mineralization of samples**

R2 = Mixture of Seawater and Crude oil; K2 = Mixture of Seawater, Crude oil and Rigwash 'Dispersant'; L2 = Mixture of Seawater, Crude oil and Eco-Remover Dispersant

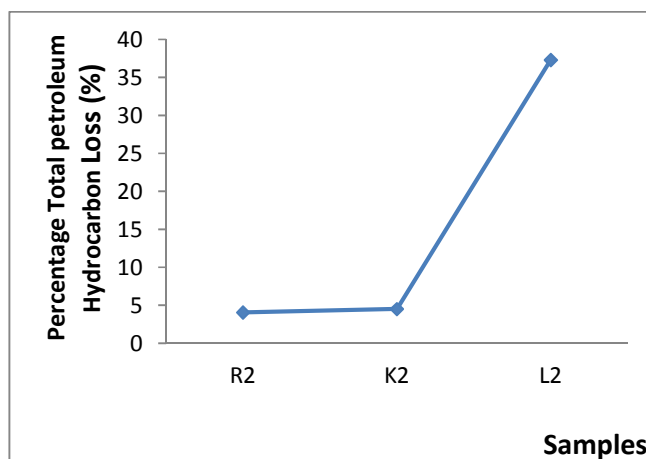


**Fig. 2. Percentage primary biodegradation of samples**

R2 = Mixture of Seawater and Crude oil; K2 = Mixture of Seawater, Crude oil and Rigwash 'Dispersant'; L2 = Mixture of Seawater, Crude oil and Eco-Remover Dispersant

In this study, salinity affected the rate of biodegradation. The biodegradation of R2 mainly increases with decrease in Salinity whereas in K2, it slightly decreases with increase in salinity. The result of sodium chloride

precipitations in this study agrees with previous studies [8,23], that the biodegradation of oil spill dispersants used in the Nigerian petroleum industries decreases with increase in salinity.



**Fig. 3. Percentage total petroleum hydrocarbon loss of samples**

*R2 = Mixture of Seawater and Crude oil; K2 = Mixture of Seawater, Crude oil and Rigwash 'Dispersant'; L2 = Mixture of Seawater, Crude oil and Eco-Remover Dispersant.*

#### 4. CONCLUSION

The evidence provided by this study revealed that Rigwash oil spill 'dispersant' may have negative impact on the biodegradation of Bonny light crude oil and thus may portends great danger to health, safety and environment of aquatic floras and faunas of marine systems. The reason for this adverse effect may be due to the chemical compositions of Rigwash which may have led to the inhibition of microbial growth and development. Considering the mean of microbial loads present in the samples, L2 with the highest number of hydrocarbon-dispersant utilizing bacterial and fungal counts showed the ability to be utilized by microorganisms more than the K2 mixture. However, this study is limited, in that the use of other water sources can present a higher microbial activity on K2 mixture than L2. The observed effect may also be because of the non-compliance of most physico-chemical parameters of the K2 sample such as the pH with that of the DPR's limit. This is further supported by the outcome of the percentage primary and ultimate biodegradation of Rigwash-crude oil mixture within the experimental period. The percentage primary and ultimate biodegradation of K2 are the lowest when compared with that of L2 and R2.

#### 5. RECOMMENDATIONS

The authors of this research work wish to recommend:

- Further study on crude oil and its combinations with Rigwash to determine whether the test substances are readily biodegradable or not. Aquatic toxicity test for short-term exposures is very necessary during the study.
- That the chemical compositions of Rigwash should be determined; and the manufacturing companies of oil spill dispersants should make available on the Material Safety Data Sheets (MSDS) the chemical compositions of dispersants to inform users about the cleaning agents and to encourage research.
- That the samples studied should be investigated over a longer period (at least 42 days) to prove beyond the reasonable doubt the outcome of the 21 days' experimental period.
- That a highly competitive oil/water treatment technology such as the combination of Advanced Oxidation Processes (APOs) and biological treatments should be considered for the degradation of oil spill dispersants and crude oil not treatable by conventional methods, may be, because of their high chemical stability and/or low biodegradability [24].
- That the DPR should ensure that oil industries operating in Nigeria strictly obeys its rules and regulations guiding the use of oil spill dispersants and adoption of response strategies. Erring companies should be sanctioned to serve as a deterrent to others.



## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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