International Journal of Physical Sciences Vol. 7(38), pp. 5477-5484, 9 October, 2012
Available online at http://www.academicjournals.org/IJPS
DOI: 10.5897/IJPS11.525
ISSN 1992 - 1950 ©2012 Academic Journals

Full Length Research Paper

Biodegradation of oil spill dispersants in natural aquatic ecosystem

Uzoigwe, C. I.* and Okpokwasili, G. C.

Department of Microbiology, University of Port Harcourt P. M. B5323 Choba Port Harcourt, Rivers State, Nigeria.

Accepted 9 March, 2012

The biodegradability of three oil spill dispersants (teepol, sodium dodeocyl sulphate (SDS) and corexit 9527) in a brackish water were monitored using the ratio of dissolved organic carbon (DOC) to total organic carbon (TOC) (primary biodegradation), the ratio of inorganic carbon to total organic carbon (mineralization) and total microbial (bacteria and fungi) population. The study lasted for 20 days. Sampling was done every 5 day period (0, 5, 10, 15 and 20 days). The biodegradation setup containing corexit 9527 dispersant showed the highest primary biodegradation (98.3%), followed by teepol (69.2%) and SDS (58.3%) at the 20th day. The percentage mineralization of the biodegradation setup containing teepol, SDS and corexit 9527 was 19.2, 7.8 and 3.3%, respectively, at the 20th day. The total heterotrophic bacterial (THB) and fungal counts were slightly decreased compared to the control with increasing time interval. However, SDS dispersant supported the highest THB and dispersant-utilizing bacteria, followed by teepol and corexit 9527, while teepol showed the highest fungal count. Teepol was the most biodegradable of the test dispersants in this study, followed by SDS and corexit9527. This study reveals the toxic implications of some dispersants used in oil spill control on the growth of aquatic microbial population and the biodegradability of the dispersants, and therefore suggests that, ecotoxicological analysis of oil spill dispersants should be carried out before field application on any natural ecosystem since some of them are not completely biodegradable and persist in the such environments.

Key words: Ecosystem, oil spill, biodegradation, dispersants, brackish water.

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

*Corresponding author. E-mail: ifybest_4@yahoo.com.

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., 2011). 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. Petroleum fractions containing asphalt components are not degraded quantitatively. 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. Floating tar globules are encountered in the marine environment in increasing quantities. 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).

Much has been published on the effectiveness, toxicity and biodegradability of dispersants for dispersing oil spills on water (NRC, 1989; Vasquez and Nunez, 1988). The effect of dispersant and dispersant-oil mixtures on the growth of microorganisms also has been reported. The important issue when discussing dispersants is toxicity both of the dispersant itself and of the dispersed oil droplets (that is, the oil/disperant mixtures).

Toxicity became an important issue in the late 1960s and early 1970s when application of toxic products resulted in substantial loss of sea life. For example, the use of dispersants during the Torrey Canyon episode in Great Britain in 1968 caused massive damage to intertidal and sub-tidal life (Fingas and Punt, 2000). A version of corexit disperant used widely after the 1989 Exxon Valdez spill contain 2-butoxyethanol, a compound that is associated with headaches, vomiting and reproductive problems at high doses. According to a literature review performed by Alaska community Action on Toxics, this dispersant was later linked with health impacts in people including respiratory, nervous system, liver, kidney and blood disorders, but the National Research Council (NRC) report makes it clear that the dispersants available today are much less toxic (often one hundredth as toxic) than earlier products (Lustgarten, 2010; NRC, 2005) A key factor contributing to the toxicity of dispersants meant for environmental release is their degradability (Odokuma and Okpokwasili, 1997). Biodegradability of dispersants is absolutely crucial; otherwise, they get accumulated in the environment and make the secondary cause of water contamination. Modern-day dispersants are much less toxic to sea water than those used in the past. However, concern still exists on their possible toxic effects, on fresh and brackish water organisms, especially if dispersants are used near shore waters (Venosa and Holder, 2007). This study is designed to monitor the biodegradability of three test oil spill dispersants in brackish water ecosystems.

MATERIALS AND METHODS

Collection of water sample/dispersants

The water sample which served as the inoculum in this study was collected from Nembe water front in Borokiri area of Port Harcourt Local Government Area of Rivers State of Nigeria. Nembe water front is brackish water (mixture of fresh and marine water). It serves as a source of fishing and local transportation for the inhabitant of the area. The water sample was collected by submerging a 10 liter plastic can into the water at a particular depth and transported within 2 h to the laboratory. Samples of the oil spill dispersants used and their sources were: corexit 9527 (Shell Petroleum Development Company Ltd. Port Harcourt), Teepol (Shell Petroleum Development Company Ltd. Port Harcourt) and sodium dodeocyl sulphate (National Oil and Chemical Marketing Company, Port Harcourt).

Biodegradation monitoring

Biodegradation monitoring was set for each dispersants with the concentrations, three biodegradation flasks were setup with each flask containing 0.1 mg/l concentration of each test dispersant in 99.9 ml of the brackish water. The control flask contained only

100 ml of brackish water sample. The biodegradation of each test dispersant was determined by conducting microbiological and physicochemical analyses of the samples collected from the biodegradation flasks at day 0, 5, 10, 15 and 20.

MICROBIOLOGICAL ANALYSIS

Isolation and enumeration of total heterotrophic bacteria

Total heterotrophic bacteria for each biodegradation set up were enumerated by spread plate method (Odokuma and Okpokwasili, 1992). 0.1 ml aliquot of the 10^{-1} to 10^{-4} dilutions was transferred onto well dried sterile nutrient agar plates (in duplicate) and incubated at 37°C for 24 to 48 h. 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. Discrete colonies on the plates were then transferred into nutrient 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 fungal population in the biodegradation setup (water samples and dispersants) were enumerated and isolated by inoculating 0.1 ml aliquot of the mixture onto well-dried potato dextrose agar containing lactic acid to inhibit bacterial growth. Pure cultures of the fungi isolates were enumerated and transferred onto potato agar slants as stock cultures for preservation and identification (Odokuma and Okpokwasili, 1992).

Isolation and enumeration of dispersant utilizing bacteria

The enumeration of dispersant utilizing bacteria was performed by inoculating 0.1 ml of the dilutions into mineral salt agar plates containing the dispersants (Odokuma and Okpokwasili, 1992) and colonies were counted after 48 to 72 h incubation at room temperature. The bacterial colonies on the plates after incubation were counted and sub-cultured onto fresh mineral salt agar plates (Amanchukwu et al., 1989).

IDENTIFICATION OF BACTERIAL 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 tests used include; gram staining, motility, catalase, oxidase, citrate utilization, hydrogen sulphide production, indole production, methyl red and voges-proskauer tests. The absence or presence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscropic characteristics as well as culture characteristics were used in the identification of the fungal isolates (Cheesbrough, 2006).

Physico-chemical analysis

Parameters such as pH was determined using pH meter (Jenway 3015 method), dissolved oxygen (DO) and biochemical oxygen demand (BOD) were determined by modified winkler method (APHA, 1998), total organic carbon (TOC) by method adopted from Okalebo (1993) and chemical oxygen demand (COD) was determined by permanganate oxidation method from the biodegradation set-up on various days 0, 5, 10, 15 and 20 days.

Primary biodegradation

This was calculated from the percentage ratio of the DOC to TOC (OECD, 1992) at day 0, 5, 10, 15 and 20.

Mineralization

These were calculated from the percentage ratio of the inorganic carbon (COD) to TOC (OCED, 1992) at day 0, 5, 10, 15 and 20.

RESULTS AND DISCUSSION

The bacterial and fungal isolates obtained from different mixtures of dispersants and brackish water samples were identified to be of the following genera; *Pseudomonas* spp., *Proteus* spp., *Micrococcus* spp., *Bacillus* spp. *Rhizopus* spp, *Penicillium* spp., and *Aspergillus* spp. Figures 1, 2 and 3 show the changes in total heterotrophic bacteria, total fungal count and dispersant-utilizing bacterial counts throughout the period of study.

The results showed that the total heterotrophic and dispersant-utilizing bacterial counts from the biodegradation setups were slightly decreased compared to the control from day 0 to day 20. However, SDS dispersant supported the highest THB ($2.50x10^2$ cfu/ml), followed by teepol and corexit 9527, but teepol recorded the highest total fungal counts of $6.0x10^1$ cfu/ml on the 10^{th} day and the highest dispersant-utilizing bacteria of $1.50x10^2$ on the 15^{th} day compared with the control, followed by SDS and corexit 9527. These test dispersants showed mild increases and decreases in the total microbial population in the brackish water used as inoculum. This observation is in agreement with the reports of Okpokwasili and Nnubia (1995) that, oil spill dispersants support mild increases (stimulation) and decreases (inhibition) in the growth of specific heterotrophic marine bacteria. According to Swannell and Daniel (1999), the addition of corexit 9500 dispersants on biodegradation of crude oil stimulated the growth of dispersant degraders than hydrocarbon-degraders and supported the growth of indigenous seawater bacteria confirming that bacteria could utilize the nutrients available within the dispersants even at low concentrations. Baghbaderani et al. (2012) studied the biodegradability of three dispersants; Pars1, Pars2 and Gamlen OD4000. The study showed that, the highest growth of microorganisms was documented for either Pars1 or Pars2. Pars1 dispersants showed more degradability in the first 24 h compared to others, and has more adaptability to the aquatic ecosystem. The positive effect of dispersants 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). According to Ladhari et al. (2000), a consortium of micro- and macro-organisms called the

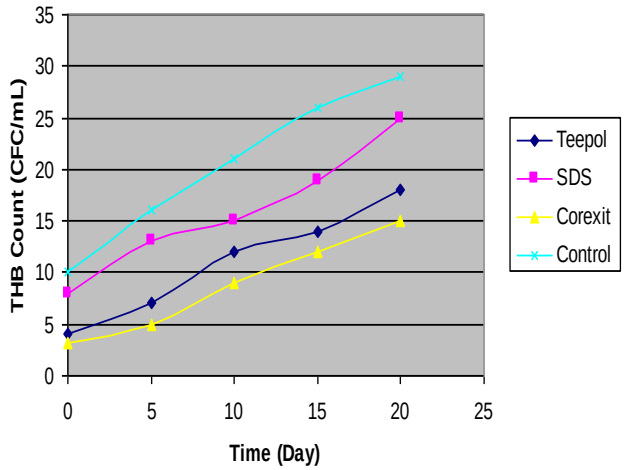

Figure 1. THB count of samples (CFU/mL).

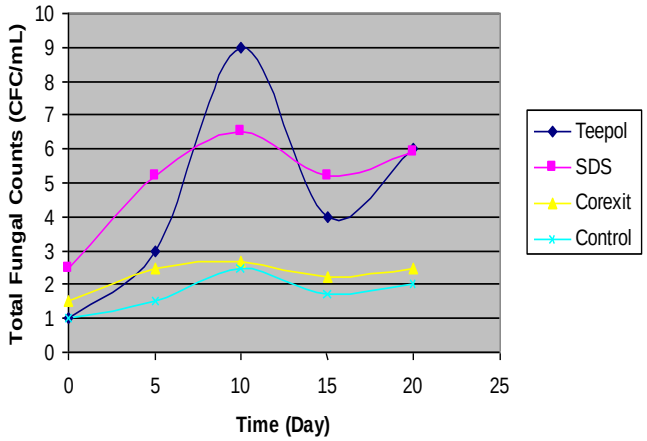

Figure 3. Total fungal counts for samples (CFU/ml).

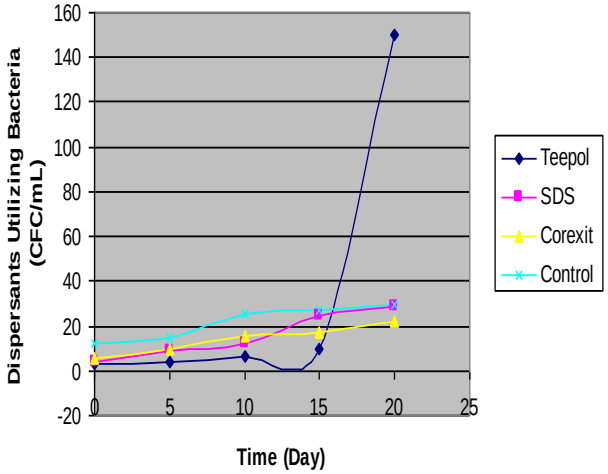

Figure 2. Dispersant utilizing bacterial (CFU/ml).

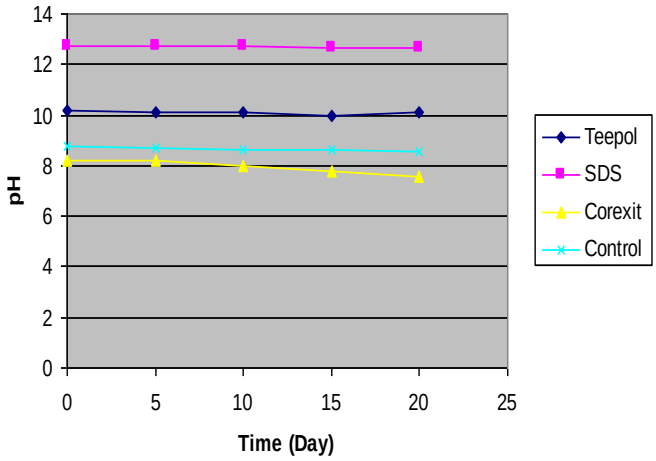

Figure 4. pH of the samples.

biomass is able to use the dispersant as food. Consequently, the polymerization degree is reduced. The most commonly identified microorganisms are heterotrophic bacteria that include genera such as *Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, and Zooglea*. These are often associated with filamentous organisms which contribute to biofloculation. The extracellular polymers produced help cells to aggregate and facilitate their attachment to solid support. The biodegradability of the dispersant is the expression with which the living organisms present in the water cause its degradation.

Biodegradation of dispersants also depends on a number of factors including pH, DO, BOD, alkalinity, TOC, DOC, NO_3, inorganic carbon, COD, PO_4 and SO_4 (Odokuma and Okpokwasili, 1992).

The results of the physicochemical analyses of the biodegradation setup are presented in Figures 4, 5, 6, 7, 8, 9 and 10. The pH of the samples in the study ranged from 7.60 to 12.78 indicating that the samples were all basic, but the biodegradation flask containing SDS recorded the highest increased in pH followed by teepol, and this could be a contributory factor to increased total bacterial counts in their biodegradation setup, since most bacteria are neutrophiles while most fungi prefer slightly acidic environment (Prescott et al., 1999). The result revealed that the dissolved oxygen in the biodegradation flasks containing the three dispersants remained constant compared with the control. This is an indication of reduced rate of biodegradation of the dispersants by the indigenous microbial population. There was decrease in BOD with increased time in the biodegradation flasks containing teepol and SDS, while BOD increased in corexit 9527 containing flask through the study. The decrease in BOD with time for teepol and SDS indicated that the concentrations of biodegradable organic carbon

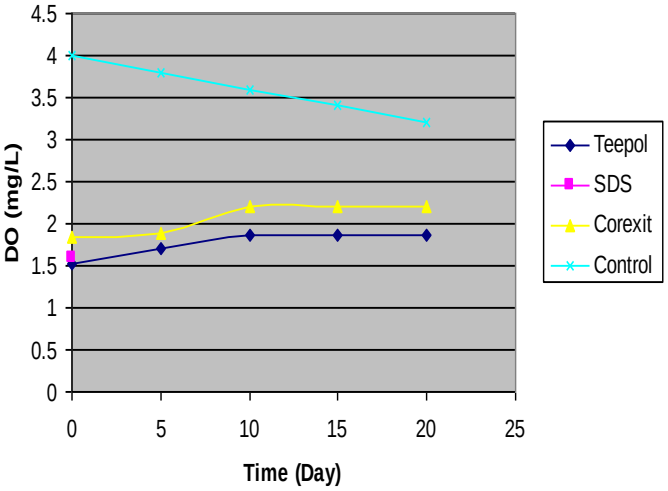

Figure 5. DO of the sample (mg/L).

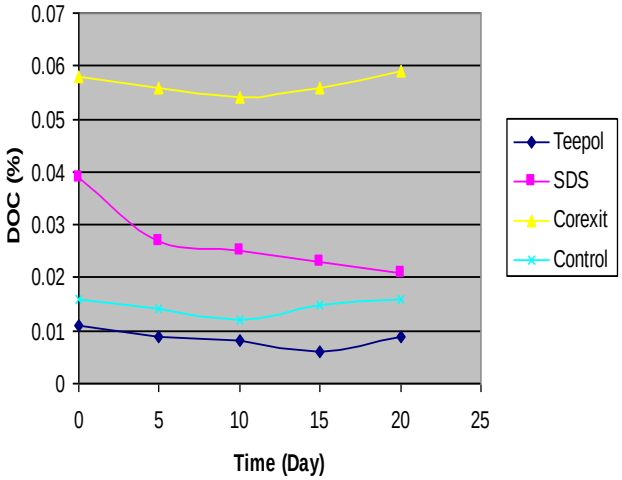

Figure 8. DOC of the sample (mg/L).

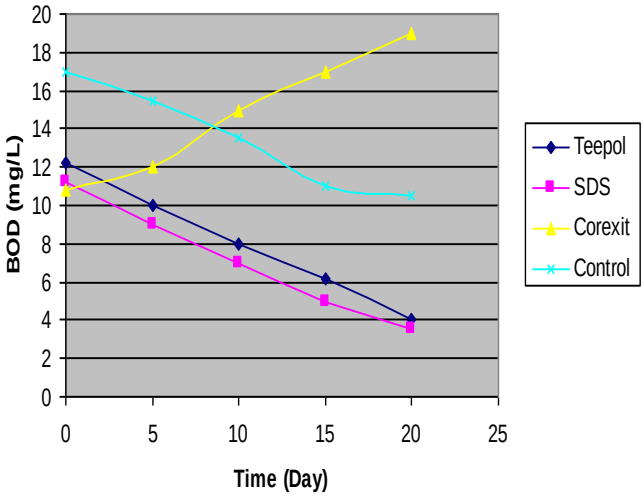

Figure 6. BOD of the sample (mg/L).

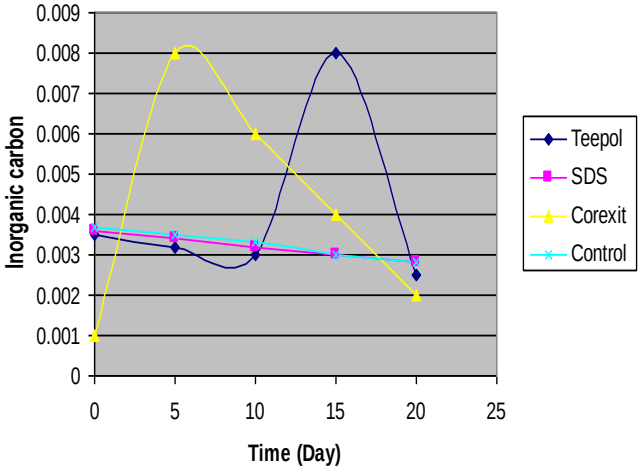

Figure 9. Inorganic carbon of the sample (mg/L).

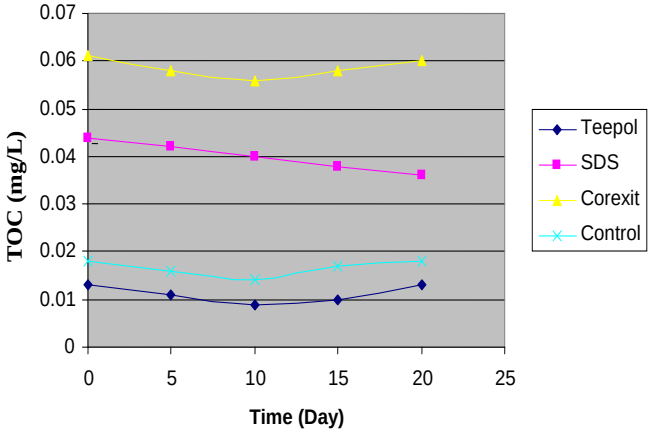

Figure 7. TOC of the Sample (mg/L).

in the monitoring were decreasing with time which could have occurred as a result of increased microbial activity in the biodegradation flasks containing these test dispersants (APHA, 1998). These test dispersants served as the sole source of organic carbon and energy for the heterotrophic bacterial and fungal population in the brackish water sample.

Both TOC and DOC decreased in SDS throughout the study period while for teepol and corexit 9527, these parameters decreased from the 15th day to the 20th day. DOC is the fraction of total organic carbon that is soluble in water. This solubility may be due to both physicochemical properties of the dispersants (compounds) and microbiological factors operating in the degradation system.

The result of the inorganic carbon analysis showed that there was a decrease in the inorganic content of the

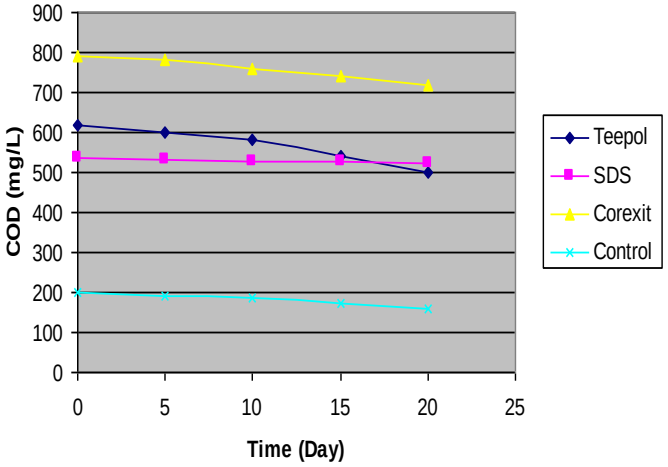

Figure 10. COD of the sample (mg/L).

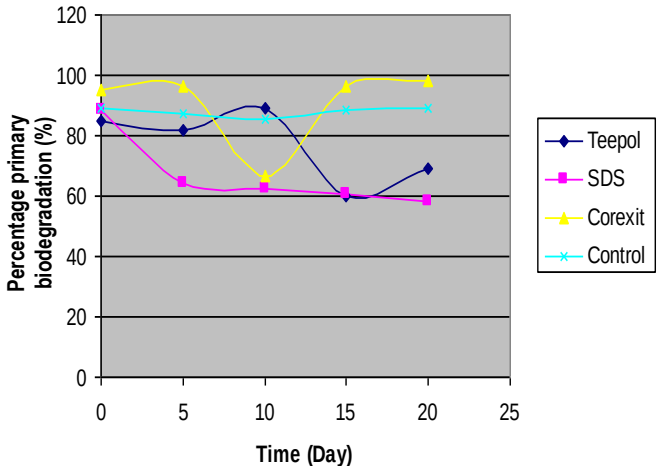

Figure 11. Percentage primary biodegradation (%).

biodegradation flasks containing the three test dispersants throughout the study. The COD of the samples decreased throughout the study with increased time in all biodegradation flasks. This agrees with the finding of Ladhari et al. (2000), who reported that COD value decreased and became stable after 30 days of biodegradation monitoring of dispersants, which can be explained to be as a result of reduction in the concentration of the dispersants due to their biodegradation. BOD and COD tests are methods for assessment of biodegradability of organic materials such as surfactants (Clesceri et al., 2005).

Primary degradation is a partial biodegradation of any compound, that is, the loss of some of its properties, for example, ability to cause foaming. Mineralization is the complete biodegradation of a compound (dispersants). It represents the ratio of inorganic carbon to total organic carbon.

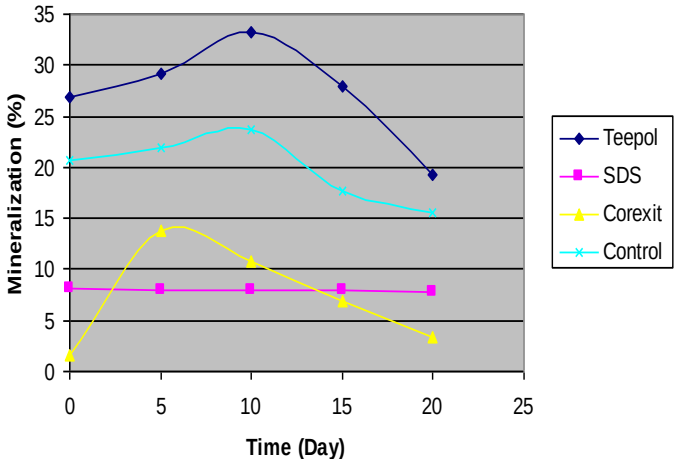

Figure 12. Mineralization of samples (%).

Figures 11 and 12 show the percentage primary biodegradation and mineralization of the samples. The result revealed that biodegradation occurred throughout the 20 days in the flasks containing the three tests dispersants. However, the biodegradation setup containing corexit 9527 dispersant showed the highest primary biodegradation (98.3%), followed by teepol (69.2%) and SDS (58.3%) at the 20th day. The percentage mineralization of the biodegradation setup containing teepol, SDS and corexit 9527 was 19.2, 7.8 and 3.3%, respectively, at the 20th day.

This study revealed that teepol was the most biodegradable of the test dispersants, followed by SDS and corexit 9527; this was due to the fact that the level of mineralization in teepol was higher than the other test dispersants in the analysis within the 20 days study period. The information that an oil spill dispersant is biodegradable in fresh water habitats may not be completely relied upon brackish water or marine environment as was earlier reported by Okpokwasili and Nwabuzor (1988). The properties of the compounds of the dispersants may therefore render them resistant to biodegradation. Corexit 9527 dispersant used in this study showed the least rate of mineralization (ultimate biodegradation) in the brackish water used for the biodegradation monitoring. This could be due to the chemical ingredients in the dispersant formulation. The fate of sorbitan oleate, glycol (polysorbate 80) and butoxyethanol in aquatic environment have been studied. It was reported that these chemicals (which are ingredients in dispersant formulations) have low potential for bioconcentration in aquatic organisms, and do not adsorb to suspended solids and sediments, they are therefore expected to biodegrade rapidly in aquatic environment (Lyman, 1990). Furthermore, studies have shown that a river-die away screen test of sodium sulfosuccinate in river water demonstrated 97.7% biodegradation of the chemical in 3 days at 12.9 ppm.

However, the aquatic fate of octyl methoxycinnamate (a component of some dispersants, comestics and plastic products) was also studied and the report showed that this chemical adsorbs to suspended solids and sediment. The estimated volatilization half-lives for a model river and model lake was 5.0 and 60 days respectively, and volatilization from water surfaces is expected to be attenuated by adsorption to suspended solids and sediment in the water column. The volatilization half-life from a model pond is about 1 year when adsorption is considered, and the potential for bioconcentration in aquatic organisms was found to be very high (Seidlova-wuttke et al., 2006).

In this study, corexit 9527 could not support the growth of THB, total fungal and dispersant-utilizing bacteria compared with the control. This could be due to the toxic effect of the chemicals used in the dispersant formulation on the microbial species in the brackish water. This is in agreement with US Environmental Protection Agency (EPA) findings (2010). The use of corexit 9527 was discontinued because it was considered toxic. US EPA reported that the corexit products used in the Gulf of Mexico spill depressed the growth of a wide range of aquatic species ranging from phytoplanktons to fish. In June 2010, the EPA reported toxicity tests of 8 dispersants (not combined with crude oil) including those used in the BP spill. The dispersants were roughly similar to one another in toxicity when tested on the EPA-standard test organisms, mysid, shrimp and silversides fish but the dispersants were also generally less toxic than oil, and they were expected to biodegrade in weeks or months rather than years as is the case for oil. As a result of the widespread public concern on the use of dispersants, the EPA and Nalco (a company that produces corexit 9500 and 9527) had both released the ingredient list for corexit 9500 which is accepted by EPA. Its constituents include butanedioic acid (a wetting agent in cosmetics), sorbitan (found in everything from baby bath to food), and petroleum distillates in varying proportions—and it decomposes almost entirely in 28 days. They reported that, all the six ingredients in the corexit 9500 formulation are also used in our day-to-day life—in mouthwash, toothpaste, ice cream, pickles, and therefore, it is believe that corexit 9500 is very safe."

According to NRC (2005), dispersant application represents a conscious decision to increase the hydrocarbon load (resulting from a spill) on one component of the ecosystem (for example, the water column) while reducing the load on another (for example, coastal wetland). Decisions to use dispersants, therefore involve trade-offs between decreasing the risk to water surface and shoreline habitats while increasing the potential risk to organisms in the water column and on the seafloor. The NRC report recommends that “Relevant state and federal agencies, industry, and appropriate international partners should develop and fund a series of focused toxicity studies to determine the mechanisms of both acute and sublethal toxicity to key organisms exposed to oil spill dispersants and dispersed oil.”

Conclusion

There is growing acceptance worldwide that use of dispersants to counter the effects of oil spill offers many advantages and can often result in a net environmental benefit when considered in relation to other response options. A major reason for this growing support and increased reliance on dispersants is the advent of improved dispersant products that are low in toxicity to marine life and more effective at dispersing heavy and weathered oils; oils previously believed to be undispersible. However, the observation from this study has revealed that ecotoxicological analysis of these dispersants should be carried out before field application since some of them are not completely biodegradable.

REFERENCES

APHA (1998). Standard Method for the Examination of Waste Water and Water. American Public Health Association. 19th Ed. Washington D.C.

Amanchukwu SC, Obafemi A, Okpokwasili GC (1989). Hydrocarbon Degradation and Utilization by Palm wine Isolates, FEMS Microbiol. Lett. 57:151-154.

Baghbaderani AZ, Emtayazjoo M, Porsafa P, Mehrabian S, Bijani S, Farkhani D, Mirmoghtadee P (2012). Effects of three types of oil dispersants on biodegradation of dispersed crude oil in water Cheesbrough M (2006). District Laboratory Practice in Tropical Countries, p. 2.

Clesceri LS, Berg AEG, Trusell R (2005). Standard Methods for the Examination of Water and Waste Water, American Public Health Association, Washington, DC, USA, 17th edition, pp. 376-410.

Fingas M, Punt M (2000). In-situ burning: A clean up technique for oil spills on water. Environment Canada, Ontario, Canda. pp. 102-114.

Fingas M, Fieldhouse B, Mullin J (1995). Water-in-oil emulsions. How they are formed and how they broken. In: Proceedings of the 1995 International Oil spill conference, Long Beach, California. pp. 829-830

Hamdan LJ, Fulmer PA (2011) Effects of Corexit EC9500 on bacteria from a beach oiled by the Deepwater horizon spill. Aquat. Microb. Ecol. 63:101-109.

Ladhari N, Hayet G, Harrabi L (2000). Study of the biodegradability of sodium salt from the condensation product of Naphthalene sulphonic acids and formaldehyde. Unit Textile Res. pp. 1-10.

Lustgarten A (2010). Chemicals meant to break up BP oil spill present new environmental concerns, ProPublica, 12:275-282.

Lyman WJ (1990). Handbook of Chemical Property Estimation Methods Washington DC: Am. Chem. Soc. 3:4-29.

Milinkovitch T, Godefroy J, Théron M, Thomas-Guyon H (2011). Toxicity of dispersant application: biomarkers responses in gills of juvenile golden grey mullet (*Liza aurata*). Environ. Poll. 159(10):2921-2928.

National Research Council (NRC) (1989). Using Oil Spill Dispersant on the sea, National Research council. National Academy Press, washington, D.C.

National Research Council (NRC) (2005). Oil spill dispersants. The National Academic Press, Washington, DC, USA.

Okpokwasili GC, Odokuma LO (1997). Response of Nitrobacter to toxicity of oilfield dispersants and domestic detergents. Trop. Freshw. Biol. 6:65-75.

Okpokwasili GC, Nnubia C (1995). Effects of oil spill dispersants and drilling fluids on substrate specificity of marine bacteria, Waste. Manage. 7:515-520.

Odokuma LO, Okpokwasili GC (1992). Role of Composition in Degradability of Oil spill Dispersants, Waste. manage. pp.590-660.

Okpokwasili GC, Nwabuzor CN (1988). Primary Biodegradation of

Anionic Surfactant in Laundry Detergents. Chemosphere 17:2175-2182.
Okpokwasili GC, Odokuma LO (1990). Effect of Salinity on Biodegradation of oil Spill Dispersant, Waste. Manage. 10:141-146.
Odokuma LO, Ibor MN (2002). Nitrogen fixing Bacteria Enhanced Bioremediation of Crude oil Polluted Soil. Global J. Pure Appl. Sci. 8(4):453-468.
Organization of Economic Cooperation and Development (OECD) (1992). Ready Biodegrability: Carbon (iv) oxide Evolution (Modified Sturm Test) Guideline for Testing of Chemicals OECD, Paris. pp. 245-400.
Pekdemir K, Copur M, Urum K (2005). Emulsification of crude oil—water systems using biosurfactants. Proc. Safety. Environ. Protect. 83:38-46.
Prescott L, Hardley JP, Klein DA (1999). Microbiology. 4th ed. Mc Graw Hill, New York.
Ron EZ, Rosenberg E (2002). Biosurfactants and oil bioremediation, Curr. Opin. Ophthal. 13:249-252.
Seidlova-wuttke D, Harry H, Christoffel J, Rimoldi G, Wuttke W (2006). Analysis of comestic products, Toxicol. Appl. Pharmacol. 20:210-246.
Swannell RPJ, Daniel F (1999). Effect of dispersants on oil biodegradation under simulated marine conditions. In: Proceedings of the 1999 International Oil spill Conference, Seattle, Washington. American Petroleum Institute, Washington, DC pp. 166-176.
US Enivronmental Protection Agency (EPA) (2010). EPA Requirement for quality assurance project plans. US Environmental Protection Agency, Office of Environmental Information, Washington DC.
Varadaraj R, Robbins ML, Bock S, MacDonald D (1995). Dispersion and Biodegradtion of oil spills on water. In: Proceedings of the 1995 International oil spill conference, American Petroleum Institute, Washington DC.
Vasquez UG, Nunez GMJ (1988). Provide title in the reference section Eur. J. Appl. Microbiol. Biotechnol. 18:n5-315.
Venosa AD, Xueqing Z (2003). Bioremediation of Crude oil contaminating marine shorelines and fresh water wetlands. Spill Sci. Technol. Bull. 8:163-178.
Venosa AD, Holder EL (2007). “Biodegradability of dispersed crude oil at two different temperatures, Mar. Poll. Bull. 54(5):545-553,
Ventikos NP, Vergetis EO, Psaraftis HN, Triantafyllou G (2004). A high—level synthesis of oil spill response equipment and countermeasures, J. Hazard. Mat. 107:51-58.
Watanabe K (2001). Microorganisms relevant to bioremediation, Curr. Opin. Biotech. 12(3):237-241.