Biodegradation Potential of 4-Ester Based Drilling Mud Base Fluids under Microaerophilic and Anaerobic Conditions

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Abstract: Biodegradation potential of 4 – Ester based drilling fluids were monitored at various depths of the sediment over a period of 120 days with the aim of quantifying the roles of microaerophilic and strict anaerobic bacteria species in the degradation of the various ester based fluids. The redox potential at various depths of the sediment were measured to determine the extent of anaerobiosis or otherwise of the sediment. On the average, the three Ester based fluids used in the experiment (BR-EST, CH-EST and PFB-009) recorded about 62% degradation after 30 days of exposure within the 5cm depth where aerobic and microaerophilic microorganisms were observed to be very active and over 95% degradation was recorded at day 120 when the experiment was terminated. The 4th sample PFE-008 recorded 79% degradation within the same period (30days). At the 10cm depth where microaerophilic microorganisms were observed to be predominant, less than 3% of the residual ester based fluids on the average were available for degradation at day 60 and no traces of these compounds were found thereafter in the sediments after the 90th day, an indication of complete degradation by microaerophilic microorganisms. Only very little traces of the fluids (less than 0.5%) were found in the anaerobic zone (15cm depth) in two of the sediment samples analysed. The present study have shown that the Ester based drilling fluids used in the experiment diffused very slowly into the sediment and the bulk of the degradation of fluids in the sediment were carried out by aerobic and microaerophilic microorganisms located within the 5cm depth of the sediment. Degradation of the ester based fluids by strict anaerobes in the sediment was very negligible.
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1. Introduction

There are 3 main catigories of drilling fluids namely; the oil based fluids (OBF), the water based fluids (WBF) and the synthetic based fluids (SBF). OBF have been traditionally used in the past because of their high performance drilling characteristics but they have poor environmental performance in terms of their ecotoxicity and their tendency to persist in cutting piles in the sea floor (poor biodegradability). The WBF provides the best environmental performance in terms of their non toxic nature and enhanced ability to biodegrade rapidly on the seafloor but they generally do not provide optimal performance in more challenging drilling conditions. The SBF was developed most recently to combine the advantages of OBF and WBF in the sense that they provide similar drilling performance as OBF but with improved ecotoxicity and biodegradability as WBF (Cobby, 2002).

Synthetic based fluids are therefore new class of materials that are presently used to provide safe and effective technology for drilling mostly offshore oil and gas wells. Their enhanced drilling performance decreases drilling time and provides advantaged safety, human health and in some cases environmental performance above other non-synthetic drilling fluids. The SBF were therefore developed as alternatives to mineral and diesel based oil muds for the purpose of reducing the environmental impact of discharging cuttings to the seafloor.

Synthetic-based drilling fluids include; Linear Olefins, Synthetic Parrafins, Internal Olefins and Esters. These new class of drilling fluids provides lubricity, maintains pressure during drilling, protects, supports and stabilizes the bore hole wall and are generally environment friendly. Cuttings generated while drilling with SBF can be discharged into the marine environment without any harm to the marine ecosystem (Cognis, 2000).

According to Tapavicza (2005), Ester based fluids deliver outstanding performance under extreme bore hole and formation conditions and their overall benefits includes; faster drilling, reduced drilling costs, superior lubricity, excellent borehole cleaning, protection of drilling formations and proven track record on performance. In addition, Ester based fluids strongly protects the geological formations, preventing the swelling of the reactive clay and shale formations. The polar Ester groups and the balanced C-chain are the main factors conferring these good properties (Tapavicza, 2005).

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 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 Ester based fluid in the sediment
b. Using redox potential measurements to monitor the level of oxygen at various depths of the sediment where the ester based fluids were found.
c. Isolating various groups of organisms that participate in the degradation of ester based fluids at various depths of the sediment.
d. Further clarifying the issues of anaerobic degradation in the sediment by distinguishing the activities of microaerophylic microorganisms, facultative anaerobic and obligate anaerobic microorganisms, which most investigators in the past have consistently grouped under anaerobic activity.

The major objective of the present study therefore 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 ester based drilling fluids deposited at various depths of the Gulf of Guinea sediments.

2. Materials and Methods:

Experimental Design:

The experimental test set up consists of a series of 4 easily assessable rectangular shaped glass indoor basins called benthic chambers measuring approximately 25x30cm. Each of the glass containers was filled with the wet sediment (with moisture content ranging between 35 and 55%) collected from Escravos river (Located within the Gulf of Guinea) up to 25cm depth followed by the introduction of 150 mls of each of the Ester based fluids to 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 organic carbon and microorganisms capable of utilizing the Ester based fluids 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-BR-EST, 2. SE-CH-EST, 3. SE-PFB-009 , and 4. SE- PFB-008 depending on the type of ester based fluid added to the sediment

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

The Ester based fluids samples which were collected from the Nigerian Department of Petroleum Resources (DPR) were coded and have the following descriptions.

1. BR-EST (BAROID ESTER)
2. CH-EST (CHEVRON ESTER)
3. PFB-009 (Mixture of Ester and Olefin)
4. PFE-008 (Ester of Aliphatic acid).

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), $MgSO_4.7H_20$ (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 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^{0}C for 4 days in a candle Jar for microaerophiles and anaerobic incubator for obligate anaerobes.

Enumeration of Microaerophylic bacteria

The candle jar method described by Cheesbrough (1992) was used. 0.1mls of the inoculums 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^{0}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 indicatosr 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 colomel 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^{o}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 Ester based fluids

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 Ester based fluids

Degraded organic carbon 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 27.15min 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^{0}C while the injection port temperature was 305^{0}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 µl was injected into the GC.

Identification Microorganisms capable of utilizing the Ester based fluids.

Cultural characteristics of the various bacterial cultures were noted in the selective media used, this was followed by staining of bacterial cultures using grams staining procedure and final identification was done using a computerized BBL Enterotube identification test kits, manufactured by Becton Dickson Microbiology systems Inc. USA.

3. Results;

Physicochemical properties of Gulf of Guinea sediments and Biodegradation profile of various ester based fluids used in the study

The physicochemical parameters recoded at various depths of the sediment (5, 10, 15, 20 cm) were the residual concentrations of the various ester based drilling fluids used in the study, the redox potential, and the endogenous nutrient concentrations. After spiking the sediment with the fluids followed by proper mixing, the residual concentration of the ester based fluids were measured at the various depths and the monitoring interval was 30 days for a total duration of 120 days. All the ester based fluids tested showed drastic reduction in their concentrations for the first 30 days at the 5cm depth. Diffusion of the fluids into the inner most portions of the sediment was however very slow as very little concentrations were found in some sediments after 90 days of exposure. In two of the sediments tested (CH-EST and PFE-008), the residual fluids only diffused up to the 10cm depth of the sediment, the detailed results are shown on Tables 1a-1d).

The redox potential were also measured at various depths of the sediment and as can be seen on Tables 1a-1d, normal aerobic environment were only observed at the 5cm depth, at 10cm depth, the oxygen level was very low and the environment changed to anaerobiosis at depths 15 and 20.

Total bacterial counts with the ability to utilize the ester based fluids as the sole carbon source in the sediment included both aerobic, microaerophilic and strict anaerobic bacteria and the results are shown in Tables 1a-1d.

The level on endogenous nutrients in the sediment were also measured to establish whether the sediment have enough nutrients for microbial growth and proliferation. The levels of Phosphorus, Potassium and Ammonium were all estimated at various depths of the sediment and the results are shown in Tables 1a-1d. The level of Sulphate was also estimated to monitor the fate of anaerobic organisms that might require sulphate as alternative electron acceptor in the absence of oxygen and the results are shown in Tables 1a-1d

4. Discussion:

The degradation of 4 Ester-based drilling fluids under natural conditions were monitored at various depths of the sediment with the aim of establishing the exact roles of microaerophilic and strict anaerobic microorganisms in the degradation of these compounds. The moisture content of the sediment used ranged between 35 and 55%.

Previous research works have shown that over 80% ester based fluids deposited on the surface of sediments are degraded by aerobic microorganisms that inhabit the surface of the sediment (Okoro, 2011, Alan and Debora, 2006, Benka-Coker and Olumagin, 1995), the remaining concentrations that diffused into the subsurface sediment are expected to be degraded by microaerophilic and anaerobic microorganisms. Few data on the distribution of anaerobes in the sediment are available but microbial data and counts concerning microaerophilic microorganisms in the sediment are scarce. Microaerophilic bacteria which uses molecular oxygen as terminal electron acceptor for their respiratory metabolism are not able to grow under high atmospheric oxygen concentrations because such bacteria need oxygen depleted environment (0.2-2.4%) to thrive and therefore, the upper layers of the marine sediment could be advantageous to them (Ferrara-Guerrero and Bianchi, 1990). Some investigators have established that the number of microaerophiles in the sediment are usually higher than those of obligate anaerobes (Sugita *et al.* 2002, Tar and Frenchel, 2005).

All the ester based fluids used in the study could not diffuse beyond the 10cm depth in two of the samples and very negligible concentrations were found in the other two sediments samples at the 15cm depth after the 120 days the experiment lasted. The bulk of the degradation and microbial activity were carried out within the 5cm depth of the sediment where aerobic and microaerophilic microorganisms dominate and this is also the depth where over 85% of the spiked Ester based fluids were concentrated. At the 10cm depth, less than 5% of the residual Ester based fluids were found and because of the very low oxygen concentration at this zone, the microaerophilic microorganisms were the predominant microbial flora.

In a similar study conducted by Ferrera-Guerrero and Bianchi, 1990 on the sandy clay Gulf of Mexico sediments, microaerophilic bacterial counts were less than 1% of the total aerobic population densities at 0-10mm upper layer of the sediment but in the 10-15mm zone, the two microbial groups had equivalent population densities (1×10^5 cells/ml^{-1}) but beyond 20mm, anaerobes prevail. This is expected because the Gulf of Mexico sediments used had a sandy clay sediment which naturally have slower ester fluid and oxygen penetration capability than the loose sandy Gulf of Guinea sediment we used in the present study. Catello (1999), have also demonstrated that sediment redox potential vary on hourly basis in response to microbial activity, flooding and draining.

Table 1a. Physicochemical properties of Gulf of Guinea sediments and biodegradation profile of BR-EST (Baroid Ester)

	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 BR-EST(ppm)	26,400	ND	ND	ND	8650	ND	ND	ND	3550	680	ND	ND	1260	130	45	ND	65	ND	ND	ND
Redox-Potential (mV)	+ 220	+45	-65	-120	+180	+35	-60	-180	+120	+60	-85	-220	+85	+55	-80	-240	+76	+60	-90	-220
BR-EST utilizing microbial counts (cfu/g x 10^6)	0.08	0.026	0.011	0.0036	0.120	0.076	0.036	0.0028	0.260	0.160	0.087	0.0036	0.140	0.056	0.066	0.0011	0.032	0.0018	0.0020	0.00016
Sulphate (mg/g)	84	68	62	36	76	52	41	32	63	51	36	16	66	43	32	28	68	36	27	16
pH	6.80	6.70	6.30	6.60	6.40	6.90	6.50	6.60	6.80	6.70	6.70	6.40	6.60	6.70	6.50	6.60	6.90	6.40	6.70	6.60
Phosphorus(mg/g)	68	46	48	28	62	43	44	28	82	48	43	36	66	43	46	32	60	41	42	32
Potassium (mg/g)	**43**	**41**	**36**	**32**	**48**	**38**	**16**	**22**	**44**	**38**	**21**	**30**	**28**	**13**	**23**	**12**	**23**	**13**	**19**	**32**
Amonium - Nitrogen(mg/g)	**3.42**	**2.28**	**1.30**	**0.86**	**2.65**	**1.87**	**0.48**	**1.26**	**3.88**	**2.11**	**1.86**	**0.75**	**3.35**	**2.67**	**1.33**	**0.45**	**1.68**	**0.77**	**1.25**	**0.88**

ND = NOT DETECTED

Table 1b. Physicochemical properties of Gulf of Guinea sediments and biodegradation profile of CH-EST (Chevron Ester)

	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 CH-EST(ppm)	22,100	ND	ND	ND	9350	ND	ND	ND	2650	420	ND	ND	860	110	ND	ND	35	ND	ND	ND
Redox-Potential (mV)	+ 210	+65	-75	-140	+170	+55	-40	-170	+140	+60	-75	-240	+75	+65	-90	-260	+66	+50	-90	-240
BR-EST utilizing microbial counts (cfu/g x 10^6)	0.18	0.036	0.021	0.0016	0.130	0.066	0.034	0.0025	0.230	0.140	0.067	0.0026	0.130	0.056	0.056	0.0021	0.022	0.0028	0.0060	0.00015
Sulphate (mg/g)	88	63	72	46	75	62	49	22	73	61	66	26	56	33	42	23	78	26	47	11
pH	6.90	6.80	6.30	6.70	6.40	6.60	6.50	6.80	6.70	6.70	6.90	6.50	6.60	6.70	6.20	6.60	6.70	6.30	6.70	6.30
Phosphorus(mg/g)	63	41	23	38	65	23	34	58	85	48	47	36	62	43	45	32	60	43	22	31
Potassium (mg/g)	**41**	**31**	**26**	**42**	**43**	**38**	**16**	**32**	**44**	**38**	**31**	**36**	**28**	**23**	**23**	**32**	**23**	**23**	**39**	**22**
Amonium - Nitrogen(mg/g)	**3.22**	**2.28**	**1.20**	**0.83**	**2.45**	**1.37**	**1.48**	**1.16**	**3.88**	**2.16**	**1.80**	**1.72**	**3.35**	**2.65**	**1.23**	**1.45**	**1.68**	**0.67**	**1.25**	**0.68**

ND = NOT DETECTED

Table 1c. Physicochemical properties of Gulf of Guinea sediments and biodegradation profile of PFB-009 (Mixture of Ester and Olefin)

	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 PFB-009(ppm)	26,200	ND	ND	ND	9820	ND	ND	ND	4650	860	ND	ND	1820	210	55	ND	85	36	ND	ND
Redox-Potential (mV)	+ 230	+70	-78	-120	+190	+60	-60	-160	+170	+65	-85	-260	+85	+75	-80	-220	+60	+70	-60	-280
BR-EST utilizing microbial counts (cfu/g x 10^6)	0.10	0.016	0.041	0.0036	0.140	0.060	0.024	0.0055	0.210	0.170	0.057	0.0016	0.110	0.066	0.046	0.0071	0.032	0.0068	0.0030	0.00018
Sulphate (mg/g)	108	93	82	76	85	72	69	43	70	61	65	56	86	53	72	53	78	66	67	41
pH	6.90	6.70	6.20	6.70	6.40	6.50	6.90	6.80	6.70	6.30	6.40	6.50	6.60	6.70	6.50	6.60	6.80	6.30	6.70	6.70
Phosphorus(mg/g)	43	31	43	28	45	33	34	48	35	48	43	36	60	45	35	32	60	53	42	31
Potassium (mg/g)	**61**	**41**	**36**	**42**	**48**	**58**	**36**	**32**	**44**	**38**	**36**	**56**	**58**	**43**	**33**	**22**	**23**	**23**	**39**	**28**
Amonium - Nitrogen(mg/g)	**3.42**	**2.38**	**2.20**	**1.83**	**2.45**	**1.37**	**1.45**	**1.26**	**3.58**	**2.26**	**1.88**	**1.62**	**3.25**	**2.65**	**2.23**	**1.40**	**1.68**	**1.67**	**1.20**	**0.88**

ND = NOT DETECTED

Table 1d. Physicochemical properties of Gulf of Guinea sediments and biodegradation profile of PFE-008 (Ester of Aliphatic acid)

	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 PFE-008(ppm)	27,400	ND	ND	ND	5820	ND	ND	ND	950	160	ND	ND	80	ND	ND	ND	ND	ND	ND	ND
Redox-Potential (mV)	+ 220	+60	-75	-160	+180	+55	-80	-180	+160	+40	-45	-260	+65	+95	-80	-270	+60	+80	-160	-280
BR-EST utilizing microbial counts (cfu/g x 10^6)	0.60	0.036	0.041	0.0026	0.680	0.060	0.034	0.0045	0.610	0.270	0.067	0.0036	0.120	0.046	0.046	0.0021	0.052	0.0011	0.0040	0.00028
Sulphate (mg/g)	88	73	52	36	85	72	63	43	75	66	62	56	80	57	52	53	78	65	67	44
pH	6.90	6.60	6.40	6.70	6.40	6.60	6.70	6.80	6.50	6.30	6.40	6.50	6.60	6.70	6.50	6.60	6.80	6.80	6.20	6.70
Phosphorus(mg/g)	40	41	33	38	45	33	35	48	36	45	43	36	62	35	38	32	62	53	43	31
Potassium (mg/g)	**51**	**44**	**33**	**42**	**45**	**56**	**56**	**32**	**44**	**35**	**36**	**51**	**53**	**48**	**43**	**32**	**43**	**53**	**49**	**58**
Amonium - Nitrogen(mg/g)	**3.22**	**2.48**	**2.40**	**1.63**	**2.45**	**1.77**	**1.55**	**1.66**	**2.88**	**2.56**	**1.88**	**1.62**	**3.25**	**2.45**	**2.23**	**1.30**	**1.38**	**1.57**	**1.20**	**1.50**

ND = NOT DETECTED

The present study have shown the real anaerobic zones of the sandy Gulf of Guinea sediments to be 15-20cm depth and at these depths, little or no traces of the residual ester fluid were found suggesting that the anaerobes isolated from these zones had little or no role to play in the biodegradation sequence. Bothner *et al* (1992) observed that SBF introduced at the sediment penetrated up to a depth of 5cm below the sea floor while Soetaert *et al*, 1996 observed that SBF introduced into the sediment penetrated up to 5cm depth but at 10cm depth, they observed an exponential decrease in bioturbation and very little traces of the compound were found at that depth.

Some of the predominant microaerophilic microorganisms isolated from the sediment subsurface within the depths of 5 and 10 cm are *Vibrio* sp., *Campylobacter* sp., and *Streptococcus* sp. and they all have the capability under laboratory conditions to utilize the ester based fluid as their sole carbon source. Anaerobic microorganisms isolated within the 15cm and 20cm depth were predominantly *Actinomyces* sp., *Clostridium* sp. and *Desulfovibrio* sp. Under laboratory conditions, they all have the capability to utilize the ester based fluids as their sole carbon source but since the residual ester base fluids used in the present study did not diffuse up to that depth, their direct roles in biodegradation of the ester based fluids in the sediment could not be properly established.

Conclusion:

The present study have clearly demonstrated that though the Gulf of Guinea sediments used are loose and sandy in nature, the ester based fluids used to spike the sediments diffused poorly and over 85% of the original fluid spiked were concentrated within the 5cm depth of the sediment where they were degraded by aerobic and microaerophilic microorganisms. Very little concentration (less than 5%) of the residual fluid diffused into the 10cm depth and degradation at this zone was mostly by microaerophilic microorganisms and possibly some facultative anaerobes. Strict anaerobes that inhabit the 15 and 20cm zone practically played little or no direct roles in the biodegradation of these organic compounds so it can be safely concluded from the analytical data obtained from this study that degradation of ester based fluids in the subsurface sediment where oxygen concentration is very low was probably carried out by microaerophilic and facultative anaerobic microorganisms. Degradation of the ester based fluids by strict anaerobic microorganisms in the Gulf of Guinea sediments is not very likely because the fluids were either degraded considerably before they reach such zones or are hardly diffused to such zones.

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