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THE EFFECTS OF THE OIL DISPERSANT COREXIT 9527 ON BIODEGRADATION OF CRUDE OIL AROMATIC FRACTIONS

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ABSTRACT

The effects of oil spill dispersant Corexit 9527 on the rate of biodegradation of aromatic fractions benzene, toluene, and xylene (BTX) were monitored in this study. Seawater from Hartepool (Northeast United Kingdom) containing small flagellates (Bodo saltans) and gymnamoebae was spilled with the aromatic fractions, BTX, and then treated by spraying with different combination of compounds used in the formulation of the oil spill dispersant Corexit 9527 and rate of reduction in concentrations monitored for 59 days using SPME-GC/MS method with calibration graphs obtained from a new designed method. The results indicated that the oil spill dispersant Corexit 9527 did not have much effect on the natural biodegradation process of the aromatic fraction, BTX and also, Corexit 9527 was not significantly toxic to the microorganisms involved in this biodegradation processes even though the solvent, 2-butoxyl ethanol, in the formulation of Corexit 9527 only had slight negative effect.

Keywords: Benzene, Biodegradation, Corexit 9527, Dispersant, Toluene, Xylene

INTRODUCTION

Chemical method using oil spill dispersants is a frequently used method in crude oil spill response operations due to the advantages associated with its usage [1]. However, there are calls for review of dispersants usage due to their believed toxic effects on microorganisms involved in biodegradation processes of crude oil fractions [2-3]. Most of the toxic effects of these dispersants are associated with the solvents used in their formulations [4]. Corexit 9527, manufactured by Nalco, is a frequently used oil spill dispersant in such crude oil spill cleaning operations [5]. Laboratory test on Corexit 9527 and Corexit 9500 shows that their effectiveness is dependent on oil weathering state, seawater salinity and temperature [6]. Study on the effect of Corexit 9527 on microbial biodegradation of aromatics and saturates in crude oil from Prudhoe Bay oil samples from a previous study by reanalyzing using capillary gas chromatography with a sulfur-specific detector indicated inhibitory effect of Corexit 9527 on degradation of sulphur heterocycles (such as benzothiophenes and dibenzothiophenes), depended upon dispersant concentration and nutrient supplementation [7].

Of the Corexit series, Corexit 9527 has been reported to be less toxic compared to Corexit 9500 on green hydra (*Hydra viridissima*) as determined mean (SE) 96 h LC50 values for Corexit 9527 and Corexit 9500 were 230 (4.8) ppm and 160 (2.3) ppm. The 7-day no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) values based on population growth rates were <15 and 15 ppm for Corexit 9527; while 13 and 43 ppm were for Corexit 9500 [8]. All the studies on Corexit series are on the whole formulations even though solvents used in the formulations of these dispersants are reported to be the components of concern [9].

Based on the need to have simpler but effective methods for studying the role of the different compounds used in formulations of dispersants on crude oil fractions and their toxicity on microorganism, Godwin [10] designed an experimental apparatus that can be used for this type of studies using the analytical technique Solid Phase microextraction (SPME)-Gas Chromatography/Mass Spectrometry. This study used extraction, separation, identification and quantification of BTX in the different mixtures of seawater in BTX, seawater/BTX/2-butoxy ethanol and seawater/BTX/dispersants at different incubation intervals. The quantification of BTX was done using calibration graphs obtained using the designed SPME.GC/MS methodology.

This research is aimed at evaluating the effects of the dispersant Corexit 9527 formulation components on the biodegradation rate of aromatic fractions BTX in seawater. The objectives are to compare effects of this dispersants Tween 85 and Span 80 (main ingredients) and the solvent 2-butoxy ethanol in the formulation of Corexit 9527 on biodegradation of BTX and also provide an analytical method for carrying out such evaluation in related studies.

MATERIALS AND METHOD

All SPME fibres and holders were supplied by *SUPELCO*. *Fluka Chemical* supplied SPAN 80. *Aldrich* supplied TWEEN 85, Toluene, and 2-Butoxy ethanol. *Fissions Chemicals* manufactured sodium chloride, potassium chloride, calcium sulphate, magnesium sulphate, calcium carbonate, and magnesium chloride used. *Fishers Ltd* manufactured chlorobenzene used. All of the manufacturers are in the United Kingdom. *Thirty six* (36) constructed experimental vessels. Composition of *Corexit 9527* (Tween 85, Span 80, and sodium di-isooctyl sulphosuccinate (*Aerosol OT*), with solvent 2-butoxyethanol were supplied by *Ivar Singaas* of SINTEF.

Instrument: Varian Saturn 3400CX GC-MS (Quadrupole Ion Trap), Column: SP-2330^{TM}, 30 m by 0.32 mm, and 0.20 um film. Injector at constant temperature of 190 °C and operated in Split mode.

A litre of artificial seawater water containing 29.42 g sodium chloride, 0.5 g potassium chloride, 3.22 g magnesium chloride, 0.56 g sodium bromide, 1.36 g calcium sulphate, 2.40 g magnesium sulphate and 0.11 g calcium carbonate was prepared based on the composition of the seawater at Naples [11]. The artificial water, 0.5 L, was mixed with 1 litre of seawater from Hartepool (North east of England) to get the sample seawater for the study. Bioassay was done to ascertain types of microorganisms in the seawater.

Thirty-six (36) constructed experimental vessels were labeled as follows: Control Benzene/Artificial Seawater, Benzene/Seawater (Replicate 1, 2, 3), Benzene/Seawater/Solvent (1, 2, and 3) Benzene/Seawater/Dispersants (1, 2, and 3). The same was done for toluene and xylene. Exactly 40 µL/L of the analytes in seawater was put in the experimental vessels as labeled. The vessels labeled Analytes/Seawater/Solvent was sprayed with 200 µL/L of 2-butoxy ethanol and those labeled Analytes/Seawater/Dispersants were sprayed with 200 µL/L of 10 µL/mL of surfactants (Tween 85 and Span 80) in 2-butoxy ethanol. The vessels were then pressurized with a manual pump and the aqueous phase collected in a sample vial. 1 mL internal standard from a prepared solution of 2.5 mL/L of chlorobenzene was added to vial and shaken properly. These vials were then preconcentrated with a 7 µm polydimethylsiloxane coated fibre. For each run the SPME was removed after 5 minutes and cleaned in GC injector pot at 220 °C for 10 minutes. The column was baked out with a baking out programme and always checked to be clean before samples are analyzed to eliminate problem of interference of carryover components in column from other analysis done.

The calibration graphs from aqueous phases analysis in the designed method was used to calculate the concentrations of benzene, Toluene and xylene after 11 days and 59 days. The results were statistically analysed using p-test for significant differences [12].

RESULTS AND DISCUSSION

The results from the bioassay showed that the seawater had small flagellates (*Bodo saltans*) and gymnamoebae (*small naked amoebae*). Although, other amoebas and protozoa, which are predators of bacteria, were seen, their real identity was not observed. This indicated that their population was small in the sample seawater.

The concentrations of benzene, toluene and xylene in day 1, 11 and 59 and statistical analysis of the results are shown in Tables 1-6, while Figures 1-3 are bar charts showing percentage of benzene, toluene and xylene lost after 11 and 59 days.

Table 1: Results of Concentration of Benzene from Day 1 – Day 59 in the different solutions

Sample	Benzene concentration (µL/L) (Mean ± Standard Deviation of Mean)		
	Day 1	Day 11	Day 56
Benzene/Artificial Seawater	39.46 ± 0.02	39.08 ± 0.51	39.05 ± 0.02
Benzene/Seawater	39.87 ± 0.10	0.48 ± 0.00	0.00
Benzene/Seawater/Solvent	39.94 ± 0.25	1.79 ± 0.09	0.17 ± 0.02
Benzene/Seawater/Dispersants	39.92 ± 0.20	0.18 ± 0.02	0.12 ± 0.02

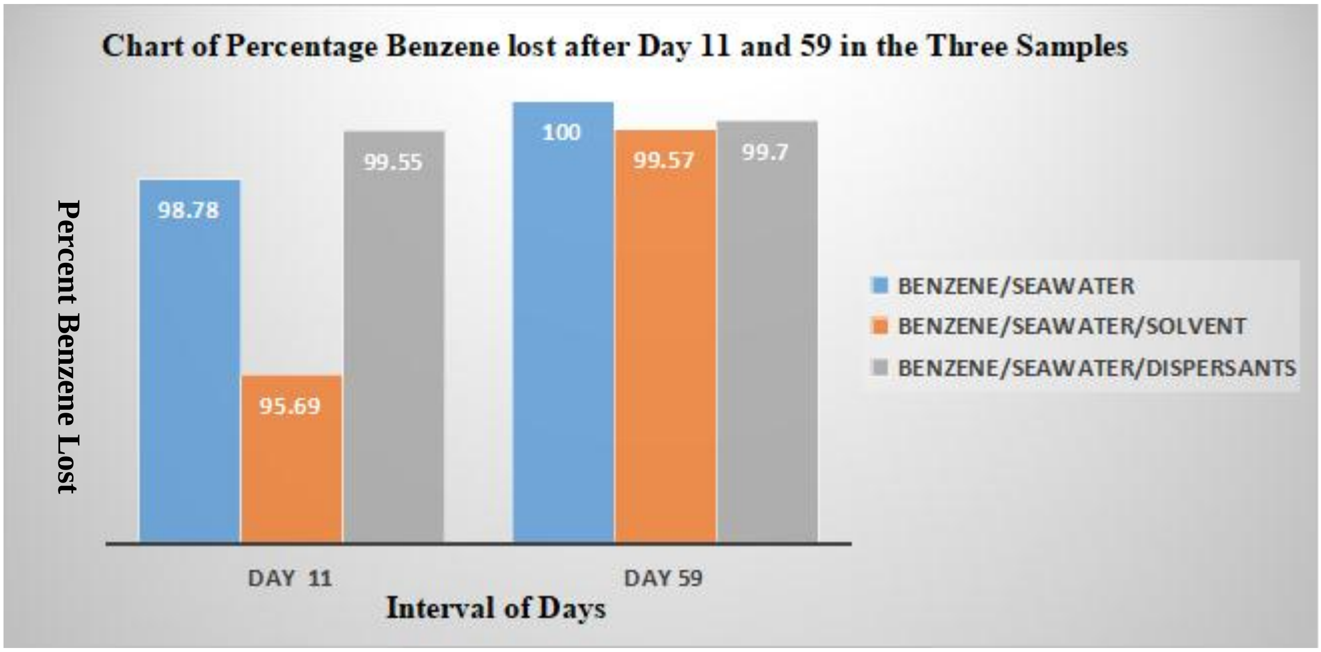

Figure 1: Percentage of benzene lost at intervals in the three samples

Table 2: statistical results for benzene showing p values

	DAY 1 BS	DAY 11 BS	DAY 56 BS	DAY 1 BSS	DAY 11 BSS	DAY 56 BSS	DAY 1 BSD	DAY 11 BSD	DAY 56 BSD
DAY 1 BS		0.000143	0.000143	1	0.000143	0.000143	1	0.000143	0.000143
DAY 11 BS	382.6		0.1001	0.000143	0.000143	0.6382	0.000143	0.6807	0.4136
DAY 56 BS	387.2	4.628		0.000143	0.000143	0.9849	0.000143	0.9771	0.9993
DAY 1 BSS	0.6474	383.2	387.8		0.000143	0.000143	1	0.000143	0.000143
DAY 11 BSS	369.8	12.72	17.35	370.5		0.000143	0.000143	0.000143	0.000143
DAY 56 BSS	385.5	2.946	1.683	386.2	15.67		0.000143	1	1
DAY 1 BSD	0.4855	383.1	387.7	0.1618	370.3	386		0.000143	0.000143
DAY 11 BSD	385.4	2.848	1.78	386.1	15.57	0.09711	385.9		1
DAY 56 BSD	386	3.463	1.165	386.7	16.18	0.5179	386.5	0.615	

Significantly different when $p < 0.05$ and shown in red and Not significantly different when $p > 0.05$.

From Figure 1, the percentage of benzene lost after 11 days was in the order BSD>BS>BSS, showing dispersants were playing a significant role in the availability of benzene to microorganisms for biodegradation. The Day 59 results indicate however, that it is possible for total biodegradation without any enhancement as percentage of benzene lost was in the order BS>BSD>BSS. The percentage of benzene lost in BSS mixture was above 95% from Day 11. This might indicate that the solvent did not impair the biodegradation of benzene but statistically Table 2 shows that even though benzene/seawater day 11 result is not significantly different from that for benzene/seawater/dispersants with $p = 0.6807$, the result is significantly different from that for benzene/seawater/solvent with $p = 1.43 \times 10^{-4}$. Day 56 results show no significant differences between the groups with all $p > 0.05$. These results indicated that the solvent 2-butoxyl ethanol had some negative effect on the biodegradation of benzene when applied alone but its' effect was not significantly negative in the formulation with the dispersants in Corexit 9527 and the negative effect is lost with time as shown in day 56 results.

Table 3: Results of Concentration of Toluene from Day1 – Day 59 in the different solutions

Sample	Toluene Concentrations µL/L (Mean ± Standard Deviation of Mean)		
	Day 1	Day 11	Day 59
Toluene/Artificial Seawater	39.14 ± 0.14	39.21 ± 0.43	38.92 ± 0.90
Toluene/Seawater	39.64 ± 0.13	0.11 ± 0.02	0.00
Toluene/Seawater/Solvent	39.87 ± 0.07	0.30 ± 0.02	0.03 ± 0.00
Toluene/Seawater/Dispersants	39.02 ± 0.21	0.24 ± 0.00	0.00

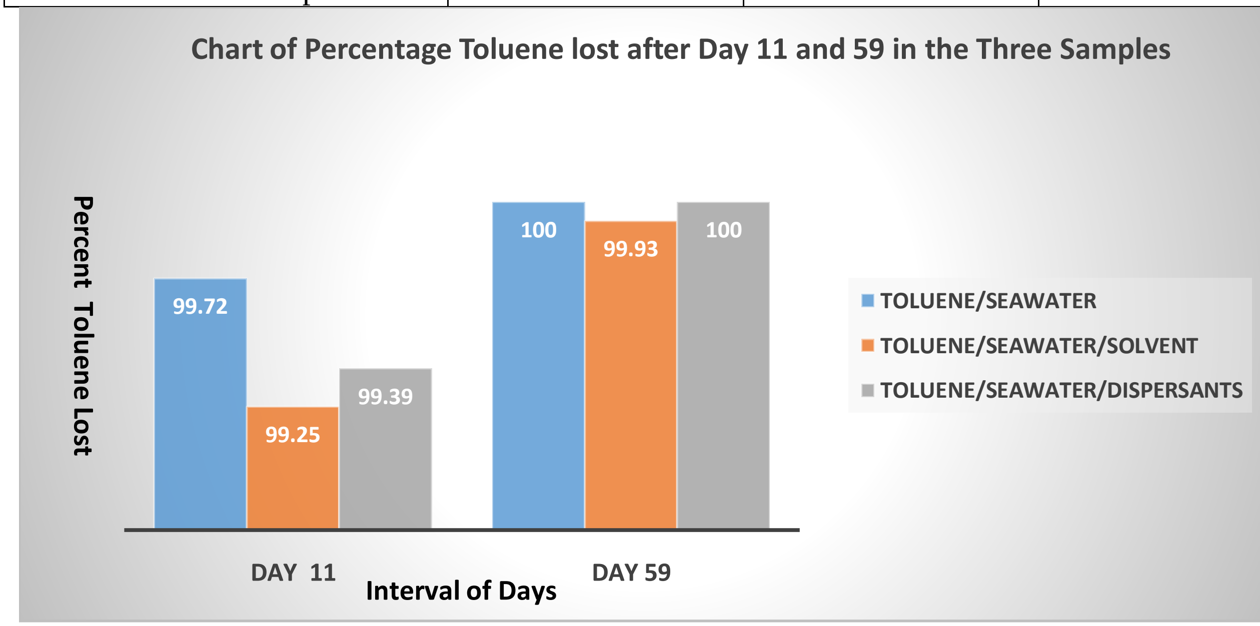

Figure 2: Percentage of toluene lost at intervals in the three samples

Table 4: Statistical Results for Toluene showing p values

	DAY 1 TS	DAY 11 TS	DAY 59 TS	DAY 1 TSS	DAY 11 TSS	DAY 59 TSS	DAY 1 TSD	DAY 11 TSD	DAY 59 TSD
DAY 1 TS		0.000143	0.000143	0.9952	0.000143	0.000143	0.2173	0.000143	0.000143
DAY 11 TS	257.9		1	0.000143	0.9984	1	0.000143	1	1
DAY 59 TS	258.6	0.6957		0.000143	0.9526	1	0.000143	0.9922	1
DAY 1 TSS	1.457	259.3	260		0.000143	0.000143	0.02682	0.000143	0.000143
DAY 11 TSS	256.6	1.283	1.979	258.1		0.9768	0.000143	1	0.9526
DAY 59 TSS	258.4	0.5001	0.1957	259.8	1.783		0.000143	0.9975	1
DAY 1 TSD	4.044	253.8	254.5	5.501	252.6	254.3		0.000143	0.000143
DAY 11 TSD	257	0.848	1.544	258.5	0.4349	1.348	253		0.9922
DAY 59 TSD	258.6	0.6957	0	260	1.979	0.1957	254.5	1.544	

Significantly different when $p < 0.05$ and shown in red and Not significantly different when $p > 0.05$

Table 5: Results of concentration of xylene from Day 1 – Day 59 in the different solutions

Sample	Xylene Concentration µL/L (Mean ± Standard Deviation of Mean)		
	Day 1	Day 11	Day 59
Xylene/Artificial Water	39.56 ± 0.24	40.11 ± 0.24	39.45 ± 0.04
Xylene/Seawater	39.46 ± 0.24	1.22 ± 0.10	0.00
Xylene/Seawater/Solvent	39.36 ± 0.03	3.40 ± 0.38	0.029 ± 0.00
Xylene/Seawater/Dispersants	39.35 ± 0.36	1.28 ± 0.067	0.027 ± 0.00

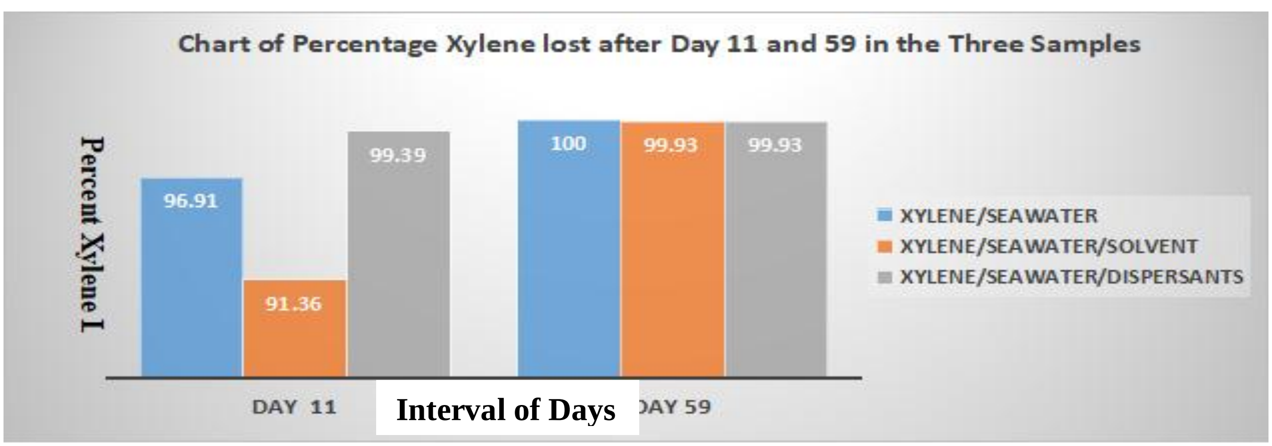

Figure 3: Percentage of xylene lost at intervals in the three samples

The results of percentage toluene lost by Day 11 from Figure 2 showed that toluene lost in Toluene/Seawater was highest, although percentage of toluene lost in TSD was higher than that for the TSS. Day 59 results had above 99% for all three mixtures showing again that the solvent did not affect biodegradation in seawater. The results of percentage of xylene lost by Day 11 and Day 59 were similar with those obtained for toluene. The statistical analysis for toluene results on Table 4 show that there was no significant differences between the three parameters as p values were all > 0.05. However, statistical analysis for xylene results shown in Table 6 show results were similar to those for benzene with Day 11 xylene lost for Xylene Seawater significantly different from that for Day 11 xylene lost in Xylene/Seawater/Solvent with $p = 1.43 \times 10^{-4}$ and Day 11 xylene lost for Xylene Seawater not significantly different from that for Day 11 xylene lost in Xylene/Seawater/Dispersant with p = 0.997. Xylene/Seawater/Dispersant result was also significantly different from that for Xylene/Seawater/Solvent with $p = 1.43 \times 10^{-4}$ also confirming the negative effect of the solvent 2-butoxyl ethanol when alone on the biodegradation of xylene.

Table 6: Statistical results for xylene showing p values

	DAY 1 XS	DAY 11 XS	DAY 59 XS	DAY 1 XSS	DAY 11 XSS	DAY 59 XSS	DAY 1 XSD	DAY 11 XSD	DAY 59 XSD
DAY 1 XS		0.000143	0.000143	0.9493	0.000143	0.000143	0.000143	0.000143	0.000143
DAY 11 XS	791		0.000143	0.000143	0.000143			0.997	0.000143
DAY 59 XS	816.2	25.17		0.000143	0.000143	1	0.000143	0.000143	1
DAY 1 XSS	2	789	814.2			0.0001429	0.000143	0.000143	0.000143
DAY 11 XSS	745.9	45.09	70.26	743.9		0.0001429	0.000143	0.000143	0.000143
DAY 59 XSS	815.6	24.62	0.5515	813.6	69.71		0.000143	0.000143	1
DAY 1 XSD	188.4	602.6	627.8	186.4	557.5	627.2		0.000143	0.000143
DAY 11 XSD	789.6	1.379	26.55	787.6	43.71	25.99	601.2		0.000143
DAY 59 XSD	815.6	24.61	0.5584	813.6	69.7	0.006895	627.2	25.99	

Significantly different when $p < 0.05$ and shown in red and Not significantly different > 0.05

The results for benzene, toluene and xylene with percentage lost > 99% for benzene and toluene and 96,73% for xylene show the surfactants (Tween 85 and Span 80 minimize the volatilities of monocyclic aromatic compounds especially benzene and toluene and enhance solubilization as demonstrated in previous studies [13-14]. The results also demonstrated that the solvent 2-butoxy ethanol did not exhibit a very toxic effect on the biodegrading microorganisms in the seawater to limit biodegradation activities to a high level as all samples containing BTX/Seawater/Solvent had above 91% loss of starting analytes from Day 1 to Day 11. This indicates 2-butoxy ethanol enhance solubilization of both surfactants and BTX but did not exert a strong diauxic effect associated with ethanol during BTX biodegradation as reported [14-15]. All results apart from Benzene Day 11 results had higher percentage loss of starting Analytes in Analyte/Seawater samples. This indicates that natural biodegradation processes are better in environment with the presence of microorganisms that can degrade the oil components causing contamination and as such bioremediation is usually recommended for very sensitive ecosystems [16].

Bodo Saltans seen in the bioassay are of similar size and abundance as the flagellate *Heteromita globosa,* which had been demonstrated to increase biodegradation rate of toluene by > 7.5 times as a result of it's grazing on toluene degrading bacteria [17]. Although BTX components have similar chemical structure, isolated strains of bacteria show different substrate ranges and no one strain has been shown to degrade all of BTX components [18]. However, in this study BTX were all degraded to a very large extent by the microorganisms even in the presence of 2-butoxy ethanol and nonionic surfactants (Tween 85 and Span 80). These results suggest that the flagellate *Bodo saltans* and other microorganisms found close to them should be studied for their prospects in bioremediation techniques.

CONCLUSIONS

The method, SPME/GC-MS, can be used for testing of dispersants formulations for their effects on biodegradation of crude fractions. The dispersant, Corexit 9527, did not show significant negative effects on the biodegradation of benzene, toluene and xylene. The solvent 2-butoxyl ethanol in Corexit 9527 did not show significant toxic effect in most cases. Natural biodegradation in the absence of the dispersants formulation components was relatively high and as such should be recommended for very sensitive ecosystems.

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REFERENCES

1. Lessard, R.R., & Demarco, G. (2000). The Significance of Oil Spill Dispersants, *Spill Science & Technology Bulletin*, 6, 59 - 68.
2. Epstein, N., Bak, R.P.M., & Rinkevich, J. (2000). Toxicity of Third Generation Dispersants and Dispersed Egyptian Crude Oil on Red Sea Coral Larvae, *Marine Pollution Bulletin,* 40 (6), 497-503.
3. Puremba K., Gumkel W., Lang S., & Wagner F. (1991). Toxicity testing of synthetic and biogenic surfactants on marine microorganisms, *Environmental Toxicity and Water Quality', An International Journal*, 6, 157 - 163.
4. Kingham, J.D. (1983). Oil spill chemicals: Environmental implications and use policy. *In The Petroleum Industry and the Nigerian Environment Proceedings of the 1983 International Seminar*, Nigerian National Petroleum Corporation, Lagos, 179 - 184.
5. George-Ares, A. & Clark, J.R. (2000). Aquatic Toxicity of Two Corexit Dispersants, Chemosphere, 40 (8), 897-906.
6. Moles, A., Holland, L., & Short, J.W. (2002). Effectiveness in the Laboratory of Corexit 9527 and 9500 in Dispersing Fresh, Weathered, and Emulsion of Alaska North Slope Crude Oil under Subarctic Conditions, *Spill Science & Technology Bulletin 7(5), 241-247.*
7. Foght, J.W., Fedorak, P.M., & Westlake, D.W.S. (2011). Effect of the dispersant Corexit 9527 on the microbial degradation of sulfur heterocycles in Prudhoe Bay oil, *Canadian Journal of Microbiology*, 29(5),623-627.
8. Mitchell, F.M., & Holdway, D.A. (2000). The acute and chronic toxicity of the dispersants Corexit 9527 and 9500, water accommodated fraction (WAF) of crude oil, and dispersant enhanced WAF (DEWAF) to Hydra viridissima (green hydra), *Water Research*, 34(1),343-348.
9. Song, D., Liang, S., Zhang, Q., Wang, J., & Yan, L. (2013). Development of High Efficient and Low Toxic Oil Spill Dispersants based on Sorbitol Derivants Nonionic Surfactants and Glycolipid Biosurfactants, *Journal of Environmental Protection*, 4(1B), 16-22.
10. Godwin, J. (2008). An experimental apparatus for biodegradation of fraction of crude oil studies, *International Journal of Chemical Sciences*, 1(1), 156-162.
11. Mc Kean, R.C., & Ivimey Cook, W.R. (*1973), Plant Science Formulae*. 1st Edition. Macmillan and Company Limited, St. Martin's Street, London, 45 - 46.
12. Sprinthall, R. C. (2011). Basic statistical Analysis. Ninth Edition, Allyn and Bacon Inc, Boston, United States. 183-213.

13. Doong, R.A., Lei, W.G., Chen, T.F., Lee, C.Y., Chen, J.H., & Chang, W.H. (1996). Effects of Anionic and Nonionic Surfactants on Sorption and Micell Solubilization of Monocyclic Aromatic compounds, *Water Science and Technology*, 34 (7-8), 327 – 334.
14. Corseuil, H.X., Hunt, C.S., Dos Santos, R.C.F., & Alvarez, P.J.J. (1998). Influence of the Gasoline Oxygenate Ethanol on Aerobic and Anaerobic BTX Biodegradation, *Water Research*, 32 (7), 2065 – 2072.
15. Corseuil, H.X., &Alvarez, P.J.J. (1996). Natural Bioremediation Perspective for BTX-Contaminated Groundwater in Brazil: Effect of Ethanol, *Water Science and Technology*, 34 (7-8), 311 – 318.
16. Atlas, R.M., & Hazen, T.C, (2011). Oil Biodegradation and Bioremediation: A tale of the two worst spills in U.S. history, *Environmental Science Technology*. 45(16), 6709-6715.
17. Mattison, R.G. & Harayama, S. (2001). The Predatory Spoil Flagellate *Heteromita Globosa* Stimulates Toluene Biodegradation by a *Pseudomonas Sp*., *Fems Microbiology Letters*; 194 (1), 39 – 45.
18. Lee, C.Y., & Lin, C.H. (2006). Bacterial growth and substrate degradation by BTX-oxidizing culture in response to salt stress, *Journal of Industrial Microbiology and Biotechnology*, 33, 37 – 44.