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Full Length Research Paper

Enhanced remediation of an oily sludge with saline water

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This study investigates the potentials of saline (that is, brackish) water to enhance the remediation of an oily sludge, which was part of the waste stream from the improvement project of the Tank Farm at the Bonny Island in the Niger Delta region of Nigeria. Twice weekly, five separate laboratory-scale reactors (labeled A, B, C, D and O), each containing 2.0×10^{-2} m^3 of the diluted sludge samples, received 170 g of liquid 20:10:10-NPK-fertilizer (corresponding to an application rate of approximately 4.3 kg-N-m^{-3}, 2.1 kg-P-m^{-3} and 2.1 kg-K-m^{-3} of diluted sludge). On a weekly basis, control reactors A and B received 5.0×10^{-4} and 1.5×10^{-3} m^3 of fresh water respectively while 'treatment' reactors C and D received 5.0×10^{-4} and 1.5×10^{-3} m^3 of saline water (containing 4.54 g/L of NaCl) respectively. Reactor O, which served as a counterfactual, was only rain-fed. Equal oxygen exposure levels, through regularly scheduled tilling, was maintained in all five reactors. After 12 weeks of treatment (that is, from May to August, 2007), sludge physicochemical characteristics showed distinct variations. The saline water treated-reactor D, had a 7-fold increment in bacterial population while the fresh water treated-reactor B, had an approximately 3-fold increment in bacterial population. The drop in the hydrocarbon content of the saline water-treated reactors ranged from 41.7 to 55.9% whereas in the fresh water-treated reactors, the hydrocarbon losses ranged from 17.3 to 25.0%. These results showed the possibility of enhanced biodegradation of oily sludge by hydrocarbon utilizing bacteria (*Bacillus subtilis*) at salinity (NaCl concentration) of 4.54 g/L.

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Key words: Bioremediation, biostimulation, oily sludge, saline water*, Bacillus subtilis*.

INTRODUCTION

A huge amount of oily sludge is often generated during the cleaning up of crude oil storage tanks, maintenance of associated facilities and pre-export processing activities of crude oil at ocean terminals (that is, tank farms). Because oily sludge contains toxic substances like aromatic hydrocarbons (benzene, toluene, ethyl benzene and xylene), poly-aromatic hydrocarbons (Swoboda-Colberg, 1995) and high total hydrocarbon content (Ayotamuno et al., 2007), its disposal without adequate treatment leads to environmental, particularly soil, pollution. Apart from recent socio-economic problems like militancy and kidnapping, occasioned by the neglect of their corporate social responsibilities, the treatment of this oily sludge has become one of the major problems facing crude oil-producing multinational companies operating in developing countries like Nigeria. This is because, the officially recommended (DPR, 2002) treatment method, incineration is prohibitively expensive (Shkidchenko et al., 2004) and exposes personnel and equipment to the resulting fugitive dusts. Consequently, it is important to adopt a cheaper and much more ecologically sound treatment technique for this type of petroleum waste.

In recent times, several literatures have shown that bioremediation has high potentials for restoring polluted media with least negative impact on the environment at relatively low cost. Bioremediation, the basis of which may date back to the work of Atlas and Bartha (1972), is the use of microorganisms (bacteria and fungi) to

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accelerate the natural decomposition of hydrocarbon-contaminated waste into nontoxic residues. This technology has been used in bio-treating exploration and production (E&P) wastes (McMillen et al., 2004). However, intrinsic bioremediation has been observed to be a very slow process, which could take years to yield the desired results (Mitchell et al., 2000; Wills, 2000). To remedy this situation, taking into account overall costs and operational time, numerous researchers have demonstrated high bioremediation efficiency for oil polluted soils by adopting various strategies to aid bioremediation (Antai, 1990; Amadi et al., 1993; Onwurah, 1996; Odokwuma and Dickson, 2003; Obire and Akinde, 2004; Ebuehi et al., 2005; Adenipekun and Fasidi, 2005; Okolo et al., 2005; Ayotamuno et al., 2006; Abu and Atu, 2007; Adoki and Orugbani, 2007).

However, these methods have limitations for an oily sludge, which is mainly characterized by extremely high pollution levels and contaminants recalcitrant to bioremediation such as polycyclic aromatic hydrocarbons (PAHs) with more than five rings (Allard and Neilson, 1997). This may be partly because indigenous bacteria in the soil can degrade target constituents of oily sludge only to an extent dependent on their abundance or deficiency in the medium especially, when the contaminants are present at high concentrations (Mishra et al., 2001).

Therefore, to pave the way for the effective bioremediation of a heavily polluted oily sludge by bacteria, it is necessary to bio-augment the indigenous bacterial population with bacterial isolates and/or to create necessary conditions that will encourage microbial degradation such as nutrient addition, exposure to oxygen, and maintaining an optimal soil moisture, temperature and pH. Ayotamuno et al. (2007) had adopted bio-augmentation strategy to enhance the bioremediation of a sludge containing hydrocarbons. On the other hand, despite the assumption in the literature (Oren et al., 1992) that microbial activity and thus, the rate of biodegradation decreases dramatically with rising salinity, Al-Mahruki et al. (2006) used saline water to enhance land-farm performance in the remediation of a hydrocarbon-contaminated drilling mud/sand mixture. However, the effectiveness of saline water in the bioremediation of hydrocarbon-contaminated sludge has yet got little attention. The present study, therefore, aimed to investi-gate the potentials of saline (that is, brackish) water to enhance the bioremediation of a sludge containing hydrocarbons.

MATERIALS AND METHODS

Experimental materials

The oily sludge used for this study was obtained from the Ocean Terminal at the Bonny Island ($4^0$27'N, $7^0$14'E). The fresh water was collected from Orashi River ($4^0$57'N, $6^0$31'E) and the saline water from Elelenwo Creek ($4^0$50'N, $7^0$04'E), all in Rivers State in the Niger Delta ($5^0$19'N, $6^0$28'E) of Nigeria. The Niger Delta region is located in the Southern Nigeria coastal zone and is Africa's largest delta covering some 70,000 km^2. The region is home to Nigeria's oil and gas industries with most of the country's oil and gas fields located in the area (Niger Delta Environmental Survey, 1995), making the region most vulnerable to environmental pollution arising from petroleum waste discharges.

Sludge sampling

From the uppermost, 5 cm layer of the composite sludge, random samples were collected every 3 days for 3 weeks in air-tight plastic containers and put in Ziploc bags. Some samples were taken to the laboratory for microcosm studies while others were taken to the Research Campus of the Rivers State University of Science and Technology, Port Harcourt, for treatment. Samples for microbial analyses were conveyed to the laboratory in a cooler and refrigerated at 4°C until analysis.

Cultivation and enumeration of heterotrophic bacteria

The bacteria were cultivated by serial dilution of the sludge sample with normal saline-solution. The normal saline solution was prepared adopting the methods described in Ayotamuno et al. (2007). Using nutrient agar and oil agar (prepared in the Microbiology Laboratory of the University), 1.0 g (dry weight) of fine sludge was homogenized with 1.0×10^{-6} m^3 of sterile distilled-water to give 10^{-1} dilutions; and 1×10^{-6} m^3 of the 10^{-1} dilutions was transferred into the next test tube containing 9.0×10^{-6} m^3 normal saline (diluent) and diluted serially in one tenth steps up to 10^{-3} dilution (Harrigan and McGrane, 1990 for further details). From the dilutions of 10^{-2} and 10^{-3} of each sludge sample, a 0.1×10^{-6} m^3 aliquot was transferred aseptically onto nutrient agar-plates, supplemented with oil agar, and spread with a bent glass-rod. The inoculated plates were incubated at 37°C for 24 h. Thereafter, the plates were examined for any growths on them. Discrete colonies of heterotrophic bacteria in the sludge were recorded.

Isolation and identification of heterotrophic bacteria

Culturable bacteria were prepared by aseptically streaking representative colonies of different cultural types, which appeared on the culture plates, onto nutrient agar-plates and incubated at 28^0C for 24 h. The nutrient agar plates were stored in a refrigerator and served as pure stock culture for subsequent characterization and identification tests. Standard characterization tests (such as gram staining, motility, methyl-red, Vogues Proskaver, indole, citrate utilization and sugar fermentation) were performed. The pure culture was identified on the basis of its cultural, morphological and physiological characteristics (Cowan, 1974; Buchanan and Gibbons, 1974).

Experimental procedure

Before treatment commenced, the oily sludge was diluted with sandy-loam soil in the ratio of three parts of oily sludge to one part, by volume, of the topsoil to achieve a THC of 64,494 mg/kg. This was done to enhance aeration and the infiltration of nutrients since oily sludge is highly compact and tends to retard nutrient infiltration and aeration. The diluted sludge sample was then left to incubate for 72 h before transferring 2.0×10^{-2} m^3 of the sludge sample into five separate laboratory-scale reactors (O, A, B, C and D) and quickly homogenized with 170 g of 20:10:10-NPK-fertilizer in turn. The fertilizer was applied in liquid form twice a week until the end of the remediation period. This was to provide nitrogen, phosphorus

Table 1. Physicochemical characteristics of the oily sludge.

Parameter	Initial sludge sample	Sludge sample 3 days after dilution with sandy-loam soil	DPR (2002) limits
Moisture content (%)	2.5 ± 1.9	5.5 ± 2	-
pH (sludge: Water = 1:2.5)	5.44 ± 1.2	5.51 ± 1.86	-
Total hydrocarbon content (THC) (mg/kg)	98,032 ± 320	64,494 ± 150	50,000
Available phosphorus (mg/kg)	14.8 ± 2	31.5 ± 3	-
Total organic carbon (TOC) (%)	0.95 ± 0.09	0.72 ± 0.04	-
Total nitrogen (TN) (%)	0.20 ± 0.05	0.25 ± 0.01	-
Carbon: nitrogen ratio (C/N)	4.75 ± 0.6	2.88 ± 0.5	-

Values are given in mean ± standard deviation of three readings.

and potassium, which are major limiting nutrients in the growth of sludge microorganisms. As may be inferred from previous study (Ayotamuno et al., 2006), this quantity of fertilizer corresponded to a fertilizer application rate of 8.5 kg/m^3 of diluted sludge sample and provided each reactor with approximately 4.3 kg of nitrogen, 2.1 kg of phosphorus and 2.1 kg of potassium per application. In addition, an equal level of oxygen exposure was maintained in the five reactors. This was achieved through tilling, which was done five times a week throughout the remediation period. Control reactors A and B received 5.0 × 10^{-4} and 1.5 x 10^{-3} m^3 of fresh water respectively while 'treatment' reactors C and D received 5.0 × 10^{-4} and 1.5 x 10^{-3} m^3 of saline water (containing 4.54 g/L of NaCl) respectively on a weekly basis. Reactor O, which served as the counterfactual, was only rain-fed. At six weeks intervals, triplicate composite sludge samples were randomly collected from each reactor for laboratory analyses.

Chemical and instrumental analysis

The pH of the sludge extract (sludge: Water = 1:2.5) was determined using a WTW Multi-340 pH-meter according to ASTM (1999) method D4972. Total organic carbon (TOC) was determined according to BS 1377 (FUGRO-PRODEC Laboratories Ltd., 2007). Total nitrogen (TN) was determined according to APHA (1998) method 4500-NO_3B. Available phosphorous was determined according to APHA (1998) method 4500-PO_4^{3-}. Cl^- was analyzed by silver nitrate titration according to ASTM (1999) method D512-04 Na^+ was analyzed using a UNICAM-969 Atomic Absorption Spectrophotometer according to APHA (1998) method 3111C. Total hydrocarbon content (THC) was determined using a SHIMADZU Infrared Spectrophotometer according to ASTM (1999) method D3921 by measuring light absorbance at the wavelength range of 3333 to 3704 nm. Bonny light crude was used to calibrate the equipment before use. The total heterotrophic bacteria (THB) were determined according to ASTM (1999) method D5485.

Statistical analysis

Standard deviation (SD), using the STDEV function in Microsoft® Excel 2007, and simple percentages were calculated.

RESULTS

Sludge physicochemical characteristics

Table 1 shows the initial physicochemical characteristics of the oily sludge. From the table, it is evident that the THC level of the oily sludge sample (bulked and unbulked) far exceeded the discharge-limit of the Nigerian Government Department of Petroleum Resources (DPR).

While the sludge moisture content, available phosphorus, TN and pH increased, the TOC and C/N decreased after dilution with the topsoil. Tables 3 and 4 showed that after the 6th and 12th week of treatment, sludge physicochemical characteristics in all the reactors showed distinct variations. Table 5 showed that the reduction in the THC of the saline water-treated reactors varied between 41.7 and 55.9% and in the fresh water-treated reactors, it varied between 17.3 and 25.0%.

Microbial population

The changes in the bacterial population during the 12 weeks remediation period are depicted in Table 2. From the table, it is seen that treatment reactor D, which received 1.5 × 10^{-3} m^3 of saline water, had a 7-fold increment in bacterial growth within a period of six to twelve weeks while control reactor B, which received 1.5 × 10^{-3} m^3 of fresh water had an approximately 3-fold increment in bacterial growth within the same period. However, only a 2-fold increment in bacterial population was observed in the counterfactual, reactor O, which was only rain-fed.

Identification of heterotrophic bacteria

The cultural and colonial characteristics showed that the colonies were indented, flat, whitish, opaque, and dry with serrated edge and smooth surfaces. Morphological characterization showed gram positive rods. Other identifying characteristics showed that the colonies were catalase positive, motile, nitrate reduced, produced acid from glucose and starch hydrolyzed; suggesting *Bacillus subtilis*.

Table 2. The total heterotrophic bacterial population (× 10^6 Cfu/g) in relation to stated time of treatment.

Reactor	Weeks after treatment	
	6	12
O	10.2	21.5
A	6.7	10.5
B	10.2	26.1
C	7.2	18.5
D	7.5	55.6

Table 3. Physicochemical characteristics of the oily sludge 6 weeks after remediation.

Reactor	pH (1:2.5)	THC (mg/kg)	Phosphorus (mg/kg)	TOC (%)	TN (%)	C/N
O	6.12±2	58759±180	1.89±0.2	0.88±0.4	0.24±0.5	3.67±0.07
A	6±3	56070±250	1.08±0.4	0.80±0.2	0.23±0.2	3.48±0.03
B	6.2±1.9	52190±100	1.24±0.2	0.93±0.6	0.22±0.1	4.23±0.04
C	6±2.5	49470±230	1.20±0.6	0.84±0.3	0.25±0.3	3.36±0.02
D	7±2	47560±150	1.68±0.9	0.90±0.4	0.26±0.3	3.46±0.09

Values are given in mean ± standard deviation of three readings.

Table 4. Physicochemical characteristics of the oily sludge 12 weeks after remediation.

Reactor	pH (1:2.5)	THC (mg/kg)	Phosphorus (mg/kg)	TOC (%)	TN (%)	C/N
O	6.62±2.5	54277±150	0.88±0.07	0.16±0.05	0.05±0.2	3.2±0.5
A	6.2±3.7	53349±220	0.73±0.08	0.14±0.06	0.06±0.9	2.3±0.3
B	6.87±3.0	48389±120	0.79±0.06	0.13±0.04	0.09±0.8	1.4±0.7
C	6.58±2.1	37620±180	0.76±0.06	0.14±0.07	0.09±0.9	1.6±0.8
D	7.30±1.1	28474±100	0.85±0.05	0.13±0.06	0.08±0.6	1.6±0.6

Values are given in mean ± standard deviation of three readings.

DISCUSSION

Changes in sludge physicochemical characteristics

The THC of the oily sludge of 98,032 mg/kg for the unbulked sludge and 64,494 md/kg for the bulked sludge sample were way too far from the 50,000 mg/kg discharge limit prescribed by the Government Department of Petroleum Resources (DPR). This implies that the oily sludge is not safe to be discharged on land without prior treatment. However, after the 12 weeks of treatment, Tables 3 to 5 showed that there were varying decreases in the total hydrocarbon content (THC) in all the reactors including the counterfactual, which although received the same level of treatment as other reactors but was not watered except for natural precipitation. The THC level of the oily sludge dropped by 15.8% in reactor O, 17.3% in reactor A, 25.0% in reactor B, 41.7% in reactor C, and 55.9% in reactor D (Table 5). This shows that the saline water-treated reactors C and D performed relatively better than the fresh water-treated reactors A and B as well as the rain-fed reactor O. As evident from Table 4, the THC levels of the oily sludge in the saline water-treated reactors were reduced to the acceptable discharge-limit of the DPR of the Nigerian Government. Thus, the saline water significantly enhanced the remediation of the oily sludge.

The initial low moisture content, according to several studies, has been attributed to the fact that water droplets adhere to the hydrophobic surface layer formed therefore, precluded from infiltrating into the inner parts of the sludge aggregates. Nevertheless, the moisture content of the oily sludge was adjusted to 18% since in previous study (Ayotamuno et al., 2006), it has been found out that moisture content of 18% would be optimal for this type of treatment.

Sludge samples collected for analyses after 6 and 12 weeks of remediation showed an increase in the pH

Table 5. Percent THC (w/w) reduction in relation to stated time of treatment.

Reactor	Weeks after treatment	
	6	12
O	8.9	15.8
A	13.1	17.3
B	19.1	25.0
C	23.3	41.7
D	26.3	55.9

values in all the reactors, which suggested that there was a release of by-products during the degradation of hydrocarbons (Tables 3 and 4). As evident from Tables 3 and 4, the pH value of reactor D (treated with 1.5×10^{-3} m^3 of saline water) fluctuated between 7 and 7.3. These pH values were well within the pH range of 7 to 8, which is reported to be the optimal range for degrading microflora (Chaerum, 1995). This indicates that the bio-treatment of the oily sludge was amenable to saline water.

The TOC in the five reactors increased by almost equal amount in the 6th week (Table 3) before plummeting by almost an equal amount again in the 12th week of treatment. The increase in TOC observed in the five reactors in the 6th week may be attributed to trapped leachate and the accumulation of organic carbon (OC) due to evaporation in the reactors. However, the drop in TOC observed in the 12th week suggested that the environmental bacterial population in the diluted sludge may have used the carbons as substrates in decontaminating the oily sludge.

The TN of the various reactors decreased with increasing period of remediation (Tables 3 and 4), which was not expected considering that nitrogenous fertilizer was periodically applied to the degrading sludge and apparently would have increased the total nitrogen of the sludge. This huge loss of nitrogen might largely be due to the biochemical activities of denitrifying bacteria, *Bacillus subtilis,* isolated from the degrading sludge. This observation is corroborated by a previous study on crude oil-polluted agricultural soil (Ayotamuno et al., 2006).

Tables 3 and 4 showed that after treatment commenced, the C/N ratio increased until the 6th week before dropping again in the 12th week in all the reactors except in the control where this drop was dismal. Apparently, the change in C/N within the remediation period seemed very much dictated by the changes in the total organic carbon and nitrogen. The initial increase in the C/N might be linked with trapped leachate and the accumulation of organic carbon (OC) due to evaporation in the reactors and the partial degradation of hydrocarbons by microbes while the drop in the C/N towards the end of the remediation periods might be attributed to the use of the carbons as sources of energy by the environmental bacterial population.

As evident from Tables 3 and 4, there was a rapid reduction of the available phosphorus in all the reactors despite the periodic application of nitrogenous fertilizer to the degrading sludge. Since there is a paucity of information in the literatures at the moment to account for such situation, it may be hypothesized that the bacterial population utilized the available phosphorus for contaminant attenuation.

Biodegradation of oily sludge in relation to amount of salinity

The 7-fold increment in the heterotrophic bacterial population within a period of 6 to 12 weeks witnessed in reactor D, which received 1.5×10^{-3} m^3 of saline water, and the corresponding highest level of THC reduction of 55.9% in the reactor showed that: biodegradation was evident at the salinity (NaCl concentration) of 4.54 g/L and to this extent the heterotrophic bacteria may be said to be halotolerant. This might be because hydrocarbon utilizing bacteria may have become immobilized on sludge matrix at this level of salinity becoming stable and significantly enhanced the biodegradation rates of the hydrocarbons compared with free-living cells in the fresh water-treated reactors. This observation highlights the views in the literature (Diaz et al., 2002) that at rising salinity, bacterial consortium is highly stable in immobilized systems and is not greatly affected by increments in salinity. Diaz and others (2002) reported that the bacterial consortium, MPD-M, isolated from sediment associated with Colombian mangrove roots was effective in the treatment of hydrocarbons in water with salinities varying from 0 to 180 g/L. They reported evidence *of* biodegradation of crude oil even at the highest salinity of 180 g/L. Also, Al-Mahruki *et* al. (2006) used saline water to achieve the biodegradation of hydrocarbons in the remediation of a hydrocarbon-contaminated drilling mud/sand mixture in a study conducted in Oman.

Conclusion

Evidently, the study showed that the THC level of 64,494 mg/kg for bulked and 98,032 mg/kg for unbulked oily sludge sample were way too far from satisfying the discharge limit of 50,000 mg/kg set by the Nigerian Government Department of Petroleum Resources (DPR). While some sludge physicochemical characteristics such as moisture content, available phosphorus, TN and pH increased, the TOC and C/N decreased after dilution with the topsoil and after 6 to 12 weeks of treatment; these characteristics varied in no particular pattern. The reduction in the THC was highest in the saline water-treated reactors and varied between 41.7 and 55.9% while it was least in the fresh water-treated reactors and varied between 17.3 and 25.0%. Correspondingly, the reactor, which received $1.5 \times 10^{-3} m^3$ of saline water, had a 7-fold increment in bacterial population within a period

of 6 to 12 weeks while control reactor, which received 1.5 × 10^{-3} m^3 of fresh water, had an approximately 3-fold increment in bacterial population within the same period. These results showed the possibility of enhanced biodegradation of oily sludge by hydrocarbon utilizing bacteria at salinity (NaCl concentration) of 4.54 g/L. The bacterial strain was identified as *B. subtilis.*

RECOMMENDATION

For enhanced attenuation of THC of an oily sludge, it might, therefore, be advisable to maintain salinity (NaCl concentration) of 4.54 mg/L in the saline water to be applied in the reclamation process.

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