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BIO-REMEDIATION OF A SLUDGE CONTAINING HYDROCARBONS

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ABSTRACT

Bio-augmentation has been used as a bioremediation option for hydrocarbon-contaminated, oily-sludge restoration. This sludge was obtained from the Bonny-Terminal Improvement Project (BTIP) for Bonny Island, near Port Harcourt, Nigeria. Its total hydrocarbon-content (THC) was 69,372mg/kg of sludge. Three treatment reactors (X, Y and Z) and one control reactor (A) were charged with 1500g of oily sludge and 250g of agricultural soil (i.e. an oily sludge to soil ratio of 6:1), the mixture homogenized and allowed to settle for seven days before various CFUs were added to reactors X, Y and Z. Reactor A did not receive any bio-preparation. The agricultural soil served both as a nutrient and a microbe carrier. With regularly scheduled mixing and watering, the THC reduction in the oily sludge varied between 40.7 and 53.2% within two weeks as well as between 63.7 and 84.5% within six weeks of applying the bioremediation. The CFU counts of the added bio-preparation varied between 1.2×12^{12} and 3.0×10^{12} CFU/g of sludge and decreased to 7.0×10^{11} CFU/g of sludge by the end of the sixth week. The pH of the degrading sludge fluctuated between 6.5 and 7.8 during the same period. When compared with the performance of the indigenous microbes in the control sample, the added bio-preparation evidently increased the THC reduction rate in the oily sludge.

Key words: Bioremediation, Oily sludge, Bio-augmentation, Terminal Operations.

ABBREVIATIONS

APHA	American Public-Health Association
ASTM	American Society for Testing and Materials
BTIP	Bonny-Terminal Improvement Project
CFU	Colony-Forming Units
DPR	Department of Petroleum Resources
FMA	Federal Ministry of Aviation
HUB	Hydrocarbon-Utilizing Bacteria
IR	Infrared
THC	Total Hydrocarbon-Content

ml $\equiv 10^{-6}\ m^3$

1.0 INTRODUCTION

Tank farms are used as transit depots for the storage of crude oil and natural gas. Prior to its export, crude oil is temporarily housed in storage tanks, dehydrated and fiscalised. These facilities require scheduled maintenance. Maintenance and improvement projects at these terminals generate huge (in terms of pollution potential) volumes of oily sludge during the cleaning of the storage tanks and heaters. Oily sludge, from the improvement project of the Bonny Terminal in the Niger Delta region of Nigeria, is characterized by high THC levels, i.e. of toxic substances such as aromatic hydrocarbons (benzene, toluene, xylene and ethyl benzene) and poly aromatic hydrocarbons (Swoboda-Colberg, 1995; DPR, 2002). Because of this, land and /or sea disposal of this oily sludge without previous remedial treatment poses serious threats to the environment and human beings The treatment of this oily sludge usually has been achieved through incineration as recommended by the Department of Petroleum Resources (DPR). However, incinerating petroleum wastes, apart from being prohibitively expensive (Shkidchenko *et al*, 2004), also results in the use of enormous amounts of additional fuel (Mrayyan and Battikhi, 2005), which contributes to the already fast-depleting rates of fossil-fuel resources. Again, personnel and equipment are exposed to the resulting fugitive dusts. Concern about these drawbacks of the incineration method and the need to detoxify and reclaim this oily sludge prompts a need to adopt a treatment method that is cheap, simple and environmentally friendly. Recently, a plethora of research has shown that bioremediation offers an excellent means for reclaiming soil that has become polluted. In Nigeria, various methods of bioremediation have been used to reclaim soil polluted by crude oil (Amadi *et al*, 1993; Onwurah, 1996; Odokwuma and Dickson, 2003; Obire and Akinde, 2004; Ebuehi *et al*, 2005; Ayotamuno *et al*, 2006). However, there is a dearth of research concerning the use of bio-augmentation to treat oily sludge.

In this study, bio-augmentation, which is the addition of micro-organisms like bacteria and fungi to detoxify the polluted media (Atlas and Bartha, 1972), is employed to decontaminate oily sludge from the Bonny Terminal Improvement Project (BTIP). Although, intrinsic bioremediation is

inherently a slow process (Mitchell *et al*, 2000; Wills, 2000), bio-augmentation involves the use of a microbial consortium to enhance the degradation of the contaminated media (Noyes, 1994). Bio-augmentation offers a viable means of seeding, with the competent micro-flora, to ensure the effective breakdown of targeted contaminants in the polluted media. The present study assessed the effectiveness of using *bacillus* and *pseudomonas*, which are the constituents of the bacteria consortia, in the bioremediation of the BTIP oily-sludge.

2.0 MATERIALS AND METHODS

2.1 Study Area

The oily sludge for this study was obtained from the Bonny Island Terminal, Rivers State, Niger Delta region of Nigeria. It forms part of the southern Nigeria coastal zone, and Rivers State falls within the lowland rain-forest ecological expanse. The micro-climatology, according to the Meteorological Department of the Federal Ministry of Aviation (2003), indicates that the State has a mean annual rainfall of 2369mm and a mean maximum daily-temperature of 28^{o}C.

2.2 The Sludge

Using plastic containers, samples were taken randomly every three days from the uppermost 5cm layer of the composite sludge. The containers were then properly sealed with polyethylene materials to preserve the integrity of the smples. Some samples were immediately transferred to the laboratory for initial characterization, while the others were taken to the research campus for treatment.

2.3 Preparation of Medium for Enumeration of Heterotrophic Bacteria

The medium used for the serial dilution of the sample was normal saline-solution. In preparing this, all diluents and media were sterilized in an autoclave at 121^{o}C for 15 minutes and all glassware sterilized in a dry hot-air oven at 160^{o}C. Then 0.85g of sodium chloride (NaCl) was weighed out and transferred into 100ml of distilled water inside a beaker and mixed thoroughly. Subsequently, 9ml of the solution was dispensed into a test tube

and sterilized at 121^{o}C for 15 minutes. After which, it became the solution for serial dilution of the oil-sludge sample.

2.4 Cultivation and Enumeration of Viable Heterotrophic Bacteria

In culturing the bacteria, use was made of nutrient agar and oil agar already prepared in the Microbiology Laboratory of the University. To do this, 1.0g (dry weight) of fine sludge was homogenized with 1.0 x $10^{-6}m^3$ of sterile distilled-water to give 10^{-1} dilutions; and 1 x $10^{-6}m^3$ of the 10^{-1} dilution was transferred into the next test tube containing 9.0 x $10^{-6}m^3$ normal saline (diluent) and diluted serially in one tenth steps up to 10^{-3} dilution (Harringan and McGrane, 1990). From the dilutions of 10^{-2} and 10^{-3} of each sludge sample, a 0.1 x $10^{-6}m^3$ 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^{o}C for 24 hours. Thereafter, the plates were examined for any growths on them. Discrete colonies of heterotrophic bacteria in the sludge were recorded.

2.5 Isolation and Identification of 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^{o}C for 24 hours. 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 grain 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).

2.6 Experimental Design

Four plastic containers (i.e. the reactor vessels) consisting of three treatment-reactors and one control-reactor were used for this study. Control reactor A was not to be subjected to any treatment while reactors X, Y, and Z were labelled to receive different levels of treatment. Agricultural soil containing indigenous microbes and nutrients was obtained from within the

Research Campus. Then 1.5kg of oil sludge and 0.25kg of this agricultural soil were added to each of the reactors in turn. This mix ratio (i.e. sludge: soil = 6:1) corresponded with that used by Ouyang *et al* (2005). The mixture was thoroughly mixed and allowed to settle for seven days so that microbial activity could ensue before the application of the working solution of the bio-preparation (including HUB), which was added to reactors X and Y at cell densities of (7.6×10^{11} CFU/ml) and (7.4×10^{11} CFU/ml) respectively. Reactor Z was treated with the mixed culture (1.5×10^{12} CFU/ml). Reactor A did not receive any bio-preparation. The experiments lasted for 42 days under an ambient temperature of 30^{o}C. Mixing and watering were repeated every three days.

2.7 Chemical Analysis

The THC was determined using a PRESTIGE-21 IR-Spectrophotometer by measuring light absorbance at wavelengths of 3704 to 3333nm according to ASTM (1999) methods. Bonny light crude-oil was used to calibrate the equipment beforehand. Other physico-chemical parameters; namely pH, moisture content, organic carbon-content, carbon-nitrogen ratio, and available phosphorus were determined according to the APHA (1998) standards.

3.0 RESULTS AND DISCUSSION (See Tables 1 – 8)

3.1 THC Reduction

The effects of microbial addition on the THC reduction in the oily sludge is shown in **Figure 1**. The THC reductions in the oily sludge were 40.7 to 53.2% and 63.7 to 84.5% within two and six weeks of bio-augmentation respectively. The indigenous micro-organisms (as in Reactor A) could reduce the THC by a limited amount (i.e. 6 to 12.8%) within the same period of bio-augmentation despite being aerated by mixing and watering. This indicates that the bio-preparation addition achieved a significant advantage over just having the indigenous microbes alone in the THC reduction.

3.2 Change in pH

It has been stated that, in the presence of excess nutrients and oxygen, the pH value of an oily sludge under microbial degradation would drop (Marn and Khodijah, 2004). In the present study, the initial pH value of the oily sludge was 5.8 (**Table 1**) but varied between 6.5 and 6.8 at the end of the second week (**Table 5**) and between 7.7 and 7.8 at the end of the sixth week (**Table 6**) of bioremediation. The pH value of the control specimen (in Reactor A) varied from 6.0 to 6.7 during the same period of bioremediation. From these results, the pH of the oily sludge increased during the remediation period, which implied that the nutrient and oxygen levels were not in excess. The final pH range (i.e. 7.7 to 7.8, **Table 6**) did not fall out of the range 7 to 8, which is said to be the optimal range for degrading micro-flora (Chaerun, 1995). This result also corroborated that obtained by Ouyang *et al* (2005).

3.3 Identification of Bacteria Isolates

Based on their cultural and colonial characteristics, two different bacteria species were identified on the inoculated plates, namely *bacillus and pseudomonas* (**Table 2**). The initial population of the colony forming hydrocarbon-utilizing bacteria identified is shown in **Table 3**. The *bacillus* species was slightly more populous than the *pseudomonas* species.

3.4 Microbial Activity

Table 7 shows the variation of microbial counts during the bioremediation of the oily sludge. The microbial counts varied from 1.2 x 10^{12} to 3.0 x 10^{12} CFU/g of sludge within two weeks and decreased to 7.0 x 10^{11} CFU/g of sludge at the end of the sixth week. In the control reactor A, microbial counts varied from 3.0 x 10^{5} to 3.2x10^{5} CFU/g of sludge within two weeks and decreased to 2.8 x 10^{5} CFU/g of sludge at the end of the sixth week. Thus, seeding with HUB increased the microbial counts and this had a profound influence on the rate of THC reduction. Evidently, the mixed culture in reactor Z had the highest influence on the degradation of the oily sludge (**Table 8**). The reduction in microbial counts by the sixth week was partly due to the process of eutrophication of the micro-flora.

4.0 CONCLUSIONS

The use of bio-augmentation in the bioremediation of the BTIP oily-sludge reduced the THC of the sludge from an initial 69,372mg/kg of sludge to 10,734mg/kg of sludge (representing an 84.5% reduction) after six weeks of treatment. But in the control reactor, only a 12.8% THC reduction was achieved during the same period. This shows that the addition of microbes enhanced the degradation of the oily sludge, which implies that bio-augmentation is an effective bioremediation technology for dealing with an oily sludge. The performance, in terms of THC reduction, of the two strains of bacteria (*bacillus* and *pseudomonas*) used in the mixed culture during the bio-augmentation process, was better than the performance of individuals strains in the pure culture. Between the two strains, *pseudomonas* appears to be the better degrader

5.0 RECOMMENDATIONS

Because of the spate of environmental-degradation incidents, occasioned by increased crude-oil and natural gas activities in the Niger Delta region of Nigeria, urgent efforts are necessary to implement remedial activities such as discussed in this report, but on a large scale. There is too much talk about the pollution, but so little implementation commercially. However, bioremediation is fast becoming one of the most economic and environmentally-friendly technologies for hydrocarbon- contaminated site restoration. To this end, it is recommended that hydrocarbon-contaminated oily-sludge resulting from terminal improvement projects and other spillages be treated by bio-remediation using bio-augmentation.

Table 1: Initial physicochemical parameter of BTIP oily sludge

Parameter	Value
pH	5.8
Total hydrocarbon content, mg/kg	69,372
Moisture content, %	20.48
Organic carbon content, %	0.49
Total nitrogen content, %	0.13
Carbon/Nitrogen ratio	4
Available phosphorus, μg/g	1.02

Table 2: Identification of bacteria isolates

Organism	Cultural/Colonial Characteristics	Other identifying characteristics
Bacillus species	Colonies are indented, flat, whitish, opaque, dry with serrated edge and smooth surfaces.	Gram positive rods*, catalase positive, motile**, nitrate reduced, produced acid from glucose, starch hydrolyzed.
Pseudomonas species	Colonies are circular, flat, opaque, creamy, moist with edge not serrated, dull surfaces and light green pigmentation.	Gram negative rods*, catalase positive, motile**, nitrate reduced, produced acid from glucose, starch hydrolyzed.

**In microbiology, gram staining technique is used to distinguish bacteria into two major groups (morphologically) – gram positive and gram negative bacteria.*
***Motile – motile bacteria move about when in liquid culture.*

Table 3: Initial microbial count of HUB in sludge after soil addition

Microbe	Population (CFU/g mix)
Bacillus	2.83×10^5
Pseudomonas	2.35×10^5

Table 4: Composition of material in the specified reactor

Content	Reactors			
	X	Y	Z	A
Oil Sludge (g)	1500	1500	1500	1500
Soil (g)	250	250	250	250
Added HUB (CFU/ml)	7.6×10^{11}	7.4×10^{11}	1.5×10^{12}	0

Table 5: Physicochemical parameters of the oil sludge after 2 weeks of bio-augmentation

Parameter	Values			
	X	Y	Z	A
pH	6.5	6.7	6.8	6.0
Moisture content (%)	21.76	21.76	23.46	19.76
Total hydrocarbon content (mg/kg)	41.126	40,026	32,487	65,231
Organic carbon content(%)	2.62	2.60	2.64	2.63
Total nitrogen content (%)	0.42	0.43	0.62	0.28
Carbon/nitrogen ratio	6	6	6	14
Available phosphorus (μ g/ g)	2.80	2.56	3.01	1.20

Table 6: Physicochemical parameters of the oil sludge after 6 weeks of bio-augmentation

Parameter	Reactors			
	X	Y	Z	A
pH	7.8	7.8	7.7	6.7
Moisture content (%)	23.46	19.76	19.76	21.21
Total hydrocarbon content (mg/kg)	25,190	24,231	10,734	60,498
Organic carbon content (%)	3.41	3.54	3.73	3.62
Total nitrogen content (%)	0.76	0.81	0.93	0.31
Carbon/nitrogen ratio	4	4	4	12
Available phosphorus (μ g/ g)	4.20	4.01	4.05	1.40

Table 7: Microbial Counts after stated period of bio-augmentation

Microbes	Population (CFU/g of Sludge)			
	2 Weeks			
	X	Y	Z	A
Bacillus	1.5×10^{12}	-	3.0×10^{12}	3.2×10^{5}
Pseudomonas	-	1.2×10^{12}	2.8×10^{12}	3.0×10^{5}
	6 Weeks			
	X	Y	Z	A
Bacillus	1.10×10^{12}	-	2.5×10^{12}	3.0×10^{5}
Pseudomonas	-	7.0×10^{11}	2.0×10^{12}	2.8×10^{5}

Table 8: Percentage THC reduction after stated period of bio-augmentation

Reactor	0 Week	2 Weeks	6 Weeks
X	0	40.7	63.7
Y	0	42.3	65.1
Z	0	53.2	84.5
A	0	6	12.8

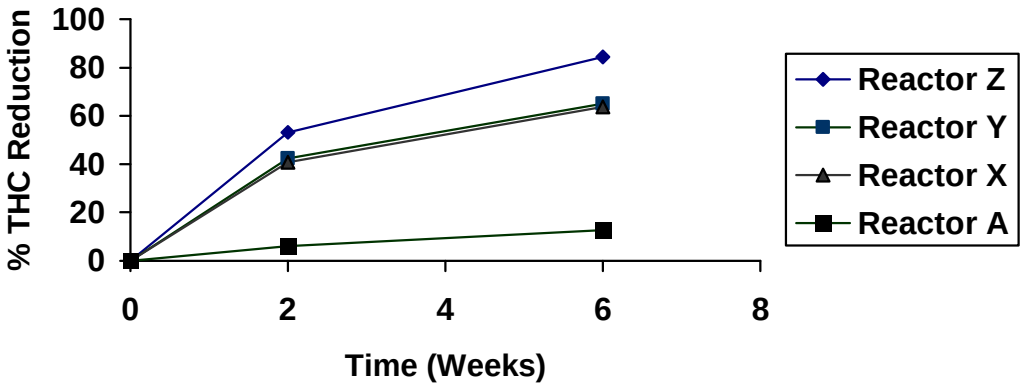

Figure 1: THC reduction history during the remediation period

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