Aerobic Degradation of Paraffin and Olefin Synthetic Based Drilling Mud Base Fluids by Gulf of Guinea Sediments under Natural Environmental Conditions

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Abstract: Aerobic biodegradation of synthetic Paraffins and Olefins in the Gulf of Guinea sediments were monitored over a 120 day period in an indoor benthic chamber basin tests measuring 18 x 30 inches. At each 30 day interval, residual hydrocarbons were measured with gas chromatograph while microbial populations were quantified with the most probable plate number method (MPN). At the end of the 120 day monitoring period, the following % degradation rates were recorded for different hydrocarbon substrates; Linear Olefin (90%), Synthetic Paraffin (82%), and Internal Olefin (86%). The overall degradation sequence showed that the Olefins degraded faster than the Paraffins but both hydrocarbon substrates were readily biodegradable by the indigenous microbial flora of the Gulf of Guinea sediments. This study demonstrated that over 85% of the degradation of Synthetic Paraffins and Olefins on the surface of sediments were carried out by aerobic microorganisms.
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Introduction:

Synthetic based fluids (SBF) are new class of fluids that are currently in use for drilling oil and gas wells. They 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). In the present study, we dealt only with synthetic Paraffins and Olefins. Ester based synthetic fluids have already been dealt with in our previous studies.

Paraffins consists 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 consists 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).

Aerobic biodegradation of Paraffin and Olefin synthetic base fluids (SBF) in the sediment is a major criterion in selecting appropriate base fluids that are suitable for drilling purposes (Gardline 1988, Neff *et al*, 2000). Ester based fluids are known to have higher biodegradation potential than the Paraffins and Olefins but their major disadvantage is that they are susceptible to calcium and acidic gas contamination as well as thermal limitations and as a result of this, some drillers do prefer synthetic paraffins and olefins (West *et al*, 2009). Toxicity tests results carried out by the American chemistry Council (2006) showed that the Olefin and Paraffin SBF are not toxic to the water and sediment dwelling organisms and on the biodegradation potential of the synthetic Paraffins and Olefins, the council advanced that they are readily biodegradable both in aerobic and anaerobic environment. Other investigators have also advanced that the synthetic based fluids in oily cuttings are biodegradable under aerobic conditions (Kjeilen, 1997, Robert and Nguyen 2006, Okoro 2011). In addition to this, Cobby (2002) advanced that biodegradation rates of SBFs in the sediment can be influenced by factors like seabed temperature, fluid concentrations and loadings, the surface area of the cuttings and the sediment particle size.

A wide variety of aerobic hydrocarbon degrading microorganisms such as *Flavobacterium* sp., *Micrococcus* sp., *Alkaligenes* sp., *Corynebacterium* sp., *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium* sp. have been isolated from the Gulf of Guinea sediments (Okoro 2010_a). In a related development, another investigation carried out by Okoro (2010_b) revealed that aerobic microorganisms are very active in the Gulf of Guinea sediments up to a depth of 2-5cm with a total heterotrophic bacterial counts of $3.20x10^6$ cfu/g and $2.20x10^4$ cfu/g respectively. Other aerobic microorganisms implicated in organic carbon degradation in marine sediments by other researchers include; *Pseudomonas* sp. (Tagger *et al*, 1990), *Flavobacterium* sp. (Okpokwasili *et al*, 1984) and *Vibrio* sp. (West *et al*, 1984). Naturally,

biodegradation occurs more rapidly under aerobic conditions than in anaerobic conditions. It is also likely that aerobic biodegradation of SBF may deplete the oxygen in sediments making the sediment anoxic if the loading of the sediment with biodegradable organic matter from SBF cuttings is high and aeration of sediment is low (CSA, 2004), at this stage microaerophilic and anaerobic microorganisms might become relevant in the degradation of the residual SBF in the sediment.

In the present study, aerobic biodegradation potential of 3 synthetic Paraffin and Olefin based drilling fluid namely; SBF-LO (Linear olefins), SBF-SP (Synthetic paraffin) and SBF-IO (Internal olefins) were tested on Gulf of Guinea sediments under natural environmental conditions over a period of time.

2. Material and Methods:

Experimental Design:

3 rectangular shaped glass containers measuring 18x30 inches (about 18 inches deep) were used for the experiment. Each of the glass container was filled with the wet sediment up to 12 inches depth followed by the introduction of 150mls of each of the representative SBF to the respective containers. The sediment/fluid mixture was mixed thoroughly 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 120 day period and at each 30-day interval, sediment samples were collected and analysed for residual hydrocarbon (SBF) and hydrocarbon utilizing bacteria.

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 Total Heterotrophic Bacterial and Fungal Counts.

Heterotrophic bacteria and Fungi were enumerated by adopting the standard plate count technique using spread plate method. Appropriate dilution of samples were plated out on nutrient agar plates for Bacteria and potato dextrose agar plates (PDA) for Fungi. The plates for Bacteria were made in duplicates and incubated aerobically at 29^0C for 24hrs while that of Fungi were incubated aerobically for 3-4 days. 2µg/L of chloramphenicol was added to PDA plates to inhibit bacterial growth as described in Eaton *et al*, 1995.

Enumeration of Hydrocarbon utilizing bacteria

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), $MgSO_4.7H_20$ (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 pH (7.2). The medium was autoclaved at 1.1 kg/cm^2 for 15 mins. The inoculated mineral agar plates were then inverted over sterile membrane filters moistened with crude oil (Escravos light) and held in the lid of the petri dishes. The dishes were wrapped round with a masking tape so as to increase the vapour pressure within the petri dishes while the plates were incubated at 29^0C for 6 days after which the growth of hydrocarbon degrading bacteria were observed and counted. For Fungal plates, 0.1g of Penicillin was added to 250 ml mineral salt medium to inhibit bacterial growth.

pH, Temperature measurement and Salinity

The pH of the sediment was measured with a portable water proof pH meter (Jenway, 3150, USA), Temperature was measured using portable thermometer (Hanana , H1-93510, USA). Salinity was measured as Chloride using the Argentometric method as earlier described in *(Eaton et al, 1995).*

Estimation of Background Nutrient Concentration of the sediment

Interstitial water samples were withdrawn with a simple apparatus as described in McKee *et al*, 1998. 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). Amonium-Nitrogen was analysed with auto analyser as described in Eaton *et al*, 1995.

Detection of heavy metals:

Heavy metals were detected using the Atomic absorption Spectrophotometer (Perlkin Elmer 5100PC, England) after sample preparation and digestion as previously described (Eaton *et al*, 1995).

Moisture content:

The moisture content of the sediment samples were measured by simple gravimetric analysis. 10grams of the sediment sample containing water was dried in the oven at a temperature of 200^oC 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 Hydrocarbon

Degraded oil 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^{0}C increasing at 3.5^{0}C per minute for 27.15 min 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^{0}C while the injection port temperature was 305^{0}C. 1 ml of the residual oil extract was dissolved in methylene chloride at the ratio of 1:1 and a sample volume of 0.2 µl was injected into the GC.

Identification Microorganisms capable of utilizing SBF

The growth and morphology of bacterial isolates in minimal salts medium and on nutrient agar plates were noted with regards to the following characteristics; form, pigmentation, texture, colour and elevation. Fungal cultures were stained with Methylene blue and observed under a microscope (x40) and each fungal culture was identified based on its morphological characteristics with the aid of an identification manual. Bacterial cultures were stained using grams staining procedure and proper identification was done using a computerized BBL Enterotube identification test kits, manufactured by Becton Dickson Microbiology systems Inc. USA.

3. Results.

Microbiological and Physicochemical properties of the Gulf of Guinea Sediments

The three sediment samples tested showed a total heterotrophic bacterial counts which ranged between 1.30 – 1.80 x 10^6 cfu/g. The hydrocarbon utilizing bacterial population among the heterotrophs ranged between 0.031- 0.066 x 10^6 cfu/g. Heterotrophic Fungal and Yeast counts in the sediment ranged between 0.001 - 0.011 x 10^6 cfu/g while the hydrocarbon utilizing Fungal and Yeast species ranged between 0.00013 – 0.00080 x 10^6 cfu/g.

The concentration of total petroleum hydrocarbons in all the sediments were less than 10ppm on the average, this is an indication that the sediments used in the experiment were all pristine and have not undergone any form of petroleum hydrocarbon pollution in the past. The endogenous concentrations of nitrogen, potassium and phosphorus in the sediment was sufficient enough to sustain microbial growth and proliferation. The detailed results are shown in table 1.

Aerobic degradation of synthetic Paraffins and Olefins in the sediment.

Varieties of aerobic microorganisms present on the sediment surface degraded considerably the synthetic Paraffins and Olefins that were used to spike the sediment within the 120 day period the experiment lasted. After the 120 -day experimental period, Sample SBF-LO which is a linear olefin recorded 90% degradation, sample SBF-SP (a synthetic paraffin) recorded 82% degradation while sample SBF-IO (an internal olefin) recorded about 86% degradation. The detailed results are shown in Table 2.

Population dynamics of hydrocarbon utilizing bacteria during biodegradation of SBFs in the sediment

The population dynamics of hydrocarbon utilizing bacteria during biodegradation of the SBFs in the sediment showed relatively low concentrations of indigenous heterotrophs with the capability to utilize the SBFs as their sole carbon source at the beginning of the experiment at day 0 when the sediments were just spiked with the SBFs. Thereafter when the microorganisms started to utilize the SBFs, their population density gradually increased and peaked at day 60. However when considerable levels of the SBFs had been degraded after day 60, the population density started to decline and the downward trend was sustained till the termination of the experiment at day 120. The trend in population dynamics were similar in all the sediment samples tested as shown in table 3.

Table 1. Microbiological and Physicochemical properties of Gulf of Guinea sediments

		SBF-LO-SD	SBF-SP-SD	SBF-IO-SD
1	Total Heterotrophic Bacterial Counts (Cfu/g x 10^6)	1.30	1.80	1.40
2	Hydrocarbon utilizing bacterial counts (Cfu/g x 10^6)	0.031	0.066	0.051
3	Total Heterotrphic Fungi/Yeast Counts. (Cfu/g x 10^6)	0.001	0.011	0.0064
4	Hydrocarbon Utilizing Fungi/Yeasts. (Cfu/g x 10^6)	0.00013	0.00022	0.00086
5	Total Petroleum Hydrocarbon (TPH) (ppm)	9.20	8.60	8.40
6	pH	6.60	6.40	6.80
7	Temperature (^{0}C)	23	25	24
8	Salinity (mg/g)	5260	5320	5540
9	Moisture content (%)	56	57	60
10	Phosphorus (mg/g)	116	121	105
11	Potassium (mg/g)	92	43	46
12	Ammonia-N (mg/g)	3.10	4.20	2.30
13	Heavy Metals detected (ppm)	Zn(0.62), Cr (0.001), Pb(0.020)	Cd(0.02), Pb(0.018)	Fe(0.022), Zn (0.004)

SEDIMENT TYPES; 1. SBF-LO-SD, 2. SBF-SP-SD, 3. SBF-IO-SD

Table 2. Aerobic Degradation of Synthetic Paraffin and Olefin based drilling fluids in the Sediment (in ppm) after a time period of 120 days

	SBF-LO	SBF-SP	SBF-IO
DAY 0	22,400	23,240	22,280
DAY 30	19,200	20,600	19,800
DAY 60	8,850	15,260	10,250
DAY 90	4,800	8,950	5,200
DAY 120	2,200	4,250	3,100
% Degradation after 120 days	**90**	**82**	**86**

SBF-LO (Linear Olefin), SBF-SP (Synthetic Paraffin), SBF-IO (Internal Olefin)

Table 3. Population dynamics of Hydrocarbon utilizing bacteria during biodegradation of Synthetic based fluids in the sediments. (Bacterial Population x 10^6 cfu/g)

SEDIMENT	DAY 0	DAY 30	DAY 60	DAY 90	DAY 120
SBF-LO-SD	**0.026**	**0.420**	**1.180**	**0.620**	**0.056**
SBF-SP-SD	**0.010**	**0.230**	**1.210**	**0.640**	**0.260**
SBF-IO-SD	**0.053**	**0.380**	**1.680**	**1.060**	**0.610**

Aerobic microorganisms isolated from the Gulf of Guinea sediments with the capability to utilize the SBFs as their sole carbon source.

Varieties of aerobic microorganisms that showed capability to utilize the SBFs in the sediment were isolated and identified from 3 different sediment samples tested. In sediment sample SBF-LO-SD which was spiked with linear olefin, the predominant microbial flora were *Pseudomonas* sp., *Achromobacter* sp., *Aspergillus niger* and *Penicillium* sp., and the total percentage of indigenous heterotrophs that utilized the SBFs as their sole

carbon source was 2.38%. Sediment sample SBF-SP-SD which was spiked with synthetic paraffin was dominated by the *Alkaligenes* sp., *Pseudomonas* sp., *Flavobacterium* sp and *Aspergillus niger* with about 3.66% of the indigenous heterotrophic microbial population having the capability to utilize the SBFs as their sole carbon source. Sediment sample SBF-IO-SD which was spiked with internal olefin showed its predominant microbial flora as *Micrococcus* sp., *Pseudomona*s sp., *Penicillium* sp., and *Aspergillus niger*. 3.64% of the indigenous heterotrophic population possessed the capability to utilize the SBFs as their sole carbon source. The detailed results are shown in Table 4.

4. Discussion:

Aerobic degradation of synthetic Linear Olefins, Paraffins and Internal Olefins by the indigenous microbial flora of Gulf of Guinea sediments were monitored over a period of 120 days. Incubation at hydrostatic pressure was not necessary because previous research have shown that incubation at such deep offshore pressure had no effect on the hydrocarbon substrate degradation (Benka-Coker and Olumagin, 1995, Alan *et al*, 2006, Robert and Nguyen, 2006). The analytical data derived from the present study on the Gulf of Guinea sediments showed that they are populated with vide varieties of indigenous microbial populations that have the capability to utilise the SBF as their sole carbon and energy sources. The background nutrient composition of Gulf of Guinea sediments used in the present study also showed that the sediments have fairly good nutrient composition that can sustain microbial growth and proliferation.

Previous investigations have also shown that the Gulf of Guinea sediments which is mostly sandy in nature have considerable background nutrient composition and is populated with a wide variety of indigenous microbial flora with the capability to utilise the organic carbons in the sediment as their sole carbon and energy sources (Okoro, 2010_a).

Table 4. Microorganisms isolated from Gulf of Guinea Sediments with the capability to utilize the SBF

	SBF-LO-SD	SBF-SP-SD	SBF-IO-SD
DAY 0	*Pseudomonas* sp.,, *Achromobacter* sp., *Acinetobacter sp.*, *Alkaligenes sp.,Flavobacterium sp.*, *Aspergillus niger*, *Penicillium* sp., *Penicillium crysogenum*	*Acinetobacter lwoffii*, *Pseudomonas* sp., *Vibrio sp., Micrococcus sp.*,, *Alkaligenes* sp., *Flavobacterium* sp. *Bacillus* sp., *Aspergillus niger* *Aspergillus Fumigatus, Penicillium sp.*	*Micrococcus sp.*, *Pseudomonas* sp.,. *Alkaligenes* sp. *Pseudomonas mallei. Penicillium* sp *Aspergillus fumigatus* *Rhizopus sp. Candida sp.*,
DAY 30	*Pseudomonas sp.* *Acinetobacter sp.*, *Achromobacter sp.,Flavobacterium sp.*, *Aspergillus niger*, *Penicillium* sp.,	*Pseudomonas* sp, *Alkaligenes* sp., *Flavobacterium* sp., *Aspergillus niger* *Penicillium sp*	*Pseudomonas* sp.. *Alkaligenes* sp., *Pseudomonas mallei, Micrococcus sp.* *Penicillium* sp.,*Candida sp.* *Aspergillus niger* *Aspergillus fumigatus*
DAY 60	*Pseudomonas sp.*, *Achromobacter* sp., *Aspergillus niger*, *Penicillium* sp.,	*Pseudomonas* sp, *Alkaligenes* sp., *Flavobacterium* sp. *Aspergillus niger*	*Pseudomonas* sp.. *Alkaligenes* sp,. *Micrococcus sp.*, *Penicillium* sp., *Aspergillus niger* *Aspergillus fumigatus*
DAY 90	*Pseudomonas* sp *Achromobacter* sp., *Aspergillus niger*, *Penicillium* sp.,	*Alkaligenes* sp.,*Pseudomonas sp.*, *Flavobacterium* sp *Aspergillus niger*	*Pseudomonas* sp.. *Alkaligenes* sp,. *Penicillium* sp., *Micrococcus sp* *Aspergillus niger, Aspergillus fumigatus*
DAY 120	*Pseudomonas* sp. *Achromobacter* sp., *Aspergillus niger*, *Penicillium* sp.,	*Alkaligenes* sp.,*Pseudomonas sp.*, *Flavobacterium* sp. *Aspergillus niger*	*Micrococcus sp., Pseudomonas sp* sp,. *Penicillium* sp., *Aspergillus niger, Aspergillus fumigatus*
% of Indigenous heterotrophs utilizing SBF	**2.38%**	**3.66%**	**3.64%**

The American Chemistry Council (2006) have equally observed that sediment dwelling microorganisms are able to utilise Paraffin and Olefin SBF as a source of nutrition and aerobic biodegradation of SBF in the sediment results in decrease in sediment oxygen concentration.

The sediments used in the present study had a total heterotrophic microbial population that ranged between 1.30 – 1.80 x 10^6 cfu/g but only about 2.38-3.64% of the indigenous heterotrophic microbial population in the sediment had the capability to utilise the spiked SBFs as their sole carbon and energy sources. Robert and Nguyen demonstrated that the indigenous aerobic microbial populations of the Gulf of Mexico sediments ranged between 1.0 x 10^8 – 1.4 x 10^9 cfu/g but less than 10% of this had the capability to utilise the SBF as their sole carbon and energy source. In a similar study conducted by Okoro (2011) using Ester based fluids and the Gulf of Guinea sediments, about 0.92-3.30% of the indigenous heterotrophic microbial population in the sediments were able to utilise the Ester based SBF as their sole carbon and energy source. All these investigations points to the fact that in marine sediments, the heterotrophic microbial populations that have the capability to utilise hydrocarbons as their sole carbon and energy source are naturally present in small numbers but their numbers increases astronomically as the microorganisms starts acting on the exogenous hydrocarbons as their input increases.

We observed in the present study that 90% of the original Linear Olefin concentration that was used to spike the sediment degraded after 120 days. Synthetic paraffins recorded 82% degradation while Internal Olefins recorded 86% degradation within the same period. An evaluation of the overall degradation sequence showed that Linear Olefins and Internal Olefins degraded faster than Synthetic Paraffins. American Chemistry Council (2006) have also observed that Synthetic Paraffins degrade slowly and may persist in the environment for longer periods than the Olefin based SBF. A similar investigation by Okoro (2011) showed that Ester based fluids degraded faster than the Paraffin and Olefin based SBFs under the same experimental conditions. The assertion that the Ester based SBF degrade faster than the Paraffins and the Olefins have also been confirmed by other investigators such as Robert and Nguyen (2006) and OGD (2003).

Another important observation in the present study is the unique roles played by mixed microbial populations in the degradation of the SBFs. The predominant aerobic microbial flora that were associated with the degradation of the SBFs used in the present study in the Gulf of Guinea sediments were *Pseudomonas* sp., *Achromobacter* sp., *Alkaligenes* sp., *Flavobacterium* sp., and *Micrococcus* sp. among the bacterial species and *Penicillium* sp., *Aspergillus niger* and *Aspergilus fumigatus* among the fungal species. These microbial flora were very active in the utilisation of the SBFs from the initial period they were used to spike the sediment up to the time the experiment was terminated. In a similar study conducted by Okoro (2011) using Ester based SBF and the Gulf of Guinea sediments, the predominant microbial flora that utilised the SBF in the sediment were found to be *Pseudomonas* sp., *Alkaligenes* sp., *Micrococcus* sp., and *Achromobacter* sp. among the bacterial species and *Aspergillus niger* and *Penicillium crysogenum* among the fungal species but in another related study conducted by Ben-Coker and Olumagin (1995) in the Gulf of Guinea sediments, the predominant microbial flora with the capability to utilise the SBF in the sediment were found to be *Alkaligenes* sp. and *Micrococcus* sp. among the bacterial species and *Penicillium* and *Cladosporium* species. among the fungal species. This study also demonstrated that about 6% of the total heterotrophic population possessed the ability to utilise the SBF in the sediment as their sole carbon and energy source. Khodja (2008)and Khoja *et al*, (2010) have also demonstrated the roles of mixed microbial culture comprising of *Enterobacter* sp., *Citrobacter freundii*, *Erogenous pseudomonas, Staphylococcus guricularis*, *Bacillus thuringensis* and *Micrococcus varians* in the degradation of drilling fluids deposited on the sediment.

Conclusion:

The present study have clearly demonstrated that the Synthetic Paraffins and Olefins used in the present study are readily biodegradable by the aerobic indigenous microbial flora of the Gulf of Guinea sediments and considerable concentrations of the original SBF used to spike the sediments were removed from the sediments after a period of 120 days. The study also showed that the Olefins degraded relatively faster than the Paraffins. We can safely conclude from this study that over 85% of degradation of SBF in the sediments were carried out by aerobic microorganisms.

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