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Full Length Research Paper

Bioremediation of hydrocarbon contaminated-oil field drill-cuttings with bacterial isolates

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The effectiveness of 2 bacterial isolates (*Bacillus subtilis* and *Pseudomonas aeruginosa*) in the restoration of oil-field drill-cuttings contaminated with polycyclic aromatic hydrocarbons (PAHs) was studied. A mixture of 4 kg of the drill-cuttings and 0.67 kg of top-soil were charged into triplicate plastic reactors labeled A1 to A3, B1 to B3, C1 to C3 and O1 to O3. These were left quiescent for 7 days under ambient conditions before adding to reactors A1 - A3 and B1 - B3 respectively, 20 ml working solution of pure cultures of *Bacillus* sp and *Pseudomonas* sp each of cell density 7.6 x 10^{11} cfu/ml. Another 20 ml working solution containing the both cultures at cell density 1.5 x 10^{12} cfu/ml was added to reactors C1 - C3. The working solution was added to each reactor (excluding the controls, O1 - O3) every 2 weeks mixing and watering of the set-ups was done at 3 days interval under ambient temperature of 30^{o}C over a period of 6 weeks. After 6 weeks of treatment, results showed that the predominant 3-ring PAHs, which made up 90% w/w, of the total PAHs concentration of 223.52 mg/kg, were degraded below detection and the 4-ring PAHs were reduced from 4 to 0.6% by the *Pseudomonas* while the *Bacillus* reduced the 3 and 4-ring PAHs respectively to 0.2 and 0.8%. This showed that the *Pseudomonas* degraded the 3 and 4-ring PAHs relatively better than the *Bacillus.* Both strains of bacteria degraded the 5 and 6-ring PAHs below detection limits. Furthermore, within the 3-ring PAHs each of the strains of bacteria reduced phenanthrene to approximately 0.2%, whereas both degraded the homologues acenaphthylene, acenaphthene and fluorene as well as anthracene below detection limits. For the 4-ring PAHs, the *Pseudomonas* degraded fluoranthene and benzo[a]anthracene while the *Bacillus* also degraded benzo[a]anthracene below detection limits. The *Pseudomonas* was able to reduce pyrene and chrysene to 0.3 and 0.2% respectively; whereas the *Bacillus* reduced fluoranthene, pyrene and chrysene to 0.1, 0.01 and 0.4% respectively. However, treatment with the mixed culture resulted in the limited degradation of the 5-ring PAHs particularly in the fourth week, which may be due to the phenomena of cometabolism and inhibition. The pseudo-first-order degradation rate constant of persistent PAHs ranged from 1.9 x 10^{-4} to 9.3 x 10^{-2} day^{-1}. Statistical analyses of results, using the 2-factor analysis-of-variance, showed that the treatments applied resulted in significant ($p < 0.05$) differences in the biodegradation of the PAHs of the drill cuttings after the 6 weeks of treatment.

Key words: Polycyclic aromatic hydrocarbons, petroleum waste, *Bacillus, Pseudomonas,* bioremediation.

INTRODUCTION

Drill cuttings are mixtures of rocks and particulates released from geologic formations in the drill holes made for crude oil drilling. Often, drilling mud and their additives are used in the drilling process and largely, influence the chemistry of the resulting drill cuttings, which are characterised by relatively high total hydrocarbon content (THC) (Okparanma and Ayotamuno, 2008) and polycyclic aromatic hydrocarbons (PAHs) (DPR, 2002). PAHs are the class of hydrocarbons containing 2 or more fused aromatic hydrocarbons. These compounds are environmentally harmful as they can be carcinogenic or mutagenic (Latimer and Zheng, 2003). Due to their abundance in the environment with comparatively simple detectability and toxicity to mammals and aquatic organisms, the USEPA has included in the priority list of pollutants 16 non-substituted PAHs as indicators of PAH pollution. They include naphthalene, acenaphthylene, acenaphthene, anthrax-cene, phenanthrene, fluorene, pyrene, benzo[a]anthraxcene, fluoranthene, chrysene, dibenzo[a,h]anthracene, benzo

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[b]fluoranthene, benzo[k]fluoranthene, benzo[a]py-rene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene (Latimer and Zheng, 2003). Therefore, it becomes imperative to have these drill cuttings treated before their disposal.

Over the years, drill cuttings have always been treated by solidification and stabilization (Akinlade et al., 1996), thermal technologies using (among others) thermal desorption units (TDUs) (Zupan and Kapila, 2000; RLC Technology Inc., 2004) and until recently, by bioremediation (KMC Oiltools, 2005). However, in a leachability test conducted after solidification / stabilization of the drill cuttings, Akinlade et al. (1996) observed that these processes were able to remove only through encapsulation of metals like arsenic, barium, cadmium, chromium and lead. Apart from the high-energy demand and prohibitive costs of thermal treatment technologies (Shkidchenko et al., 2004), personnel and equipment are exposed to the resulting fugitive dusts (DWMIS, 2004). Because of these challenges, the use of a technology similar to remediation by enhanced natural attenuation (RENA) was proposed for the treatment of oil-field drill cuttings (KMC Oiltools, 2005). But, the suggested specifications required for the successful execution of such an on-site project of land area 7.5 m^2/metric ton and land specification having high seasonal water table, are likely to impose a limitation on its application due to recent upsurge in urbanization and industrialization. An alternative technology, which takes into account short operational time, low overall cost, less land mass and eco-sound approaches, becomes a consequent tandem. To this end, bioremediation treatment like bioaugmentation may save time, incur comparatively low overall cost and could be conducted in a relatively small space resulting in sound ecological benefits. Bioaugmentation involves the site-specific application of microbes like fungi, bacteria or their enzyme preparations to increase degradation rates of target contaminants as exemplified in (Lo and Hung, 1995; Odokwuma and Dickson, 2003; Ouyang et al., 2005; Ayotamuno et al., 2007) for other contaminated media. This practice usually is employed when there is a deficiency of competent indigenous microbes in the polluted medium to degrade the contaminants as prevalent in drill cuttings contaminated with oil-based mud (OBM). Some of these specialized microbes can occur naturally while others can be reproduced in the laboratory.

Therefore, assessing the effectiveness, in terms of percentage PAHs reduction, of 2 environmental bacterial isolates (that is, *Bacillus* and *Pseudomonas),* individually and mixed, form the base of the present study. The compositional distribution of individual PAH fractions of the drill cuttings is also investigated, from which, the likely anthropogenic origin of the PAHs is deduced.

MATERIALS AND METHODS

The drill-cuttings

Using plastic containers, properly sealed afterwards with polyethylene materials, composite samples of the drill-cuttings were collected from a mud-pit close to a just-completed crude oil well in Niger Delta region (5º19≠N, 6º28≠E), Nigeria at standard atmospheric pressure for different treatment measures and analyses. Sampling was done strictly in line with DPR (2002) standards for quality assurance. The top-soil, which was obtained from within the research campus of the Rivers state university of science and technology, Nkpolu, Port Harcourt, served as both nutrients and microbes carrier.

Isolation, identification, enumeration and culture of suitable heterotrophic bacteria used in the investigation were done in the department of microbiology, Rivers state university of science and technology, Nkpolu, Port Harcourt. The working solution of the appropriate colony-forming units (CFU) was prepared using serial dilution according to the procedures described in our earlier report (Ayotamuno et al., 2007). The bacterial culture was conveyed from the laboratory to the university research campus in a cooler and stored in a refrigerator, which was maintained at 4^oC for subsequent use.

Experimental design

The bioaugmentation experiment was carried out in 4 triplicate plastic containers labelled reactors A1 to A3, B1 to B3, C1 to C3 and O1 to O3. The drill cuttings 4 kg and the top-soil 0.67 kg at cuttings to soil ratio of 6:1 (Ouyang et al., 2005) was mixed in each reactor and allowed to settle for 7 days for the commencement of microbial activity prior to the addition of working solution. The working solution 20 ml each of pure culture of *Bacillus* and *Pseudomonas* having the cell density 7.6 x 10^{11} cfu/ml was added to reactors A1 to A3 and B1 to B3 respectively. Another 20 ml working solution contain-ing the mixed culture of cell density 1.5 x 10^{12} cfu/ml was added to reactors C1 to C3. The working solution was added to each reactor (excluding the controls, O1 to O3) after every 2 weeks, whereas mixing and watering of set-ups were done at 3 days interval under the ambient temperature of 30^oC over a period of 6 weeks. Com-posite samples from each reactor were taken, using hand trowel, at 2 weeks interval for laboratory analyses.

Theory

The rate of degradation of selected PAHs was carried out by comparing their reaction rate constants of the pseudo-first- order kinetics. According to Lagergren (1898), the integrated and linearized pseudo-first- order kinetic expression is given as:

$$\log(C_o - C_t) = \log C_o - \frac{K_1}{2.303}t \quad (1)$$

The value of the reaction rate constant, K_1 was determined by regression analysis by fitting on a number of experiment-tal data points, using the LINEST function in Microsoft® Excel 2007.

Laboratory analyses

The drill cutting samples were analyzed for polycyclic aromatic hydrocarbons (PAHs) according to the procedures of USEPA (1996) method 8270B using an AGILENT TECHNOLOGY Gas Chromatogram Model 6890N equipped with a flame ionization detector (FID). The THC of the drill cutting samples was analyzed according to standards of the ASTM (1999) method D3920 using a SHIMADZU infrared spectrophotometer by measuring the light absorbance at the wavelength range of 3333 - 3704 nm. Bonny light crude oil was

used to calibrate the equipment before hand.

Statistical evaluations

Standard deviation (SD), using the STDEV function in Microsoft® Excel 2007 and simple percentages were calculated. Data analysis was performed using the 2-factor analysis of variance (ANOVA) with replication. The total variation of the data set was determined using equation (2) below:

$$V = V_{RCIE} + V + V + V \quad (2)$$

Where

$$V = \sum_{j,k,l} (X_{jkl} - \bar{X})^2 \quad (3)$$

$$V_R = bc \sum_{j=1}^{a} (\bar{X}_{j..} - \bar{X})^2 \quad (4)$$

$$V_C = ac \sum_{k=1}^{b} (\bar{X}_{..k} - \bar{X})^2 \quad (5)$$

$$V_I = c \sum_{j,k} (\bar{X}_{jk.} - \bar{X}_{j..} - \bar{X}_{.k} + \bar{X})^2 \quad (6)$$

$$V_E = \sum_{j,k,l} (X_{jkl} - \bar{X}_{jk.})^2 \quad (7)$$

The values of these variations, deduced with equations (2) to (7), were then used to set up the analysis-of-variance table. Using the appropriate degrees of freedom for each source of variability, the mean squares and *F* ratios were determined. The deduced *F* ratios were then compared with their corresponding critical *F* values. Significance of differences was evaluated at $p < 0.05$.

RESULTS

THC and PAHs characteristics of the drill cuttings

Characteristics of THC and PAHs of the untreated drill cuttings are presented in Table 1. It is evident from the Table that the level of THC in the drill cutting samples (that is, 82,195 mg/kg) far exceeded the discharge limit of 50,000 mg/kg set by Nigerian government department of petroleum resources. This implies that the drill cuttings are not safe for land disposal without prior treatment.

The PAHs are composed of 2 - 6 fused rings with molecular mass ranging from 128 g/mol in naphthalene to 278 g/mol in dibenzo[a,h]anthracene. The total PAH concentration of the drill cuttings is 223.52 mg/kg. The most abundant PAH fraction is acenaphthylene (70.7 mg/kg, dry weight) while the least abundant is fluoranthene (1.67 mg/kg, dry weight). Naphthalene and indeno [1,2,3-cd] pyrene are however, not within their laboratory detection limits. The predominant PAH ring-group in the drill cuttings is the 3-ring PAHs (representing 90% of the total PAHs) and consists of acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene.

Biodegradation of PAHs in relation to ring-group and molar-mass

The degree of degradation of individual PAHs in relation to molar-mass and ring groups over 6 weeks of bioremediation showed distinct variations as depicted in Table 3. For 2-ring PAHs, characterized by identical molar-mass of 128 g/mol, their presence was short-lived as they were not detected after the first 2 weeks of remediation. In the case of 3-ring PAHs with molar-mass ranging from 166 – 178 g/mol, the amount remained after the first 2 weeks of treatment ranged from 0.4 - 0.9%. In the remaining period of treatment, the amount of 3-ring PAHs continued to decrease though not as much as in the initial weeks before plummeting to (0.1 - 0.8%) in the last week of the treatment. On the other hand, the 4-ring homologues, benzo[a]anthracene and chrysene with the molar-mass 228 g/mol showed relatively lower losses all through the experiment of 6 weeks. For the 5-ring PAHs, having molar-mass ranging from 252 - 278 g/mol, they showed an almost similar tendency as exhibited by 4-ring PAHs. However, unlike 4-ring PAHs, 5-ring PAHs showed relatively lower loses especially towards the end of the treatment period, as their presence was undetected, except for benzo [b] fluoranthene. PAHs of 6-rings with molar-mass of 276 g/mol showed relatively lower lose as compared to 5-ring PAHs, which occurred at the initial stage of the experiment. However, 6-ring PAHs behaved almost like 5-ring PAHs in the weeks of the experiment.

Comparison of PAH degradation by *Bacillus* and *Pseudomonas*

The impact of bacterial isolates (*Bacillus* and *Pseudomonas*), in terms of percentage of the PAH degraded over time, is shown in Figures 1 to 3. The predominant 3-ring PAHs, which made up 90% w/w, of the total PAHs concentration of 223.52 mg/kg, were degraded below detection and the 4-ring PAHs were reduced from 4 to 0.6% by the *Pseudomonas* (Figure 2) while the *Bacillus* (Figure 1) reduced the 3- and 4-ring PAHs respectively to 0.2 and 0.8%. This showed that the *Pseudomonas* degraded the 3- and 4-ring PAHs relatively better than the *Bacillus.* Both strains of bacteria degraded the 5- and 6-ring PAHs below detection limits (Figure 3). Furthermore, within the 3-ring PAHs each of the strains of bacteria reduced phenanthrene to approximately 0.2%, whereas both degraded the homologues acenaphthylene, acenaphthene and fluorene as well as anthracene below detection limits (Table 3). For the 4-ring PAHs, the *Pseudomonas* degraded fluoranthene and benzo[a]anthracene while the *Bacillus* also degraded benzo[a]anthracene below detection

Table 1. THC and PAHs composition of the untreated drill cuttings sample.

Molar mass (g/mol)	Characteristics	Laboratory detection limit (mg/kg)	Value (mg/kg)	Ring group	Composition (%)	DPR (2002) Discharge-limits (mg/kg)
	THC	**-**	**82,195 ± 302.52**	**-**	**-**	**50,000**
			PAH fractions			
128	Naphthalene	1.00	nd	2-ring	1	-
	2-Methylnaphthalene	0.20	1.96 ± 0.16			
166	Acenaphthylene	1.00	70.7 ± 0.23	3-ring	90	-
	Acenaphthene	0.70	61.9 ± 0.22			
	Fluorene	0.70	36.9 ± 0.24			
178	Phenanthrene	0.20	21.4 ± 0.22			
	Anthracene	0.40	9.83 ± 0.17			
202	Fluoranthene	0.20	1.67 ± 0.19	4-ring	4	-
	Pyrene	0.20	2.27 ± 0.21			
228	Benzo [a] anthracene	0.20	2.29 ± 0.15			
	Chrysene	0.20	2.91 ± 0.22			
252	Benzo [b] fluoranthene	0.30	2.38 ± 0.16	5-ring	4	-
	Benzo [k] fluoranthene	0.20	3.03 ± 0.19			
	Benzo [a] pyrene	0.20	2.10 ± 0.21			
278	Dibenzo [a,h] anthracene	0.20	1.74 ± 0.24			
276	Benzo [g,h,i] perylene	0.20	2.44 ± 0.21	6-ring	1	-
	Indeno [1,2,3-cd] pyrene	0.20	nd			
TOTAL			223.52		100	-

Values represent mean ± standard deviation of triplicate samples; nd = not detected.

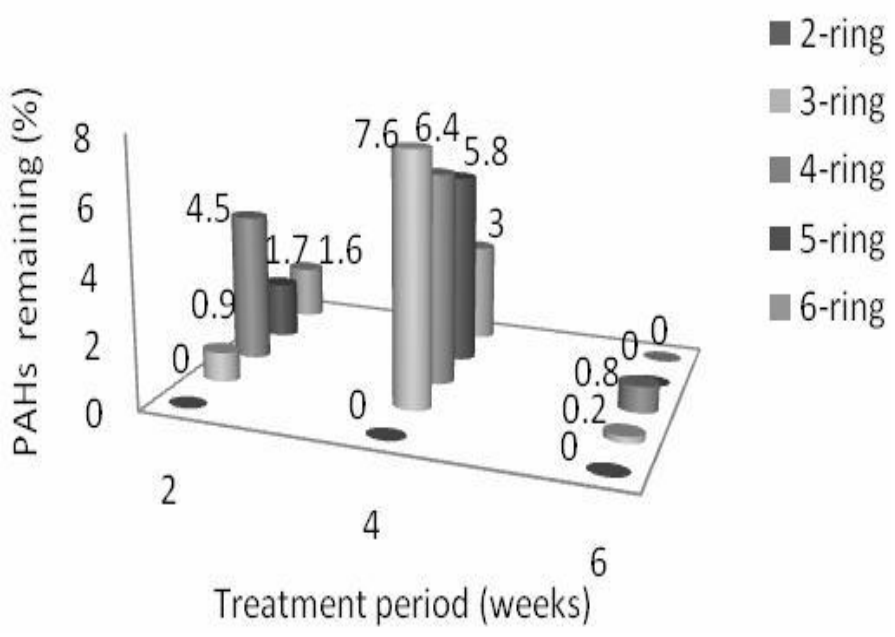

Figure 1. Performance of *Bacillus* (reaction A).

limits (Table 3). The *Pseudomonas* was able to reduce pyrene and chrysene to 0.3 and 0.2% respectively; whereas the *Bacillus* reduced fluoranthene, pyrene and chrysene to 0.1, 0.01 and 0.4% respectively (Table 3).

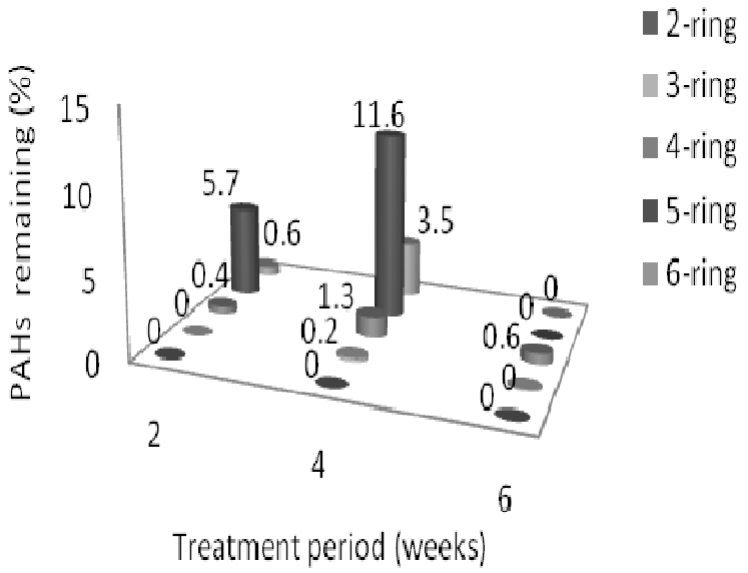

Figure 2. Performane of Pseudomonas (reaction B).

Table 2. PAH diagnostic ratio analysis.

PAHs ratio	Drill cuttings (This study)	PAHs source criteria**		PAHs molar mass
		Petrogenic	Pyrogenic	
ANT/(PHE + ANT)	0.31	≤0.10	>0.10	178
FLR/(PYR + FLR)	0.40	≤0.50	>0.50	202

**According to Hites and Gschwend (1982) cited in Okoro and Ikolo (2007)
ANT= Anthracene
PHE = Phenanthrene
FLR= Fluoranthene
PYR = Pyrene.

However, treatment with the mixed culture resulted in the limited degradation of the 5-ring PAHs particularly in the fourth week (Figure 3).

Discussion

Sources of PAHs in the drill cuttings

Yunker and McDonald (1995) described that the PAHs concentration and compositional distribution in a sample were often used to distinguish petrogenic (of petroleum) origin and pyrogenic (of combustion) origin of sources. They summarized criteria normally used in 4 strategies:

i) Number of PAH alkyl homologous series present
ii) PAH stability
iii) Number of source-specific PAHs present and
iv) Principal component analysis.

With the use of number of source-specific PAHs present criterion, it was evident from Table 1 that the PAHs in the drill cuttings were pyrogenic in nature since acenaphthylene (70.7 mg/kg) and acenaphthene (61.9 mg/kg) were more in the drill cuttings. This might be due to the possible combustion of the hydrocarbons by the heat generated during drilling. Furthermore, according to Yunker and McDonald (1995), in assessing if the PAHs is source pyrogenic the best strategy is to consider ratios of a number of PAHs of different molar-mass. To this end, a modified PAH diagnostic ratio analysis approach (Okoro and Ikolo, 2007) based on double-indicator ratio, due to source overlap, which was earlier used by Hites and Gschwend (1982) on single-indicator ratio was also used. For an environmental sample and data thus obtained are presented in Table 2. The source of the PAHs in the drill cuttings was confirmed as pyrogenic since the ANT/(PHE+ANT) ratio > 0.10 for fraction of molar-mass 178 g/mol. Another possible anthropogenic source of the PAHs in the drill cuttings was petrogenic since the FLR/(PYR+FLR) ratio < 0.50 for fraction of molar-mass 202 mg/kg. This observation strongly indicated that oil-based mud (OBM) containing petroleum fraction - diesel invert - has been used during drilling. It may therefore, be inferred that the PAHs in the drill cuttings are pyrogenic and

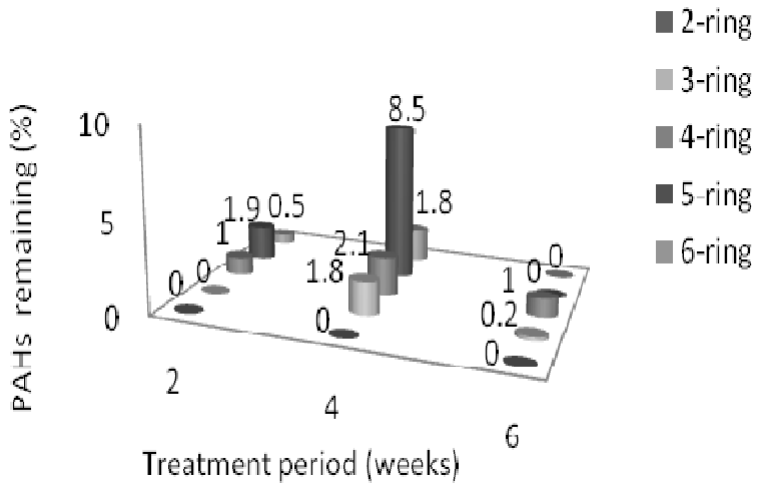

Figure 3. Performance of *Bacillus* + *Pseudomonas* (reaction C).

petrogenic in nature.

PAHs degradation in relation to molar mass and ring group

The sudden disappearance of the 2-ring PAHs (Table 3) might be due to their physical properties. These properties include high volatility, slight solubility in water (31 mg/l), high vapour pressure, 2 number of rings and low molar-mass (128 g/mol) (Latimer and Zheng, 2003). These might make them less likely absorbent by solid matter and unavailable to PAH-degrading organisms (Yunker and McDonald, 1995; Johnsen et al., 2005). The reduced rate of decrease in the concentration of the 3-ring PAHs having molar mass ranging from 166 to 178 g/mol particularly towards the end of the treatment period (Table 3) might be due to endogenous decay. The behaviour of the 3-ring PAHs was similar to the one observed by Oleszczuk and Baran (2003) in the case of soil polluted with the aircraft fuel. The 4-ring PAHs, fluoranthene and pyrene, having the molar mass 202 g/mol behaved almost in a similar fashion throughout the treatment. This might be because they are homologues. These characteristics of 4-ring PAHs were also corroborated by Oleszczuk and Baran (2003). The 5-ring PAHs showed an almost similar tendency as exhibited by 4-ring PAHs. The similarities might be due to molecular composition as suggested by Oleszczuk and Baran (2003).

The rate of degradation of the persistent PAHs showed distinct variations both in terms of their properties (molar mass and ring group) and in terms of the type of bacteria employed in the bioaugmentation (Table 4). The values of the degradation rate constant for individual PAHs va-ried between 1.9 x 10^{-4} day^{-1} in anthracene and 9.3 x 10^{-2} day^{-1} in phenanthrene. From the Table 4, the average value of K_1 for phenanthrene (3-ring) was 6.2 x 10^{-2} day^{-1} and was observed to be close to the range of 5.6 x 10^{-3} to 4.3 x 10^{-2} day^{-1} suggested by Shuttleworth and Cerniglia (1995) for phenanthrene in soils and sediments. For the higher ring PAHs, results of Table 4 showed that bezo [a] pyrene (5-ring), for instance, had an average K_1 value of 5.4 x 10^{-3} day^{-1}, which was also close to the suggested range of 5.0 x 10^{-4} to 3.0 x 10^{-3} day^{-1} for B [a] P in soils and sediments. These results also highlight the view in the literature (Kanaly and Harayama, 2000) that PAHs with less than 4 rings are particularly susceptible to bio-degradation whereas, the biodegradation of PAHs with more than 4 rings is difficult but possible (Juhasz et al., 1997).

Effect of the mixed strains on the biodegradation of PAHs

According to Bouchez et al. (1995), bacterial isolates often degrade a narrow range of PAHs, but patterns of simultaneous degradation of PAH mixtures is complex (Johnsen et al., 2005). The phenomena of co-metabolism and inhibition might be responsible for the pattern of PAH degradation observed when the mixed culture was used (Figure 3). In the case of a mixture of 2 individually degradable PAHs, Bouchez et al. (1995) observed preferential degradation of one or reduced degradation of both PAHs indicating the presence of metabolic competition. In a subsequent study, Bouchez et al. (1999) observed that the mixed culture of 2 or more strains, might possess the capacity to mineralize each of the PAHs effecting the limited degradation of a 5-ring PAH. This corroborated our observation on the cometabolism of 3- or 4-ring PAHs having no synergetic effect on 5-ring PAHs particularly in the fourth week of the experiment when inoculated with the mixed culture causing the limited degradation of 5-ring PAHs.

Table 3. Comparison of the biodegradation of the PAHs in the drill cuttings by the stated microbes.

PAHs fraction (mg/kg)	Ring group	Treatment period								
		2 weeks			4 weeks			6 weeks		
		A	B	C	A	B	C	A	B	C
Naphthalene	2-ring	nd	nd	nd	nd	nd			nd	nd
2-Methylnaphthalene		nd	nd	nd	nd	nd			nd	nd
Acenaphthylene	3-ring	nd	nd	nd	2.26±0.11	nd	nd	nd	nd	nd
Acenaphthene		nd	nd	nd	3.01±0.08	nd	nd	nd	nd	nd
Fluorene		nd	nd	nd	2.10±0.11	nd	nd	nd	nd	0.97± 0.05
Phenanthrene		0.63±0.01	nd	0.30±0.02	5.00±0.20	0.34± 0.02	nd	0.41± 0.02	0.33±0.01	0.28± 0.02
Anthracene		1.43±0.01	nd	0.52±0.02	4.55±0.13	nd	nd	nd	nd	0.57± 0.02
Fluoranthene	4-ring	3.06±0.19	nd	0.20±0.03	3.93±0.11	1.04± 0.16	2.17± 0.15	0.24± 0.02	nd	0.58± 0.02
Pyrene		3.15±0.18	0.28± 0.03	1.04± 0.13	3.80±0.20	0.86± 0.05	1.52± 0.07	0.25± 0.01	0.71±0.02	0.48± 0.02
Benzo [a] anthracene		2.81±0.11	0.31± 0.03	0.39± 0.01	0.80±0.10	0.42± 0.03	0.51± 0.03	nd	nd	0.33± 0.01
Chrysene		1.04±0.05	0.21± 0.02	0.60± 0.02	5.88±0.24	0.50± 0.10	0.50±002	1.19± 0.22	0.59±0.02	0.84± 0.05
Benzo [b] fluoranthene	5-ring	0.54±0.03	nd	0.61± 0.01	1.37±0.06	1.04± 0.06	0.56± 0.02	nd	nd	nd
Benzo [k] fluoranthene		0.48±0.01	nd	0.46±0.02	0.82±0.07	1.68±0.10	0.26± 0.02	nd	nd	nd
Benzo [a] pyrene		0.28±0.02	0.28± 0.02	0.33±0.01	1.59±0.10	0.88± 0.02	1.01± 0.10	nd	nd	nd
Dibenzo [a,h] anthracene		2.72±0.13	12.5±0.20	2.75± 0.02	9.23±0.16	22.3± 0.15	17.10±0.15	nd	nd	nd
Benzo [g,h,i] perylene	6-ring	0.70±0.10	0.44± 0.02	nd	3.29±0.10	4.26± 0.27	3.17± 0.21	nd	nd	nd
Indeno [1,2,3-cd] pyrene		2.87±0.09	0.94± 0.04	1.18± 0.13	3.34±0.07	3.53± 0.04	0.78± 0.03	nd	nd	nd
Total PAHs remaining (mg/kg)			19.71	14.96	8.38	50.97	36.85	27.58	2.09	1.63

Values represent mean ± standard deviation of three replicates;
A = Bioaugmentation with *Bacillus*;
B = Bioaugmentation with *Pseudomonas*;
C = Bioaugmentation with *Bacillus* + *Pseudomonas;*
nd = not detected;
Control is not shown

However, it was observed that acenaphthylene in the first2 weeks of the treatment with *Bacillus* was not within the laboratory detection limit, but in the fourth week increased to 2.26 mg/kg (Table 3). Such phenomenon was also observed in fluorene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[a,h,i]pyrene and indeno[1,2,3-cd]pyrene. This might be because, according to several literatures, complete biodegradation of toxic and recalcitrant organic compounds like PAHs to harmless end-products may not always occur, instead the biotransformation of some fractions may have occurred and caused the concentrations of fractions previously less than the laboratory detection limits to rise above such limits.

Statistical analyses

Table 5 shows the results of the 2-factor ANOVA performed on the results of the biodegradation of the observed across the treatments applied and did not have significant effect on the biodegradation of the PAHs after 6 weeks of treatment. PAHs. The results show that the row and column sources of variability were significant at the 0.05 probability level, which implies that there were significant differences in the biodegradation of the PAHs due to the treatments and remediation period. In order words, the treatments applied resulted in significant diffe

Table 4. Comparison of the degradation rate constants of some PAHs.

PAHs	Molar mass (g/mol)	K_1 (day^{-1})			
		A	B	C	Average
Phenanthrene	178	3.7×10^{-4}	9.3×10^{-2}	9.3×10^{-2}	6.2×10^{-2}
Anthracene	178	5.6×10^{-3}	-	1.9×10^{-4}	2.9×10^{-3}
Fluoranthene	202	1.3×10^{-2}	-	1.7×10^{-2}	1.5×10^{-2}
Pyrene	202	2.5×10^{-2}	1.6×10^{-2}	5.3×10^{-2}	3.1×10^{-2}
Benzo [a] anthracene	228	3.0×10^{-2}	1.9×10^{-2}	5.5×10^{-4}	1.7×10^{-2}
Chrysene	228	3.0×10^{-3}	5.4×10^{-3}	3.9×10^{-3}	2.0×10^{-2}
Benzo [b] fluoranthene	252	9.2×10^{-3}	2.0×10^{-2}	1.1×10^{-2}	1.3×10^{-2}
Benzo [k] fluoranthene	252	6.2×10^{-3}	-	5.9×10^{-3}	6.1×10^{-3}
Benzo [a] pyrene	252	5.1×10^{-3}	5.1×10^{-3}	6.1×10^{-3}	5.4×10^{-3}
Dibenzo [a,h] anthracene	278	2.0×10^{-2}	2.0×10^{-2}	2.0×10^{-2}	2.0×10^{-2}
Benzo [g,h,i] perylene	276	1.2×10^{-2}	2.1×10^{-2}	-	1.7×10^{-2}

A = Bioaugmentation with *Bacillus*;
B = Bioaugmentation with *Pseudomonas*;
C = Bioaugmentation with *Bacillus* + *Pseudomonas*

Table 5. 2-factor ANOVA on the result of the biodegradation of PAHs.

Sources of Variability	Variations	df.	Mean Squares	F ratio
Row	V_R = 2527.35	2	$S_R^2 = \frac{V_R}{df}$ = 1263.675	S_R^2 / S_E^2 = 2252.54 ⇒ S
Column	V_C= 8014.35	2	$S_C^2 = \frac{V_C}{df}$ = 4007.175	S_C^2 / S_E^2 = 7142.91 ⇒ S
Interaction	V_I= -1494.59	4	$S_I^2 = \frac{V_I}{df}$ = -373.648	S_I^2 / S_E^2 = -666.04 ⇒ NS
Error	V_E = 10.10	18	$S_E^2 = \frac{V_E}{df}$ = 0.561	-
Total	V = 9057.21	26	-	-

NS = Not Significant at $p<0.05$
S = Significant at $p<0.05$
df. = Degree of Freedom

ences in the biodegradation the PAHs of the drill cuttings over time. However, the interaction source of variability, according to the results, was not significant at the 0.05 probability level suggesting that significant differences were not

Conclusion

i) The chemical characteristics of the drill cuttings are by far more than the safe limits fixed by Nigerian government DPR. Therefore, the drill cuttings are unsafe to dispose off without prior treatment.

ii) The PAHs of the drill cuttings consists of 2 - 6 fused rings with molar-masses ranging from 128 – 278 g/mol. The total PAH concentration of the drill cuttings is 223.52 mg/kg. The most abundant PAH fraction is acenaphthylene (70.7 mg/kg, dry weight) while the predominant PAH ring-group in the drill cuttings is the 3-ring PAHs (representing 90% of the total PAHs) and consists of acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene.

iii) Some PAHs are pyrogenic and others petrogenic. The pyrogenic PAHs are due to possible combustion of hydrocarbons by the heat generated in the drill-bit during drilling operations. The presence of petrogenic PAHs in the drill cuttings is a strong indication of the OBM containing the diesel-invert petroleum fraction, which might have been used during drilling. This completely contravenes

the ban by Nigerian government DPR on the use of OBMs while drilling in its shore and near-shore lines.

iv) The individual PAHs like 2-ringed one having the molar mass 128 g/mol were remediated faster. In the case of predominant 3-ring PAHs with molar-mass of 166 – 178 g/mol, the amounts remained longer, 0.4 - 0.9% remained after 2 weeks of treatment, thereafter also continued to decrease, at a diminishing rate.

v) Considering the degradability of individual PAHs by *Bacillus subtilis* and *Pseudomonas aeruginosa* isolates, the 3- and 4-ring PAHs are relatively better degraded by *Pseudomonas*. Both bacterial strains degraded the 5-and 6-ring PAHs by equal proportions. Meanwhile, the use of the mixed culture of bacterial isolates led to the li-mited degradation of the 5-ring PAHs.

Abbreviations: ANOVA; Analysis of Variance, **APHA;** American Public-Health Association, **ASTM;** American Society for Testing and Materials, **CFU;** Colony Forming Unit, **DPR;** Department of Petroleum Resources, **IRS;** Infrared Spectrophotometer, **OBM;** Oil-Based Mud, **PAH;** Polycyclic Aromatic Hydrocarbons, **RENA;** Remediation by Enhanced Natural Attenuation, **THC;** Total Hydrocarbon Content and **USEPA;** United States Environmental Protection Agency.

Nomenclature: a, b, c; treatments, blocks and entries respectively $\mathbf{C_0}$, $\mathbf{C_t}$; PAH concentration at the initial and over a time period t (mg/kg) respectively, $\mathbf{K_1}$; Pseudo-first order kinetic constant (day^{-1}), **j, k, l**; row (treatment), column (block) and repetition (replication) respectively, **t**; time (days), $\mathbf{V_C}$, $\mathbf{V_E}$, $\mathbf{V_l}$, $\mathbf{V_R}$, **V** ; variation due to columns, error, interaction between rows and columns, rows and total variation of the data set respectively and $\overline{X}_j$, $\overline{X}_k$, $\overline{X}$; mean of the entries in the jth row, kth column and grand mean respectively.

Recommendations

i) Drill cuttings may be bio-treated, to bring down the contaminants level to acceptable level before its land disposal to reduce the environmental pollution.

ii) The department of petroleum resources (DPR) in Nigeria may redouble effort to enforce the ban on the use of the OBM by crude-oil producing companies operating in the country.

iii) Bioaugmentation may be adopted as a good treatment method for the drill cuttings contaminating 2- to 6-ring PAH.

iv) The choice of bacterial consortium to be used in the bio-treatment of the oil-field drill cuttings may favour *Pseudomonas* and *Bacillus.* As far as possible, the pure strains instead of the mixed stains may be made.

v) The findings may be adopted by the concerned companies in treating the drill cuttings to reduce the, cost, energy and pollution associated with the thermal treatment.

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