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Biodegradation of oil spill dispersant in brackish water ecosystem of the Niger Delta, Nigeria

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Abstract

The biodegradability of two oil dispersants (OSD/Sea care and OSD/LT) were tested in brackish water ecosystem. This study aimed at evaluating the more biodegradable oil spill dispersant frequently used in oil spill remediation in Niger Delta, Nigeria. The study lasted for 20 days and sampling was done every 5 day period (0, 5, 10, 15 and 20 days). OSD/LT supported the highest Total Heterotrophic Bacterial and fungal count (7.04 log10 cfu/ml, 5.0 log10 cfu/ml), followed by the seacare (6.72 log10 cfu/ml, 4.68 log10 cfu/ml) but OSD/seacare recorded the highest dispersants utilizing bacteria (4.83 log10 cfu/ml) than OSD/LT (4.51 log10 cfu/ml) on the day 0. These test dispersants showed mild increases and decreases in the microbial population in the brackish water used as inoculum. Conclusively, Percentage (%) Ultimate biodegradability of the two oil spill dispersant showed that OSD/Seacare (78.8%) is more biodegradable than OSD/LT (76.1%) (*Noting that slight percentage difference in biodegradation rate is of great significant impact on the environment)*. This could be due to the chemical ingredients in the dispersants formulations. Thus oil companies and government parastatals carrying out remediation in the Niger Delta should be encouraged and mandated to use OSD/Seacare due to its higher biodegradation potential over OSD/LT.

Keyword: Biodegradation, Oil spill dispersant, OSD/LT, OSD/Seacare,

Introduction

Dispersants are chemical agents that reduce interfacial tension between oil and water in order to enhance the natural process of dispersion by generating larger numbers of small droplets of oil that are entrained into the water column (Fingas *et al*., 1995). Dispersants contain surfactants, which are surface-active agents with molecules composed of groups of opposing polarity and solubility; that is, surfactants usually have both an oil- soluble hydrocarbon chain and a water-soluble group. The synthetic surfactants can be anionic, cationic, nonionic, or amphoteric; however, only anionic or nonionic surfactants are utilized as crude oil dispersants. Surfactant mixtures often include other chemical agents, such as solvents, which enhance the dispersing capability of the surfactant (Pekdemir *et al*., 2005). They are a class of chemical compounds employed in the control of oil spilled in aquatic environment (NRC, 2005).

Chemical dispersant biodegradability or the measure of the amount of oxygen required to breakdown the chemical added to the oil contaminated water, is a major environmental concern when using dispersants. Dispersant themselves exhibit

a high demand for oxygen hence their use on spills in polluted coastal bays or inland waters with limited circulation, could deplete or lower the dissolved oxygen resources, therefore causing damage to biological community in such waters (Hamdan and Fulmer, 2011). Dispersants are most effective when applied immediately after a spill, before the lightest components in the oil evaporates. The use of dispersants in nearshore areas is expected to increase the exposure of aquatic organisms to petroleum (Milinkovitch *et al*., 201 1). If a crude oil spill is not treated, it will require long period of time to naturally biodegrade. It nearly takes 22 years for complete biodegradation of one kilogram crude oil by natural processes (Venosa and Xueqing, 2003). Environmental factors, including water, salinity, temperature and conditions at sea influence the effectiveness of dispersants. Studies have shown that many dispersants work best at salinity levels close to that of normal seawater (Okpokwasili and Odokuma, 1990).

In aquatic ecosystems, dispersion and emulsification of oil in slicks appear to be prerequisites for rapid biodegradation. Large masses of mousse, tar balls or high concentrations of oil in quiescent environments tend to persist because of the limited surface areas available for microbial activity. The residues, along with polymerization products formed from free radical degradation intermediate with each other, forming tar globules. The tar is a practically oxygenate high molecular weight material resistant to further microbial degradation. An ability to isolate high numbers of certain oil- degrading microorganisms from an environment is commonly taken as evidence that those microorganisms are the active degraders in that environment. A number of well-known microorganisms are responsible for the biodegradation of oil dispersants (Ron and Rosenberg, 2002). Bacteria have evolved regulatory systems that ensure the synthesis of enzymes so that the initial attack on these compounds is induced only when required. Thus, for an organism with the genetic information for utilizing benzene as carbon source, the enzyme for degrading benzene is induced when benzene reaches the bacterial environment. Some of these organisms have evolved an additional and highly effective system for responding to a variety of potential growth substrate. The essential genes of bacteria are carried on a single chromosome but genes specifying enzymes required for the catabolism of some of these unusual substrates may be carried on plasmids (Watanabe, 2001).

When oil spill occurs, a combination of recovery, disposal and the containment of oil is performed thereafter. The conventional methods to remove oil from aquatic ecosystems include; mechanical clean up, chemical clean up and microbial degradation. Mechanical cleaning of spilled oil and dispersant is nearly impossible in "protected" ecosystems. Microbial degradation is the major mechanism for the elimination of spilled oil and dispersants from the environment (Ventikos *et al*., 2004).

This study is designed to monitor the biodegradability of two test oil dispersants in brackish water environment (under laboratory condition).

Materials and Methods

Collection of water samples and Oil spill dispersant (OSD)

Brackish water samples were collected with sterile plastic ten (10) litre containers from Azubie River, Trans-Amadi Industrial Layout near Port Harcourt zoo in Port Harcourt Local Government Area in Rivers State, Nigeria. The containers were

rinsed three times with the water samples to be collected at the site before collection was made. The river spans from Rumuogba via Woji, Azuabie down to Marine Base, Port Harcourt. The river does not only receive faecal (as the coastal dwellers traditionally defecate into the water body), other industrial chemicals, solids and waste water of domestic origin, but also serves as the sink for used drilling fluid, degreasers, dispersants and Industrial detergent waste water. Oil spill dispersant (OSD) used in the study were Oil spill dispersant—OSD/LT and OSD/Seacare

Microbiological Analysis

Diluents preparation

The diluents were prepared by dissolving 8.5 grams of Sodium chloride (NaCl) in 1000ml of distilled water. 9ml of the normal saline (diluents) was transferred using sterile pipette into different test tubes, and these test tubes were sterilized in an autoclave at 121^0C for 15 minutes at 15psi and allowed to cool. This was then used to carry out serial dilution of the water sample spiked with Oil spill dispersant (OSD).

Serial dilution of samples

One milliliter of homogenously mixed sample was transferred using sterile one-milliliter pipette into sterile test tube containing 9ml physiological saline as diluent. One milliliter of the sample was transferred to another sterile 9ml diluent and mixed properly. This ten-fold serial dilution continued until desired dilution was obtained.

Isolation and enumeration of total heterotrophic bacteria

Total heterotrophic bacteria for each biodegradation set up were enumerated by spread plate method. 0.1ml aliquot of the 10^{-1} to 10^{-4} was transferred unto well-dried nutrient agar plates and incubated at 37^0C for 24 to 48 h. after incubation, the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh nutrient agar plates using the streak plate technique. Discrete colonies on the plates were aseptically transferred into agar slants, properly labeled and stored as stock cultures for preservation and identification (Odokuma and Ibor, 2002).

Isolation and enumeration of total fungal count

The total fungi population in the biodegradation set up (Habitat water sample and oil spill dispersant) were enumerated and isolated by inoculating 0.1ml aliquot of the mixture unto well-dried potato dextrose agar containing antibiotics (Tetracycline, Penicillin and Ampicillin) to inhibit bacterial growth. Pure cultures of the fungi isolates were enumerated and transferred unto potato dextrose agar slants as stock cultures for preservation and identification (Odokuma and Okpokwasili, 1992).

Isolation and enumeration of Oil spill dispersant (OSD) utilizing bacteria

Enumeration of Oil spill dispersant (OSD) utilizing bacteria was performed by inoculating 0.1ml aliquot of the dilutions unto mineral salt agar plates containing the

TCE (Odokuma and Okpokwasili, 1992). Colonies were counted after 48 to 72 h incubation at ambient temperature. The bacterial colonies on the plates after incubation were counted and sub-cultured onto fresh mineral salt agar plate.

Identification of bacterial and fungal isolates

The cultural, morphological and biochemical characteristics of the discrete bacterial isolates were compared with the recommendation in Bergey's manual of determinative bacteriology (1994). The morphological and biochemical test include; gram staining, motility, catalase, oxidase, citrate utilization, hydrogen sulphide production, indole production, methyl red and voges proskauer tests. The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics as well as cultural characteristics were used in the identification of the fungal isolates of the biodegradation flask set up (Cheesbrough, 2006).

Biodegradation set–up

Biodegradation monitoring was set up for each Oil spill dispersant (OSD), 300ml of the brackish water sample collected from Azuabie River, Trans-Amadi was dispensed into three 500ml Erlenmeyer flask. After that, 1% (3ml) of Oil spill dispersant - OSD/LT was dispensed into the first flask and 1% (3ml) of Oil spill dispersant - OSD/Seacare liquid detergent was dispensed into the second flask. The third flask was not contaminated with any detergent and was used as a control. The flasks were perforatedly pluged to allow for aeration, and were kept at ambient temperature (28 ± 2^{0}C) for 20 days.

Sample analysis

Samples were taken at day 0, 5, 10, 15, and 20 from the Erlenmeyer flasks containing marine water contaminated with the test detergents. This was to determine the Hydrogen ion concentration (pH) using electrometric pH meter (Jenway 3015 method), dissolved oxygen (DO) and biochemical oxygen demand (BOD) were determined by modified winkler method (APHA, 1998), chemical oxygen demand (COD) was determined by permanganate oxidation method; Total Heterotrophic Bacterial Counts, Total Fungal Counts, and Dispersant Utilizing Bacteria. (Okpokwasili and Olisa, 1991).

Ultimate biodegradation monitoring using the percentage ratio of BOD to COD

The biochemical oxygen demand (BOD) of each biodegradation test set up was monitored (APHA, 1998) at 0, 5, 10, 15, 20 days. The chemical oxygen demand was determined at day 0. The ultimate biodegradability (Swisher, 1987; Nrior and Odokuma, 2015) also referred to as the percentage of carbon in the material that is potentially mineralizable was calculated from the percentage of the ratio of BOD (for day 0, 5, 10, 15, 20) to COD at day 0. The percentage of mineralizable carbon in

the test compounds that was actually mineralized was derived from this formula,

$$\frac{P}{I} \times 100$$

$$100 - M = N$$

P = percentage of potentially mineralizable carbon in the test compound
I = percentage of potentially mineralizable carbon in the test compound at day 0
N = percentage of potentially mineralizable carbon in test compound that was actually mineralized.

Result and Discussion

The bacteria and fungi isolates obtained from different mixture of dispersants and brackish water samples were identified to be of the following genera; *Pseudomonas spp, Proteus spp, Micrococcus spp, Bacillus spp, Rhizopus spp, Asprgillus spp and Penicilium spp.*

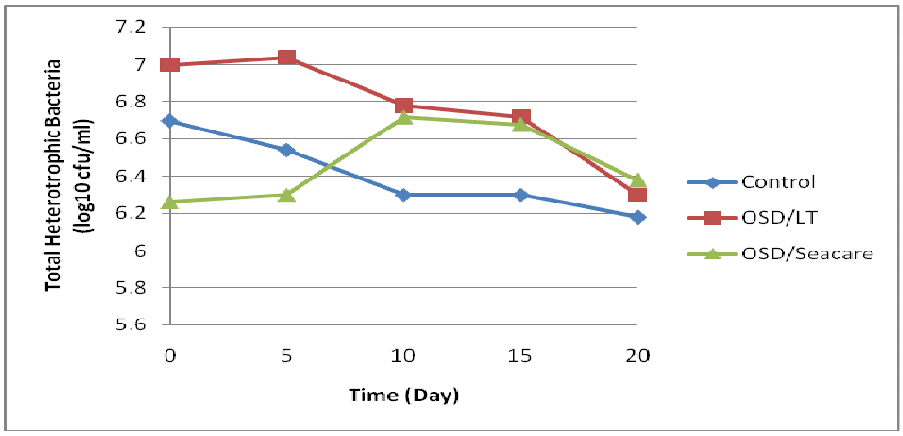

Figure 1: Total heterotrophic bacterial counts (log10 cfu/ml)

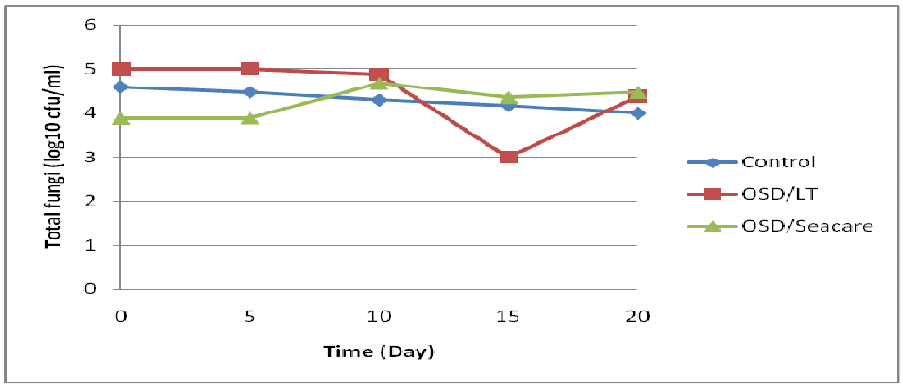

Figure 2: Total Fungal counts (log10 cfu/ml)

Figures 1-3 shows the changes in total heterotrophic bacteria, total fungi and disper-

sants utilizing bacteria counts throughout the period of study. This result showed that the total heterotrophic and dispersants – utilizing bacterial counts from the biodegradation setups were slightly decreased compared to the control from day 0 to day 20 however, OSD/LT supported the highest THB (7.04 log10 cfu/ml), followed by the seacare but seacare recorded the highest dispersants utilizing bacteria (4.83 log10 cfu/ml) on the day 0; while control has the highest fungal count (6.7 log10 cfu/ml). These test dispersants showed mild increases and decreases in the microbial population in the brackish water used as inoculum. This observation is in agreement with the report of Okpokwasili and Nnubia (1995) that, oil spill dispersants support mild increases (stimulation) and decrease (inhibition) in the growth of specific heterotrophic marine bacteria.

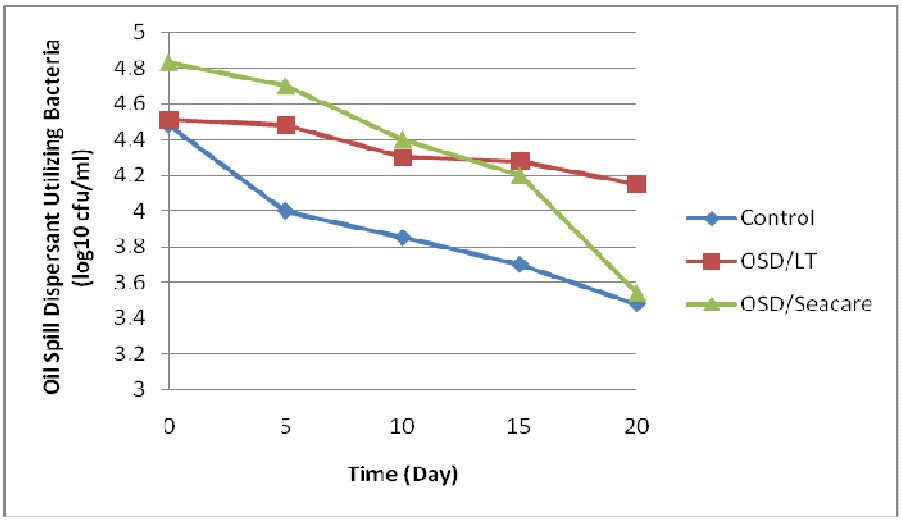

Figure 3: Oil Spill Dispersant Utilizing Bacteria (log10 cfu/ml) during biodegradation of Oil spill dispersant in brackish water ecosystem

According to Swannell and Daniel (1999), the addition of dispersants on biodegradation of crude oil stimulated the growth of dispersant degraders this hydrocarbon-degraders and supported the growth of indigenous seawater bacteria confirming that bacteria could utilize the nutrient available within the dispersants even at low concentration. Baghbaderani et al (2002) studied the biodegradability of three dispersant; pars 1, pars 2 and Gamlen OD 400. The study showed that the highest growth of microorganism documented for either pars 1 or pars 2 pars 1 dispersant showed more degradability in the first 24hrs compared to the others and has more adaptability to the aquatic ecosystem. The positive effect of dispersant on oil biodegradation is related to their ability to promote the growth of indigenous hydrocarbon – degrading bacteria and fungi as well as their ability to promote formation of small droplets (Varadaraj et al 1995).

The biodegradability of dispersants is the expression with which the living organism present in the water causes its degradation. The results of the physicochemical analyses of the biodegradation set up are represented in table 1-5

Table 1: Hydrogen ion (pH) concentration during Biodegradation of Oil spill dispersants in Brackish Water

Day	1	5	10	15	20	AVE	STDEV
Control	8.08	8.05	7.97	7.89	7.8	7.96	0.11519 5486
OSD/LT	7.37	7.58	7.44	7.39	7.56	7.47	0.09679 876
OSD/ Seacare	7.35	7.83	7.84	7.87	7.8	7.74	0.21833 4606

Table 2: Total Dissolved Solids (TDS mg/l) concentration during Biodegradation of Oil spill dispersants in Brackish Water

Day	1	5	10	15	20	AVE	STDEV
Control	1850	2370	2060	2030	1770	2016	232.1206 583
OSD/LT	1710	2260	2040	2030	1780	1964	221.4271 889
OSD/ Seacare	1600	2210	2090	2030	1770	1940	248.9979 92

Table 3: Dissolved Oxygen (DO mg/l) concentration during Biodegradation of Oil spill dispersants in Brackish Water

Day	1	5	10	15	20	AVE	STDEV
Control	10.3	9.2	7.9	4.5	4.2	7.2	2.75626 559
OSD/LT	15.8	8.9	7.9	4.7	3.8	8.2	4.74204 5972
OSD/ Seacare	15.6	7.9	7.6	6.5	3.3	8.2	4.53067 3239

Table 4: Biochemical Oxygen Demand (BOD mg/l) concentration during Biodegradation of Oil spill dispersants in Brackish Water

Day	1	5	10	15	20	AVE	STDEV
Control	27.9	24.9	21.4	12.2	11.4	19.56	7.453388
OSD/LT	42.8	24.1	21.4	12.7	10.3	22.26	12.84963
OSD/ Seacare	42.2	21.4	20.6	17.6	8.9	22.14	12.26002

Table 5: Chemical Oxygen Demand (COD mg/l) concentration during Biodegradation of Oil spill dispersants in Brackish Water

Day	1	5	10	15	20	AVE	STDEV
Control	67	60	51.5	29.5	27.5	47.1	17.85847
OSD/LT	205.3	115.6	102.6	61.1	49.4	106.8	61.60475
OSD/ Seacare	202.7	102.6	98.7	84.5	42.9	106.28	58.86257

The pH of the samples in the study (table 1) ranged from 7.65 to 10.05 indications that the samples were all basic, but the biodegradation flask containing OSD/ LT recorded highest increase in pH than the sea care and this can be a contributory factor to increased total bacteria counts in their biodegradation set up. Since most bacteria are neutrophiles which most fungi prefer slightly acidic environment (Prescott et al; 1999). The result revealed they the dissolved oxygen in the biodegradation flask containing the two dispersants remained constant compared with the control. This is an indication of reduced rate of biodegradation of dispersant by indigenous microbial population.

There was a sharp and steady decrease in the TDS value from day 5 until the end of the experiment. This decrease in the TDS value observed in the biodegradation flasks containing the test samples (dispersants) indicate that biodegradation was taking place. The decrease in the TDS value of the control sample may be due to use of dissolved nutrients in the water sample by the microbial flora of the brackish water use for biodegradation monitoring. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were higher in Oil spill dispersant OSD/LT than OSD/Seacare.

Percentage (%) Ultimate biodegradability of the two oil spill dispersant showed that OSD/Seacare (78.8%) is more biodegradable than OSD/LT (76.1%) Fig. 4.

Table 6: Percentage (%) carbon in Oil spill dispersant OSD/LT that is mineralizable in brackish water

Day	Control	OSD/LT	OSD/Seacare	
	%	%	%	
0	41.6	20.85	20.82	
5	37.2	11.74	10.56	
10	31.9	10.42	10.16	
15	18.2	6.19	8.68	
20	17.0	5.02	4.39	

Table 7: Percentage (%) Ultimate Biodegradation *(potentially mineralizable carbon)* in oil spill dispersant in brackish water

Day	Control	OSD/LT	OSD/Seacare
	%	%	%
0	0	0	0
5	10.6	44	49
10	23.4	50.2	51
15	56.2	70.3	58.2
20	59.2	76.1	78.8

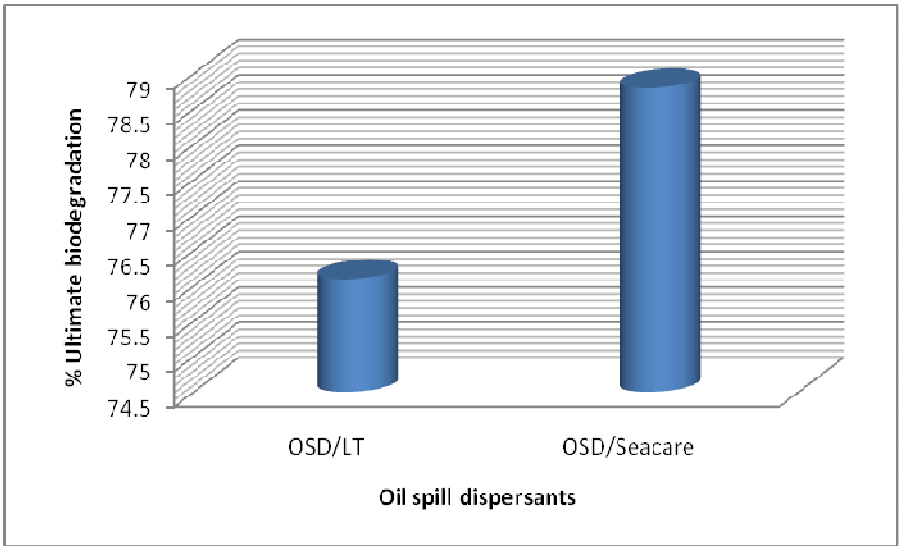

Fig.4: Percentage (%) Ultimate biodegradation of Oil spill dispersants in brackish water ecosystem at day 20.

This study revealed that OSD/Seacare was more biodegradable than OSD/LT; the result of the analysis showed that there was a decrease in the concentration of test dispersants throughout the study. The BOD, COD and percentage (%) ultimate biodegradation of the sample decrease throughout the study with increase in time in also biodegradation flasks.

Conclusion and Recommendation

Due to the growing acceptance of the use of dispersant in oil spill control; it becomes necessary that evaluation of their biodegradation potential be carried out before field application. The bacteria and fungi isolates obtained from different mixture of dispersants and brackish water samples were identified to be of the following genera; *Pseudomonas spp, Proteus spp, Micrococcus spp, Bacillus spp, Rhizopus spp, Asprgillus spp and Penicilium spp.*

Percentage (%) Ultimate biodegradability of the two oil spill dispersant showed that OSD/LT (23.9%) is more biodegradable than OSD/Seacare (21.2%) at day 20. This could be due to the chemical ingredients in the dispersants formulations. Consequently, it is recommended that oil companies and government parastatals carrying out remediation in the Niger Delta should be encouraged and mandated to use OSD/LT due to its high biodegradation potential over OSD/Seacare. Biodegradability assessment of dispersants to be used is key to environmental management.

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