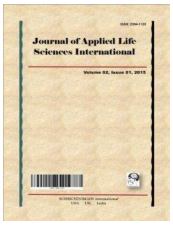

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Biodegradation of Waste Drilling Mud Using Spent Mushroom Substrate

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Authors' contributions

This work was carried out in collaboration between both authors. Author HOS designed the study. Author PMT performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PMT managed the analyses of the study. Author PMT managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Biodegradation of waste drilling muds (WDM) was carried out with the aim to investigate the efficacy and applicability of spent mushroom substrate (SMS) for enhanced bioremediation of impacted mangrove swamp. Microcosm study was carried over a 60-day period. Microbial population dynamics of waste drilling muds utilizing bacteria/fungi in the mangrove soil sediment (MSS) undergoing bioremediation was investigated. The experiment was done in four Set-ups labelled A to D: Set-up A (WDM + MSS); Set-up B (WDM+ Sterile MSS) as control; Set-up C (WDM+ MSS + Fresh SMS) and Set-up D (WDM+ MSS + Composted SMS). Physicochemical and microbiological parameters were monitored from baseline to the 60th day, following 20 days intervals. Results obtained show that the drilling muds utilizing bacterial count ranged from $0.4X10^4$–$2.5X10^4$ cfu/g, while drilling muds utilizing fungal count ranged from $0.2X10^3$ –$1.5X10^3$ cfu/g. The isolated drilling muds utilizing bacteria belong to the genera *Bacillus, Corynebacterium, Citrobacter, Klebsiella, Alcaligenes, Micrococcus* and *Pseudomonas,* and the drilling muds utilizing

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fungi belong to the genera *Candida, Cryptococcus, Rhodotorula, Saccharomyces, Trichosporium, Geotrichum, Aspergillus, Penicillium, Mucor, Fusarium, Cladosporium, Cephalosorium, Monosporium, Neurospora, Rhizopus* and *Microsporium.* The values of the pH in Set-ups were within the neutral to slightly alkaline pH range during the study period. The concentrations of nitrate, phosphate, sulphate, potassium and total organic carbon (TOC) of the Set-ups with fresh and composted SMS (treatment Set-ups) decreased significantly during the study period. The concentrations of heavy metals (Fe, Cu, Zn, Ni and Pb) in the SMS amended Set-ups also showed significant reductions. The percentage of total petroleum hydrocarbon (TPH) biodegraded was greater in Set-up D containing composted SMS (71.48%) than in Set-up C containing fresh SMS (67.93%), followed by Set-up A (22.69%)and the least was the control Set-up B with 8.36%. This study showed that both fresh and composted spent mushroom substrates are effective in the biodegradation of waste drilling muds contaminated Niger Delta mangrove soil sediment.

Keywords: Bioremediation; mangrove soil sediment; spent mushroom substrate.

1. INTRODUCTION

The mangrove swamp is the dominant wetland ecosystem in the Nigerian oil rich Niger Delta which is the main zone of oil production and exploration activities in Nigeria. Mangrove wetlands are inertial ecosystems which support wide and varied group of mobile organisms ranging from birds that nest in the trees to fishes that feed and live among submerged prop roots, and constitute important nurseries for fishes, crustaceans, sponges, algae and other small invertebrates [1,2]. The Niger Delta Mangrove Swamps provide grounds for commercial fishing, timber production, biotechnologically important microorganisms such as *Pseudomonas* sp., *Bacillus* sp., *Aspergillus* sp. etc). However, pollution caused by petroleum and its by-products has greatly impacted negatively on the mangrove leading to reduction in seafood output, increased food insecurity challenges, reduction in biodiversity of mangrove biota, youth restiveness and violence in this region.Drilling operations require the use of drilling fluids (muds) which are often discarded after use. During drilling, plumes (muddy) of turbid water are commonly seen trailing downstream from the drilling platform. The disposal of waste drilling-fluids containing surfactants, hydrocarbons and heavy metals into surrounding swamp or into burrows or waste pits in land-based operations, poses a problem to the Niger Delta environment. The concomitant effect is the accumulation of large amount of recalcitrant pollutants in the mangrove sediment [3]. Seepages and infiltration to groundwater, as well as seasonal flooding and tidal action, provide a direct communication with and, thus, pollution of ground- and surface-waters and nearby terrestrial environments [4].

Numerous studies on petroleum degradation in marine environment and soil demonstrate that organic ingredients in oily cuttings are biodegradable under aerobic conditions [5] but in the floor of some sediments for instance the Gulf of Mexico, the average oxygen concentration is 6.8 mg/L (0.21 nm) and the oxygen only diffuses a few centimetres into the sediment, an indication that oxygen availability can be limiting in deep offshore sediments [5].

Bioremediation is defined as the use of living organisms primarily microorganisms, to degrade environmental toxicants into less toxic forms. It could involve the use of naturally occurring plants and microorganisms to degrade toxicant (biostimulation) or introduction of non-native oil-degrading microorganisms are added to supplement the existing microbial population (bioaugmentation) [6]. Factors such as pH, water, nutrients, temperature, oxygen, amount and bioavailability of pollutant affect the bioremediation process by microorganisms [7].

A wide variety of aerobic hydrocarbon degrading microorganisms have been isolated from the Niger Delta sediments [8-10]. Oxygen availability can be a limiting factor in hydrocarbon degradation in sediments. Okoro [10] revealed that aerobic microorganisms are very active in sediment depth of up to 2-5 cm. Aerobic biodegradation is therefore a key criterion for selecting a base fluid for bioremediation though there may be other important factors such as availability of the base fluids, drilling environment and the operator's policy and local legislation. The objective of this study was to assess the biodegradability of waste drilling muds extracted from drill bits in drilling operations in the Niger Delta by biostimulation using SMS.

2. MATERIALS AND METHODS

2.1 Sample Collection

The waste drilling muds samples used in this study were supplied by Oando Oil Company (offshore), Rivers State, Nigeria, in wide-mouthed sterile bottles. Composite mangrove soil sediments collected at depth 0-5 cm was obtained from Eagle Island behind Nigeria Agip Oil Company in Port Harcourt using auger borer. Composite samples were obtained by mixing 5 grams of mangrove sediment collected from different areas; a portion of the composite soil (1 gram) was then placed in sterile bottles for microbiological analysis while the remainder was analysed for physicochemical parameters. Spent mushroom substrate was obtained from Bioresources Development Centre in Odi, Bayelsa State.

2.2 Experimental Treatment Design

The treatment design used was modified from [6].

Table 1. The experimental treatment design

Set-up	Experimental treatment protocol
A	WDM (50 g)+MSS (500 g)
B	WDM(50 g)+SMSS (500 g)
C	WDM(50 g)+MSS (500 g)+FSMS(50 g)
D	WDM(50 g)+MSS (500 g)+CSMS (50 g)

Key: WDM (Waste drilling muds), MSS (Mangrove soil sediment), FSMS (Fresh spent mushroom substrate), CSMS (Composted spent mushroom substrate), SMSS (Sterile Mangrove soil sediment)

2.3 Isolation and Enumeration of Bacteria and Fungi

The mineral Salt medium C of Mills et al. [11] was used for isolation of bacteria. The medium contained the following: 10.0 g NaCl; 0.42 g $MgS0.7H_2O$; 0.29 g KCl; 083 KH_2P0_4; 1.25 g $NaHPO_,$; 0.42 g NaNO in 1L deionized water. When necessary to solidify the medium, bacteriological agar was added at a concentration of 15% (w/v). The pH was adjusted to 7.4. For isolation of fungi, Sabauraud dextrose agar (SDA) was used, and pH was adjusted to 4.0.Colonies formed on media were further purified by subculturing on nutrient agar for bacteria and Sabauraud dextrose agar (SDA) for fungi. Pure colonies were identified based on morphology, Gram reactions, motility test, as well as by biochemical characteristics including catalase test, oxidase test, citrate utilization test, Voges-Proskauer test, and sugar fermentation tests. Enumeration of drilling muds utilizing bacteria and fungi was done after incubation of plate at room temperature for 2-7 days by plating out 0.1ml of samples on medium to which 1% of the drilling muds was added using the Spread plate technique [12]. All experiments were in triplicate.

The total heterotrophic bacterial count was performed in triplicate by plating out 0.1 ml of the samples in nutrient agar plates using the Spread plate technique. Samples were prepared by adding 1ml of water to 9 ml sterile saline (0.85% w/v) as diluents. Plates were enumerated after 48 hours of incubation. Also, for total culturable fungi, the same procedure was followed except that 1 ml of lactic acid was added for fungal media (SDA) to inhibit the growth of heterotrophic bacteria.

2.4 Physicochemical Analyses

The methods determined of UNEP and Association of Analytical Chemists [13,14] were used for determination of physicochemical properties including: pH, total petroleum hydrocarbon (TPH), total organic carbon(TOC), heavy metals, phosphate, moisture and nitrate content.

2.5 Statistical Analysis

The data generated in the study was subjected to statistical analysis using two-way analysis of variance (ANOVA), to determine levels of significance using the SPSS 20 software.

3. RESULTS AND DISCUSSION

The baseline microbiological and physicochemical properties of waste drilling muds, mangrove soil sediment, fresh spent mushroom substrate and composted spent mushroom substrate used in this study period are as shown in Table 2.

Table 2. Baseline microbiological and physicochemical parameters of samples

Variable	Unit	WDM	MSS	FSMS	CSMS
pH		7.32	6.87	7.12	6.92
Temperature	^{0}C	28.6	28.4	28.5	28.5
Nitrate	mg/kg	ND	12.76	252.2	213.4
Phosphate	mg/kg	ND	7.89	123.1	101.4
Sulphate	mg/kg	ND	4.23	43.31	28.67
Moisture		ND	10.2	11.3	10.9
TOC	mg/kg	5.20	ND	ND	ND
Potassium	mg/kg	3858	712.53	1210	1580
TPH	mg/kg	33400	ND	ND	ND
Fe	mg/kg	1580	2100	72.14	101.23
Cu	mg/kg	109.56	36.55	8.12	9.14
Zn	mg/kg	215.33	59.74	14.98	17.28
Ni	mg/kg	85.87	8.11	BDL	BDL
Pb	mg/kg	58.22	2.28	62.12	5.67
THB X10^4	cfu/g	-	2.0	1.8	1.6
TF X10^3	cfu/g	-	1.5	1.5	1.3
DMUB X10^2	cfu/g	-	0.3	0.1	0.1
DMUF X10^2	cfu/g	-	0.2	0.1	0.1

WDM (Waste drilling muds), MSS (Mangrove Swamp Sediment), FSMS (Fresh spent mushroom substrate), CSMS (Composted spent mushroom substrate), ND (Not detected), BDL (Below detectable limits), TOC (Total organic carbon), TPH(Total petroleum hydrocarbon), THB (Total heterotrophic bacteria), TF (Total fungi), DMUB (Drilling muds utilizing bacteria), DMUF (Drilling muds utilizing fungi)

3.1 Microbial Isolates

The isolated drilling muds utilizing bacteria (DMUB) belong to the genera *Bacillus, Corynebacterium, Citrobacter, Klebsiella, Alcaligenes, Micrococcus* and *Pseudomonas.* Reports on the degradative capabilities of these bacteria are available in literatures [15-18]. The drilling muds utilizing fungi (DMUF) isolated belong to the genera *Candida, Cryptococcus, Rhodotorula, Saccharomyces, Trichosporium, Geotrichum, Aspergillus, Penicillium, Mucor, Fusarium, Cladosporium, Cephalosorium, Monosporium, Neurospora, Rhizopus* and *Microsporium*. These fungal genera have been isolated by other workers in Nigeria [19,16].

Nnubia and Okpokwasili [20] have reported that biodegradation of drilling muds requires a microbial consortium (mixed population of microorganism). In this consortium some organism act as primary utilizers utilizing the organic carbon of the waste drilling muds while others act as secondary utilizers utilizing the breakdown products of waste drilling muds after initial attack by primary utilizers [12, 20].

3.2 Microbial Population Changes

The microbial population changes (%) during the study period are as presented in Fig. 1.In treatment set-up A, there was a drastic decrease in total heterotrophic bacteria (THB) population from 1.6X10^4 cfu/g in day zero through 1.3x10^4 cfu/g in day 20, 1.3x10^4 in day 40 and finally dropped to 1.1x10^4 cfu/g in day 60, amounting to 31.25% decrease. Set-up B gave 33.33% decrease, C 70% and D 50%. From the result, the THB decreased in all the Set-ups up till day 40, but from day 40 to the 60th day the THB in the treatment Set-ups 'C' increased and 'D' remained the same, while Set-ups 'A' and 'B' continued to reduce. This reduction could be attributed to some of the heterotrophic bacteria not being able to utilize the hydrocarbons in the environment as their sole source of carbon, but the presence of the nutrients as well as the organic carbons present in the SMS added to the treatment Set-ups and which were not added in the control Set-ups provided food for their growth and replication in the treatment Set-ups.

The total fungal (TF) population in the various treatments also changed with time and decrease

drastically. TF reduced significantly in all the Set-ups through the study period but the reduction was lesser in the 2 treatment Set-ups (C and D).In treatment A the TF decreased after 60 days by 69.23%, 42.86% for B, 41.67% for C and 70% for D.

The DMUB had percentage changes that ranged from 40 for both 'A' and 'B', 44.4 for 'C' and 33.33 for 'D'. On the other hand, the DMUF had 13.33% (A), 15.39% (B), 23.08% (C) and 30% (D). Nnubia and Okpokwasili [20] had earlier reported that as the waste drilling fluid-degrading bacteria and fungi become adaptive to the environment, they replicate faster and make use of the residual drilling muds in the mangrove sediment as their sole source of carbon and energy. The results of this study corroborated with this report as the DMUB and DMUF increased by day 40 more especially in the treatment Set-ups (C and D) which contained SMS, before they later dropped by day 60 and this could be attributed to the depletion of the nutrients and organic carbons in the system as the microorganisms used them. Percentage decreases in DMUB was greater in treatment C, lesser in treatment D and the same in A and B. Percentage decreases in DMUF were in the order D>C>B>A.

By day 60, the THB, TF, DMUB and DMUF population changed significantly ($p<0.05$) in all the amended treatment Set-up. After day 60 of nutrient amendment, there was a marked drop in microbial population. Probably the nutrient might have been over utilized; resulting to decline in microbial population [21,17]. This trend may be attributable to bioconcentration and bioaccumulation of a particular hydrocarbons fraction which was not broken down by the hydrocarbonoclastic microorganisms.

3.3 Changes in Heavy Metals Concentrations

Results of heavy metal concentration changes in all treatments are as presented in Fig. 2. The trend for the heavy metal removal rate in the different treatments Set-ups was in the order Fe<Cu<Zn<Ni<Pb. The percentage removal of all the heavy metals analysed was seen to be highest in the treatment Set-up 'C' containing fresh SMS, followed by the treatment Set-up 'D' containing composted SMS, followed by Set-up 'A', then lastly the control, 'B'. Probably, some of the *Pleurotus* mycelia still present in the fresh SMS may have contributed to the reduction in the concentrations of the heavy metals in the treatment Set-up 'C' compared to the other Set-ups. *Pleurotus ostreatus* (a white-rot fungus) have been reported to be a viable biosorbent of heavy metals in contaminated soil [22].

3.4 Changes in Physicochemical Parameters

Temperature and pH were fairly stable over the study period, ranging from 28.0 to 29.8 and6.87 to 7.78 respectively Table 3. There was a general decrease in nitrate, sulphate, phosphate, potassium, TOC and moisture levels over time (Table 3). The greatest reduction was in treatment D and the least was in treatment B. There was progressive decrease in the TOC from day 0 to day 60 in all the Set-ups, but mostly in the treatment Set-ups amended with fresh and composted SMS. The same was seen in the phosphate and nitrate levels through the biodegradation period in this study, as the waste drilling fluids utilizing microorganisms in the environment utilized the nutrients for growth. This corresponded with the report of [6], in their study using different organic wastes including spent mushroom substrate as nutrient amendments in the bioremediation of hydrocarbon contaminated soil.

3.5 Changes in Total Petroleum Hydrocarbon

Fig. 3 shows the percentages of TPH degraded in the various treatments. Microorganisms in the waste drilling fluid polluted mangrove have efficient ability in utilizing the residual muds. These observations have been reported by various researchers [23,9,8,7] and corroborate with the present finding. The result has shown that the treatment amended with composted spent mushroom substrate (D) did better in enhancing biodegradation of total petroleum hydrocarbons in the waste drilling fluids in the mangrove sediment followed by that with fresh spent mushroom substrate (C). There was a marked difference between the SMS amended Set-ups (C and D) and the unamended set-up (A) as well as the control (B). TPH removal was greater in treatment D and least in B. There was significant difference ($p<0.05$) in TPH degradation in C and D compared to A and B.

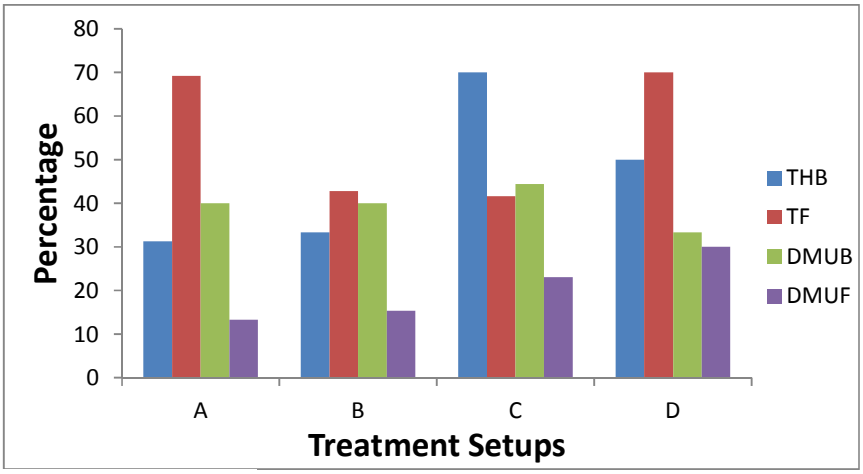

Fig. 1. Changes in microbial count

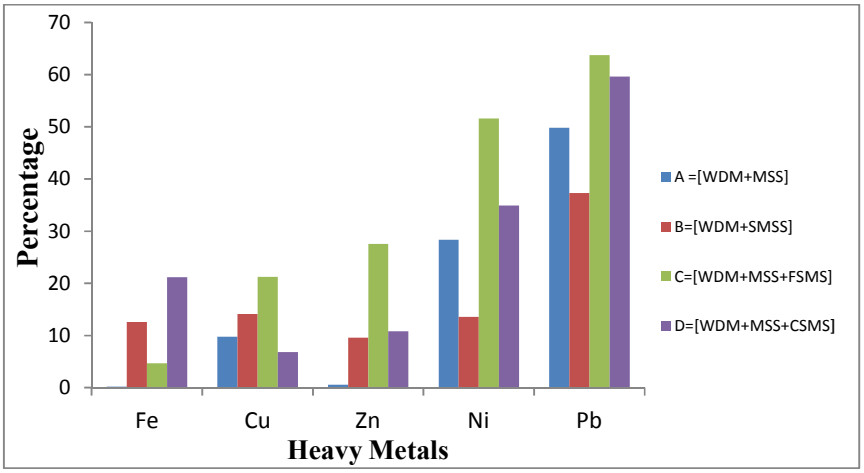

Fig. 2. Percentage removal of heavy metals in the different treatment set-ups

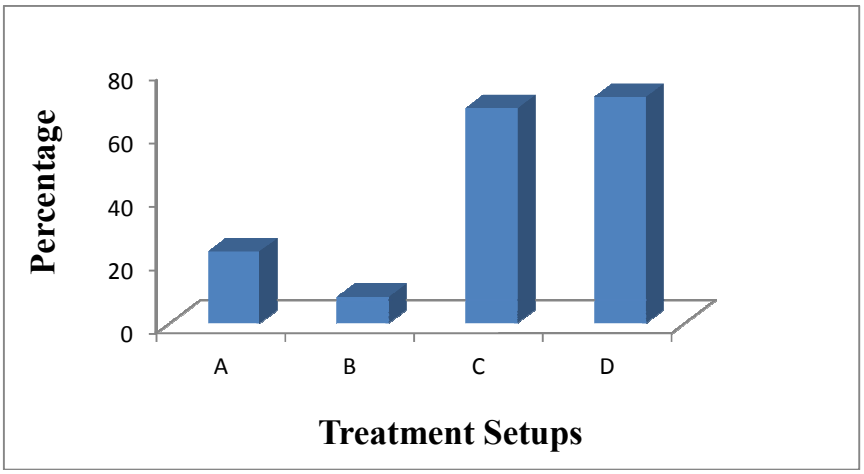

Fig. 3. Percentage degradation of total petroleum hydrocarbons

Table 3. Changes in physicochemical properties during the study period

Variables	A				B				C				D			
	Day 0	**20**	**40**	**60**	**Day 0**	**20**	**40**	**60**	**Day 0**	**20**	**40**	**60**	**Day 0**	**20**	**40**	**60**
pH	7.20	7.24	7.28	7.12	7.19	7.22	7.18	6.87	7.78	7.70	7.40	7.32	7.30	7.24	7.04	7.24
Temperature (^{o}C)	28.6	28.9	28.1	28.6	28.7	28.8	28.3	28.5	28.6	28.9	28.0	28.5	28.9	28.9	28.4	28.7
Nitrate (mg/kg)	20.13	17.73	15.43	13.33	23.46	20.73	18.35	15.15	45.03	38.23	34.03	30.73	36.87	31.87	29.97	25.17
Phosphate (mg/kg)	11.00	8.54	6.34	5.14	10.64	5.01	4.47	4.77	21.41	18.57	14.50	12.30	27.28	20.95	18.72	14.42
Sulphate (mg/kg)	24.09	22.56	20.22	19.10	14.57	15.08	13.28	11.18	18.31	24.28	20.12	18.15	30.04	36.30	32.50	28.32
Moisture	12.00	10.0	9.10	7.15	10.3	8.4	6.41	5.48	13.5	10.2	8.22	7.72	12.6	9.6	7.16	6.56
TOC (mg/kg)	3.45	2.15	1.15	1.01	2.56	1.76	0.76	0.42	1.97	1.37	0.67	0.46	1.35	0.95	0.45	0.39
Potassium (mg/kg)	1480	1510	1495	1375	1163	1118	1063	1128	2120	2059	1745	1642	2409	8148	1922	1728
TPH (mg/kg)	17540	16110	15895	13560	16280	15945	15220	14918	12820	9280	6250	4111	11970	8148	4970	3413

4. CONCLUSION

Enhanced bioremediation (in situ) offers a cost-effective means of pollutant cleanup and has been judged to be a reliable tool for modelling and optimization of waste drilling fluid bioremediation processes. The variations in total petroleum hydrocarbon and other physicochemical contents of drilling fluid bioremediation pattern with respect to fresh and composted spent mushroom substrate contents were observed to be significant ($p<0.05$). The order of biodegradation effectiveness among the various treatments used is as follows; composted spent mushroom substrate > fresh spent mushroom substrate>mangrove sediment>sterile mangrove sediment, respectively. Either fresh spent mushroom substrate or composted spent mushroom substrate can be employed for the effective and rapid biodegradation of waste drilling fluid contaminated mangrove soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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