

Biodegradation Potential of Paraffin and Olefin Synthetic Based Drilling Mud Base Fluids under Microaerophilic and Anaerobic Conditions

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Abstract: Biodegradation rates of synthetic Paraffins and Olefins used for drilling oil and gas wells were determined over a period of 120 days at various depths of the Gulf of Guinea sediments with the aim of establishing the exact roles strict anaerobic and microaerophilic bacteria play in the degradation of these substrates within the subsurface sediment. The direct approach for biodegradability tests used was the measurement of substrate disappearance by means of gas chromatography while redox potential measurements were used to predict the extent of the sediment aerobiosis or anaerobiosis as the case may be. We observed that about 80-90% of the original synthetic Paraffins and Olefins that were used to spike the sediment were degraded within the 5cm depth zone mostly by the aerobic and microaerophilic bacteria. Very negligible concentration of the residual SBFs were able to diffuse into the strict anaerobic zone (15cm depth) where partial degradation were carried out by the resident strict anaerobes. Linear and Internal Olefins showed higher biodegradation rate than synthetic Paraffins. From the available data, it can be advanced that the microaerophilic bacteria were more active and relevant than the strict anaerobes in the degradation of synthetic Paraffins and Olefins in the subsurface sediment. [Okoro Chuma. Conlette. Biodegradation Potential of Paraffin and Olefin Synthetic Based Drilling Mud Base Fluids under Microaerophilic and Anaerobic Conditions. Nature and Science 2011;9(7):81-88]. (ISSN: 1545-0740).

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Introduction:

Drillers use specialised drilling fluids referred to as muds when drilling exploration and production oil and gas wells to help maintain well control and to remove drill cuttings from the drill hole (Burke and Veil, 1995). Generally either oil based muds (OBMs) or water based muds (WBM) have been used for drilling offshore wells. Recently, in response to the current global environmental challenges in addition to strict international and local regulations on drilling waste discharge requirements, the drilling industry has developed several types of synthetic based muds (SBMs) or synthetic based fluids (SBFs) that combine the desirable operating qualities of OBMs with the lower toxicity and environmental impact qualities of WBMs (Burke and Veil, 1995, Cobby and Craddock, 1999). In addition, SBFs have the potential to drill wells more efficiently and quickly than the WBMs while also avoiding some of the disposal costs and environmental difficulties associated with the OBMs. So SBFs have the practical and economic advantages over WBMs for deep and directional drilling where torque and drag problems exist (Blanchet *et al*, 1998).

The SBFs include Linear Alpha Olefins, Internal Olefins, Synthetic Paraffins and Esters. These fluids are environment friendly and they provide lubricity, stability at high temperature and

well bore stability (American Chemistry Council, 2006). Cuttings generated while drilling with SBFs can be discharged into the marine environment without any harm to the marine ecosystem (Cognis, 2000).

In the present study, we dealt only with Synthetic Paraffins and Olefins because Ester based synthetic fluids have already been dealt with in our previous studies.

Paraffins consist of a broad class of compounds that have the general formula C_nH_{2n+2} where "n" is the number of carbon atoms which are joined by single bonds. Paraffins can be categorised as normal meaning that they are linear, iso meaning that they are branched and cyclo meaning that they consist of ring structures (American chemical council, 2006). Olefins are similar to Paraffins but contain at least two fewer hydrogen atoms providing at least one double bond between adjacent carbon atoms. Olefins with one double bond have the general formula C_nH_{2n} (American Chemistry Council, 2006).

Numerous studies on petroleum biodegradation in marine environments, sediments and soil demonstrate that organic ingredients in oily cuttings are biodegradable under aerobic and anaerobic conditions (Kjeilen, 1997, Roberts and Nguyen, 2006). In the floor of the sediments for instance the Gulf of Mexico, it was observed that the

average oxygen concentration is 6.8mg/L (0.21nm) and oxygen only diffuses a few centimetres into the sediment, an indication that oxygen availability can be limiting in deep offshore sediments (Robert and Nguyen, 2006.). In anaerobic environment, oxygen is absent and as a result of that, anaerobic microorganisms use alternative electron acceptors such as Nitrate, Sulphate and carbon dioxide if available (Simon *et al.*, 1999) but the question is, does complete anaerobic condition exists where these fluids are deposited in the sediment? Or do we have very low concentrations of oxygen in these sediments? This has been one of the issues various researchers in this area have not been able to properly address. Herman and Roberts (2005) have carried out some studies on anaerobic degradation of Esters in the sediment using the production of gas and methane from the sediment to monitor the progress of biodegradation and anaerobic degradation of esters was complete after 90 days of incubation. This work however did not demonstrate in practical terms whether the ester based fluids were capable of penetrating the anaerobic zones of the sediment where the said degradation occurred and also whether complete anaerobic condition was possible at the sediment zone where the ester based fluids were deposited. In another related study, Roberts and Nguyen, 2006 observed that SBF are poorly soluble in water and as such, their rate of diffusion in the sediment was minimal, their work on anaerobic degradation of the ester based fluids in the sediment did not still clarify the issue of whether complete anaerobic condition existed at the depths where the ester based fluids were located.

The present study was therefore aimed at achieving the following;

- a. To determine the rate and extent of diffusion of the Paraffin and Olefin synthetic based fluids in the sediment
- b. Using redox potential measurements to monitor the level of oxygen at various depths of the sediment where the Paraffin and Olefin synthetic based fluids were found.
- c. Isolating various groups of organisms that participated in the degradation of Paraffin and Olefin synthetic based fluids at various depths of the sediment especially the microaerophilic and the anaerobic types.
- d. Further clarifying the issues of strict anaerobic degradation in the sediment by distinguishing the activities of microaerophylic microorganisms, facultative anaerobic and obligate anaerobic

microorganisms, This issue have not been properly addressed by past investigators

The major objective of the present study however is to establish in practical terms the individual roles of microaerophylic microorganisms (that thrive under very low oxygen concentration), the facultative anaerobic microorganisms (that can survive with or without oxygen) and the obligate anaerobes (that survives without oxygen) in the degradation of Paraffin and Olefin synthetic based drilling fluids deposited at various depths in the Gulf of Guinea sediments.

2. Materials and Methods:

Experimental Design:

The experimental test set up consists of a series of 3 easily assessable rectangular shaped glass indoor basins called benthic chambers measuring approximately 25x30cm. Each of the glass containers was filled with the wet sediment up to 20cm depth, the moisture content ranged between 35 and 55%. The sediments were collected from Escravos river (Located within the Gulf of Guinea). 150mls of each of the representative SBFs sample were used to spike the sediments in the respective containers. The sediment/fluid mixture was mixed thoroughly within the 5cm depth by manual means using a metallic mixer. The experimental set up was allowed to settle for about 6hrs before the collection of the first sediment sample at day 0. The experiment was monitored for a period of 120 days and at each 30-day interval, sediment samples were collected and analysed for residual SBFs and microorganisms capable of utilizing the SBFs as the sole carbon source. The entire set up was similar to the simulated sea bed experiment conducted by OGP (2003). The sediment samples were labelled as follows; 1.SE-SBF-LO, 2. SE-SBF-SP, and 3. SE-SBF-IO, depending on the type of SBF added to the sediment.

Description of the Synthetic-based fluids (SBF) used for the study.

The SBF samples which were collected from the Nigerian Department of Petroleum Resources (DPR) were coded and have the following descriptions. 1. SBF-LO (Linear Olefins), 2. SBF-SP (Synthetic Paraffin) and 3. SBF-IO (Internal Olefins).

Microbiological and Physicochemical Analysis of the Sediment samples.

Enumeration of microorganisms capable of utilizing the SBF

Hydrocarbon utilizing bacterial counts were obtained by plating out at low dilutions $10^{-1} - 10^{-3}$ of samples on mineral salt medium of *Mills et al* (1978). The composition of the medium in (g/L) is as follows NaCl (10), $MgSO_4 \cdot 7H_2O$ (0.42), KCl (0.29), KH_2PO_4 (0.83), Na_2HPO_4 (1.25), $NaNO_3$ (0.42), Agar bacteriological (15), distilled water (1000 ml), and 0.5mls of the representative SBF sample. The medium was autoclaved at 1.1 kg/cm^2 for 15 mins. and after inoculation with the sample, was incubated at 28°C for 4 days in a candle Jar for microaerophiles and anaerobic incubator for obligate anaerobes.

Enumeration of Microaerophilic bacteria

The candle jar method described by Cheesbrough (1992) was used. 0.1mls of the inoculum from appropriate dilutions were introduced into sterile petri dishes and covered with 10mls of bacto anaerobic agar (Difco), the solution was mixed properly and the agar was allowed to set and this was followed by incubation in a candle jar for 48hrs at 28°C . A white smokeless candle was used and as the candle burns, the oxygen concentration was reduced leaving a carbon dioxide content of about 3-5% by the time the candle was extinguished.

Isolation of Obligate Anaerobes

The method used in isolation of Obligate anaerobes was as described by Cheesbrough (1992). 0.1mls of appropriate dilutions were introduced into sterile petridishes which was immediately covered with 10mls of bacto anaerobic agar (Difco), the solution was mixed properly and the agar was allowed to set. This was followed by incubation in an anaerobic incubator for 4-10 days incorporating both chemical and biological indicators as described in Cheesbrough (1992).

Determination of the Sediment Redox Potential and pH

The sediment redox potential at various depths were measured with bright platinum electrodes and a calomel reference electrode. Readings were taken with a portable pH/mV digital meter and the potential of the calomel reference electrode (+224mV) were added to each value to calculate the Eh as described in Patrick *et al* (1996).

Estimation of Background Nutrient Concentration of the sediment

Interstitial water samples were withdrawn with a simple apparatus as described in McKee *et al*, 1988.

The collected interstitial water was filtered and inorganic nutrients such as Phosphorus and Potassium were analysed with ICP (Inductively coupled argon plasma emission spectrometer) as described in Eaton *et al*, 1995). Ammonium-Nitrogen was analysed with auto analyser as described in Eaton *et al*, 1995.

Moisture content:

The moisture content of the sediment was measured by simple gravimetric analysis. 10grams of the sample containing water was dried in the oven at a temperature of 200°C after which, the sample was measured again and the difference in weight is the moisture content as previously described (Eaton *et al*, 1995).

Solvent extraction of Residual Hydrocarbon

One gram of the sample was introduced into a separating funnel containing 50mls of Methylene chloride, this was followed by vigorous shaking for 10mins and filtration using Watman no.1 filter paper as previously described (Eaton *et al*, 1995) and the filtrate was collected in a clean conical flask.

Gas Chromatography of Hydrocarbons in the Sediment

Degraded hydrocarbon were analyzed by gas chromatography using Hewlett Packard 5890 series 11 Gas chromatograph equipped with single flame ionization detector (FID) fitted with Perkin Elmer Nelson analog digital converter (900 series) and a Compaq deskpro computer. A J and W scientific DB-1 capillary column of 15 m length and an internal diameter of 0.32 mm wide bore of 1micron film thickness were used. A temperature program of $50-305^\circ\text{C}$ increasing at 3.5°C per minute for 27.15min was employed. Hydrogen with a flow rate of 2ml per min was used as a carrier gas while the flow rate of air was 400ml per min. The detector temperature was 325°C while the injection port temperature was 305°C . 1 ml of the residual organic carbon extract was dissolved in methylene chloride at the ratio of 1:1 and a sample volume of $0.2 \mu\text{l}$ was injected into the GC.

Identification Microorganisms capable of utilizing the SBFs in the sediment

Cultural characteristics of the various bacterial cultures were noted in the selective media used, this was followed by staining of bacterial cultures using Gram staining procedure and final identification was done using a computerized BBL Enterotube

identification test kits, manufactured by Becton Dickson Microbiology systems Inc. USA.

Results:

Physicochemical Properties of the Sediments used for the study and the biodegradation profile of the individual SBFs applied

Some physicochemical parameters such as total petroleum hydrocarbon and the levels of indigenous nutrients were measured before the commencement of the experiment to ascertain whether the sediments were pristine and also have substantial nutrient levels that can support microbial growth and proliferation. The initial sediment TPH levels of 16 ppm (SE-SBF-LO), 23 ppm (SE-SBF-SP) and 13 ppm (SE-SBF-10) showed that the sediments were pristine and have not undergone any significant form of organic pollution in the past. Concentrations of endogenous Nitrogen, Phosphorus and Potassium in the sediment samples was also an indication that the sediments used in the present study had enough nutrient concentrations that can sustain microbial growth and proliferation. Each of the sediment sample was spiked with 150 mls of the representative SBF, mixed thoroughly within the 5cm depth zone and allowed to set for 6hrs before the first set of samples were collected at Day 0. Measurement of the physicochemical and microbiological counts were carried out on all the 4 layered zones of the sediment measuring 5, 10, 15 and 20cm.

Physicochemical properties of the sediment sample SE-SBF-LO and the biodegradation profile of the spiked SBF-LO.

More than 90% of the original SBF-LO sample that was used to spike the sediment remained within the 5cm depth zone of the sediment and were subsequently degraded possibly by the aerobic and microaerophilic microbial flora that were predominant in that zone. About 7% of the sample diffused up to the 10cm depth zone after 60 days of exposure while about 0.5% diffused into the 15cm depth zone which is the anaerobic zone after 90 days of exposure. No traces of the SBF sample was found within the very strict anaerobic zone of 20cm depth. Microbial counts at various depths of the sediment showed that about 80% of the microbial activities and the corresponding hydrocarbon degradation were concentrated at the 5cm depth zone. Detailed results are shown in table 1a.

Physicochemical properties of the sediment sample SE-SBF-SP and the biodegradation profile of the spiked SBF-SP.

About 80% of the original SBF-SP sample that was used to spike the sediment remained within the 5cm depth zone. About 18.4% of the spiked SBF diffused into the 10cm depth zone after 60days of exposure while about 2.4% diffused into the anaerobic 15cm depth zone after 90days of exposure. No traces of the sample was found within the 20cm depth zone after the 120 days the experiment was terminated. The residual SBF present within the 5cm and 10cm zones of the sediment were probably degraded by aerobic and microaerophilic microorganisms while the 2.4% of the SBF that diffused into the anaerobic 15cm zone of the sediment were probably degraded by anaerobic microorganisms after day 120. The detailed results are shown in table 1b.

Physicochemical properties of the sediment sample SE-SBF-IO and the biodegradation profile of the spiked SBF-IO

Over 90% concentration of the original SBF-IO sample in the sediment were retained within the 5cm depth zone of the sediment where they were substantially degraded within the 120 day period the experiment lasted. About 8% of the residual SBF diffused into the 10cm depth zone after 60 days of exposure while 1% of the residual SBF diffused into the anaerobic 15cm depth zone after 90 days of exposure. No traces of the sample was found in the 20cm depth zone. The activities of the microaerophilic microorganisms were found to be predominant within the 5cm depth zone where over 80% of the residual SBF were concentrated and subsequently degraded within a time period of 120 days. Microaerophiles were also found to be predominant at the 10cm depth zone where about 8% of the diffused residual SBF were degraded. About 1% of the residual SBF that diffused into the 15cm depth zone were substantially degraded by the resident anaerobes. The detailed results are shown in table 1c.

Discussion

Biodegradation potential of some synthetic Paraffins and Olefins used for drilling oil wells were determined over a period of 120 days at various depths of the Gulf of Guinea sediments under microaerophilic and anaerobic conditions with the aim of establishing the exact roles strict anaerobic and microaerophilic bacteria play in the degradation of these compounds in subsurface sediments. The most direct approach for biodegradability studies which was used in the present study consists of measurements of substrate disappearance as a function of time using gas chromatographic methods.

Oxidation and Reduction (Redox) measurements were also used to determine the aerobic and anaerobic nature of the various depths of the sediment used in the study. Microaerophilic and strict anaerobic microbial activities were also monitored at the various depths of the sediment as biodegradation progressed.

Some investigators like Blanchet *et al* 1998, OGP 2003, Herman and Roberts, 2005 have noted that while information on toxicity and aerobic biodegradation of SBFs are obtained easily, that of anaerobic biodegradation is scarce and difficult. We have also observed from our literature review that little or no information was available on the activities of microaerophilic microorganisms which tend to thrive under very low oxygen tension which is a characteristics feature of most sediments. The present study examined both the roles played by this unique group of microorganisms (Microaerophiles) and strict anaerobic bacteria in the degradation of

synthetic based fluids present at various levels in subsurface sediments.

Previous research work have shown that over 80% of the SBFs deposited on the surface of sediments are degraded by aerobic microorganisms that inhabit the surface of the sediments, less than 15% of the fluids is what naturally diffused into the subsurface sediments for anaerobic degradation (Benka-Coker and Olumagin 1995, Allan and Debora 2006 and Okoro, 2011).

Bothner *et al* (1992) have observed that SBFs introduced at the surface of the sediment penetrated up to 5cm below the seafloor while Soetaert *et al*, (1996) observed that SBF introduced into the sediment penetrated up to 5cm depth, but at 10cm depth, oxygen tension was very low and they observed an exponential decrease in bioturbation and very little traces of the compound was found at that depth.

Table 1a. Physicochemical properties of Gulf of Guinea sediment (SE-SBF-LO) and biodegradation profile of SBF-LO (Linear Olefins)

	Day 0				Day 30				Day 60				Day 90				Day 120			
Depth of Sed.(cm)	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Residual Conc. of SBF-LO (ppm)	24,300	ND	ND	ND	10,650	ND	ND	ND	8530	1680	ND	ND	4260	540	120	ND	865	56	36	ND
Redox-Potential (mV)	+220	+55	-65	-130	+170	+45	-70	-160	+140	+70	-85	-210	+95	+65	-80	-220	+70	+80	-90	-210
SBF-LO utilizing microbial counts (cfu/g x 10 ⁶)*ME & ANAEROBIC	0.065	0.016	0.018	0.0016	0.220	0.066	0.046	0.0018	0.360	0.180	0.065	0.002	0.150	0.051	0.065	0.0018	0.022	0.0015	0.0010	0.00013
Sulphate (mg/g)	83	62	42	36	86	52	41	30	83	61	38	16	68	53	30	22	88	46	37	10
pH	6.70	6.70	6.50	6.60	6.40	6.80	6.50	6.30	6.80	6.70	6.70	6.80	6.40	6.70	6.50	6.60	6.80	6.40	6.60	6.60
Phosphorus(mg/g)	62	44	48	22	62	41	45	26	80	41	33	26	76	43	46	32	60	42	32	30
Potassium (mg/g)	43	31	26	32	48	32	16	22	44	30	21	18	38	23	13	10	33	20	19	12
Amonium - Nitrogen(mg/g)	3.40	2.21	1.30	0.86	2.45	1.37	0.42	1.16	3.02	2.11	1.86	0.55	3.35	2.37	1.33	0.45	1.68	0.27	1.25	0.34

ND = NOT DETECTED *ME & ANAEROBIC = Microaerophylic and Anaerobic Bacterial counts that utilized the SBFs.

Table 1b. Physicochemical properties of Gulf of Guinea sediment (SE-SBF-SP) and biodegradation profile of SBF-SP (Synthetic Paraffin)

	Day 0				Day 30				Day 60				Day 90				Day 120			
Depth of Sed.(cm)	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Residual Conc. of SBF-SP(ppm)	25,400	ND	ND	ND	14,250	ND	ND	ND	10,530	4680	ND	ND	6260	2540	620	ND	2865	1160	360	ND
Redox-Potential (mV)	+280	+65	-85	-180	+180	+55	-80	-120	+140	+80	-65	-240	+75	+85	-95	-210	+70	+60	-90	-240
SBF-SP utilizing microbial counts (cfu/g x 10 ⁶)*ME & ANAEROBIC	0.045	0.006	0.003	0.0010	0.180	0.056	0.045	0.0012	0.250	0.120	0.035	0.002	0.120	0.061	0.021	0.0016	0.022	0.001	0.0040	0.0013
Sulphate (mg/g)	103	82	42	30	96	72	48	40	83	64	38	26	68	53	40	22	88	46	37	30
pH	6.80	6.70	6.70	6.60	6.60	6.80	6.50	6.30	6.80	6.70	6.70	6.80	6.50	6.70	6.4	6.30	6.80	6.60	6.70	6.60
Phosphorus(mg/g)	42	34	28	22	32	21	25	16	40	31	23	21	36	23	16	12	30	22	12	20
Potassium (mg/g)	53	41	46	32	48	42	36	22	44	36	31	28	38	23	23	20	33	20	19	22
Amonium - Nitrogen(mg/g)	2.40	1.8	1.20	0.83	2.15	1.30	0.42	1.16	2.20	2.11	1.86	0.55	2.35	1.40	1.30	0.45	1.88	1.67	0.85	0.34

ND = NOT DETECTED. *ME & ANAEROBIC = Microaerophylic and Anaerobic Bacterial counts that utilized the SBFs.

Table 1c. Physicochemical properties of Gulf of Guinea sediment (SE-SBF-IO) and biodegradation profile of SBF-IO (Internal Olefins).

	Day 0				Day 30				Day 60				Day 90				Day 120			
Depth of Sed.(cm)	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Residual Conc. Of SBF-IO (ppm)	24,850	ND	ND	ND	11,200	ND	ND	ND	9150	1950	ND	ND	5020	880	260	ND	1,100	240	86	ND
Redox-Potential (mV)	+240	+65	-85	-160	+180	+45	-80	-140	+160	+80	-75	-240	+95	+65	-120	-220	+120	+80	-90	-260
SBF-IO utilizing microbial counts (cfu/g x 10 ⁶)*ME & ANAEROBIC	0.030	0.020	0.018	0.0016	0.260	0.045	0.026	0.002	0.340	0.160	0.05	0.006	0.120	0.031	0.025	0.0014	0.028	0.0011	0.0020	0.00019
Sulphate (mg/g)	84	72	42	36	76	52	41	30	80	62	48	36	65	56	42	24	68	46	37	20
pH	6.80	6.70	6.60	6.60	6.80	6.80	6.50	6.70	6.80	6.70	6.70	6.80	6.40	6.70	6.50	6.60	6.60	6.40	6.40	6.60
Phosphorus(mg/g)	68	54	42	32	61	43	46	36	80	42	33	26	71	43	45	32	66	42	32	36
Potassium (mg/g)	51	48	36	35	48	38	26	22	45	33	41	28	36	33	23	16	33	20	29	22
Amonium - Nitrogen(mg/g)	2.80	1.21	1.30	0.66	2.55	1.27	0.52	0.16	2.02	2.01	1.26	0.55	2.35	1.37	1.03	0.45	1.28	0.37	1.15	0.34

ND = NOT DETECTED. *ME & ANAEROBIC = Microaerophylic and Anaerobic Bacterial counts that utilized the SBFs.

The two major microbial groups that were the major focus in the present study are the Microaerophiles and the strict Anaerobes. Microaerophilic bacteria use molecular oxygen as a terminal electron acceptor for their respiratory metabolism but are not able to grow under high atmospheric oxygen conditions because such bacteria need oxygen depleted environment (0.2-2.4%) to thrive and therefore the upper layers of the subsurface marine sediment can be advantageous to them (Ferrera-Guerrero and Bianchi, 1990). Some investigators have also established that the number of microaerophiles in the sediment are usually higher than those of the obligate anaerobes (Sogita *et al* 2002, Tar and Frenchel, 2005). In the present study, about 80-90% of the residual SBF that were used to spike the sediment were degraded within the 5cm depth zone by the aerobic and microaerophilic bacteria that were almost of equal population density. The average oxygen levels within 5cm depths at the initial period of the experiment was about (15%) but as biodegradation progressed oxygen was depleted up to 5% at day 60, making way for microaerophilic microorganisms to thrive and dominate. At the 10cm depth, 7% of the Linear Olefins, 18.4% of the Synthetic Paraffins and 8% of the internal Olefins diffused into this zone after 60 days of exposure. This zone had very low oxygen tension and the microaerophiles were the predominant microorganisms isolated from this zone. The 15cm depth according to the redox-potential readings is the strict anaerobic zone and about 0.5% of the linear Olefins, 2.4% of synthetic paraffins and 1% of internal Olefins diffused into this zone after 90 days of exposure. The linear and Internal Olefins had

higher anaerobic biodegradation rates than the synthetic paraffins within the 15cm anaerobic zone.

The major highlights from this study can be summarised thus;

About 80-90% of the original synthetic Paraffins and Olefins that were used to spike the sediment were degraded within the 5cm depth of the sediment where the activities of the aerobic and microaerophilic microorganisms were found to be predominant. Similar observations have been made by some researchers like Ferrera-Guerrero and Bianchi (1990) when they observed that aerobic and microaerophilic bacteria had equal population densities at the 10-15mm layer of the Gulf of Mexico sediments but beyond 20mm zone, anaerobes prevail. The distinction between this observation and ours is that oxygen and SBF fluid penetration is likely to be faster in loose sandy Gulf of Guinea sediment than the sandy-clay Gulf of Mexico sediments. Catello (1999) have also observed that sediment redox potential vary on hourly basis in response to microbial activity which naturally determines the oxygen levels in most environments.

Another major highlight of the present study is the different diffusion rates and degradation patterns in the sediment by the various SBFs used in the experiment. While the Paraffins showed faster diffusion rates in the sediment, the linear and internal olefins showed higher degradation potential than the paraffins under microaerophilic and anaerobic conditions. At the termination of the experiment at day 120, about 0.37% of linear olefins, 5.98% of synthetic paraffins and 1.31% of internal olefins remained un-degraded within the 10 and 15cm zones. In a closed bottle anaerobic biodegradation tests carried out by Roberts and Hernman (2004), Internal

Olefins(56%) and Linear Olefins (59%) showed a better anaerobic biodegradation rates than the Paraffins and the diesel oil (30%). Shell (Unpublished data) have also carried out a 120 day closed bottle anaerobic biodegradation study showing the % degradation of the following SBFs under anaerobic conditions as, Internal olefin (55-60%), Linear Olefin (60-75%), and Synthetic Paraffin (17%). American Chemistry Council have advanced that Paraffins showed limited anaerobic biodegradability and their rate is significantly slower than Olefins.

Interestingly the predominant microaerophilic bacteria isolated from the 5 and 10cm depth such as *Pseudomonas* sp., *Streptococcus* sp., and *Vibrio* sp., all showed their ability to utilise the representative SBF as their sole carbon source under laboratory conditions. Anaerobic microorganisms isolated within the 15cm depth such as *Desulfobacter* sp., *Desulfovibrio* sp and *Actinomyces* sp. also demonstrated their ability to utilise the representative SBFs used in the experiment as their sole carbon source.

Conclusion:

We can safely conclude from the data generated from the present study that about 80-90% degradation of the synthetic paraffin and olefin deposited on the sediment surface and subsurface were carried out within the 5cm depth zone by the aerobic and microaerophilic microorganisms whose activities dominated the zone. At the subsurface (10cm), microaerophiles seems to be more relevant than the strict anaerobes in the degradation of the SBFs and degradation of synthetic paraffins and olefins by strict anaerobes in the sediment was very minimal as very negligible concentrations of the SBFs were able to diffuse into the zone. Linear Olefins and Internal Olefins degraded faster than the Paraffins in both anaerobic and microaerophilic environmental condition. Future studies should lay more emphasis on the direct roles of the microaerophiles in the degradation of SBFs in the sediment because the low level oxygen environmental condition that characterise the sediment subsurface favours them more than any other group of microorganisms.

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