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Biodegradation of Crude Oil Sludge Using Municipal Solid Waste as Bulking Agent

[1]A.A. Ibuot and [2]A. Bajhaiya
[1]Department of Science Technology, Akwa Ibom State Polytehnic, Akwa Ibom State, Nigeria
[2]Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, United Kingdom

Corresponding Author: A.A. Ibuot, Department of Science Technology, Akwa Ibom State Polytehnic, Akwa Ibom State, Nigeria Tel: +44 (0) 7778042293

ABSTRACT

Oil sludge generated during exploration and processing of crude oil could pose a serious threat to plants and animals if allowed to accumulate in the environment. In this study biodegradation of crude oil sludge using municipal waste as bulking agent was carried out. Soil samples were weighed 10 kg into vessels and polluted with 1 kg (10% pollution) and 0.5 kg (5% pollution) crude oil sludge. Municipal waste were added to the polluted soil samples as bulking agent at varying quantities ranging from 0.2 to 1 kg. The vessels containing soil with 10% crude oil sludge pollution were labeled A_1-A_6 and those with 5% crude oil pollution were labeled B_1-B_6, with A_6 and B_6 as control (crude oil polluted soil with no municipal waste added). The biodegradation process was allowed for 20 weeks. Total bacterial counts increased at week 5 and decreased as the biodegradation proceeded with less bacterial counts in the controls (A_6 and B_6). pH levels during the biodegradation period ranged between 6.7 and 7.6 for all samples. Total Petroleum Hydrocarbon (TPH) reduction ranged between 88 and 97% for samples A_1-A_5 and B_1-B_5 and 31 and 41% for samples A_6 and B_6, respectively. This research therefore shows that municipal waste can be used to enhance the biodegradation of crude oil sludge.

Key words: Oil, soil contamination, biodegradation, crude oil sludge, municipal solid waste, bulking agent

INTRODUCTION

The world is facing the challenges of environmental pollution caused by excess exploitation of petroleum and its by-products. In last few decades environmental pollution has shown remarkable increase due to pipeline leakages, accidents involving crude oil transport vehicles and leakages of storage vessels. These incidence leads to large amount of hydrocarbons being released into the environment. Also, the large volume of oily-sludge and waste oil contaminated materials produced during crude oil processing could also threaten the safety of environment (Mrayyan and Battikhi, 2005). Crude oil sludge is a complex substance characterized by high Total Hydrocarbon levels (THC), these are of toxic substances such as aromatic hydrocarbons (toluene, ethyl benzene, benzene and xylene) and poly aromatic hydrocarbons which could hazardous to plants and animals (Swoboda-Colberg, 1995; DPR, 2002).

Crude oil sludge contains toxic and carcinogenic components as such improper disposal may pose a serious threat to groundwater thereby posing threat to human life, plants and animals. Single bacterial or fungal specie has only limited capacity or may not at all degrade all the

hydrocarbon components present in crude oil, due to its recalcitrant nature this is why it persists in the environment and as such co-metabolism proves to be more efficient. The simple technique of bioremediation therefore is where natural attenuation is applied; this is an approach where contaminated sites are monitored for contaminant concentration and microorganisms in the environment biodegrade the contaminant (Bartha and Bossert, 1984; Bartha, 1986). Indigenous bacteria in the soil can degrade a wide range of target components of the oily sludge, but their population, growth rate and efficiency are affected by the high concentration of crude oil (Barbeau *et al*., 1997). The use of organic amendment can enhance microbial growth and biodegradation efficiency of microorganisms. These organic substances provide nutrients and carbon source for microorganisms to thrive in growth. Therefore this research is aimed at stimulating the growth of indigenous soil microorganisms using municipal waste so as to enhance the efficiency of crude oil sludge biodegradation.

MATERIALS AND METHODS

Collection of sample: Crude oily-sludge was collected from Shell Petroleum Development Company (SPDC), Port Harcourt, Rivers State in Nigeria. Municipal solid waste was collected from refuse dump sites in Ikot Ekpene, Akwa Ibom State, Nigeria.

Biodegradation process: Using weight/weight basis, soil sample, crude oil sludge and municipal waste were weighed into plastic containers (reactor vessels) and labeled as shown in Table 1.

All soil samples were turned and watered at intervals for 20 weeks of biodegradation. Water was sprinkled on the polluted soil during turning to maintain a favorable condition for effective biodegradation by microorganisms. Polluted soil samples were collected in triplicate 5 weekly intervals before turning for microbiological and physicochemical analysis.

Microbiological analysis: Samples were collected in triplicate at 5 weekly intervals and analyzed for microbial count during degradation.

Table 1: Description of vessels, amount of oil sludge and municipal solid waste used in experiment

	Contents of vessels (kg)		
Container labels	Soil	Crude oil sludge	Municipal solid waste
Vessel A_1	10	1.0	1.0
Vessel A_2	10	1.0	0.8
Vessel A_3	10	1.0	0.6
Vessel A_4	10	1.0	0.4
Vessel A_5	10	1.0	0.2
Vessel A_6 (Control)	10	1.0	
Vessel B_1	10	0.5	1.0
Vessel B_2	10	0.5	0.8
Vessel B_3	10	0.5	0.6
Vessel B_4	10	0.5	0.4
Vessel B_5	10	0.5	0.2
Vessel B_6 (Control)	10	0.5	

Microbial count (total heterotrophic bacterial count): Total Heterotrophic Bacterial Count (THBC) was carried out using nutrient agar. The agar was prepared according to the manufacturer's instruction (Sigma) 2.8 g of the medium was dissolved in 100 mL of distilled water. The medium was sterilized by autoclaving at 121°C for 15 min under a pressure of 15 PSI. The medium was allowed to cool to 45°C before it was poured into petri-dishes and allowed to set. A tenfold serial dilution of the samples was carried out, 1 mL of the dilution factor 10^7 was plated out on the prepared nutrient agar plates using spread plate method and this was done aseptically and in duplicate. The plates were covered, labeled and incubated in the incubator at 37°C for 24 h. After which the counting was done and the morphological characteristics of the colonies noted.

Isolation of hydrocarbon utilizing bacteria: The isolation of hydrocarbon utilizing bacteria (HUB) was carried out using the method of Zajic and Supplission (1972), using the mineral salt medium (MSM) of Zajic and Supplission (1972). The components were weighed out accordingly and were dissolved in 250 mL of distilled water in a conical flask. The mixture was supplemented with 3 g of agar-agar and shaken vigorously for the salts to dissolve in the distilled water. The medium was sterilized by autoclaving at 121°C for 15 min under a pressure of 15 PSI. The medium was allowed to cool to 45°C before it was poured into petri-dishes and allowed to set.

The hydrocarbon-utilizing bacteria were isolated by inoculating 1 mL of 10^6 dilution of the samples into the prepared plates. Using Vapor Phase Transfer (VPT) method, a sterile filter paper (Whatman No. 1.) saturated with filter sterilized Bonny Crude oil was aseptically placed on to the cover of the inverted petri-dishes. This was to provide the Bonny crude oil as sole source of carbon and energy for growth of the organisms through vapor phase transfer. The plates were then taped round with masking tape so as to increase vapor pressure within the plates after which they were incubated at ambient temperature of 30°C (±2°C) for 48 h. After the incubation, colonies were counted.

Determination of total hydrocarbon: Total Hydrocarbon (THC) was determined using the method of AOAC (1984). About 10 mL of each sample was dispensed into different separator funnels held in position by a clamp. Then 5 cm^3 of 50% H_2SO_4 was added and was allowed to stand for some hours, THC was extracted on addition of 10 mL of $CaC1_4$ (Calcium tetrachloride) followed by vigorous shaking and was allowed to settle thereby separating into two layers. The absorbance of the extract (supernatant) was read at 290 nm with Unicam UV/VIS spectrophotometer. Readings were recorded from spectrometer and using the determined curve to get figure.

Determination of pH: Using glass-electrode pH meter (Denver Instrument UB-10) 20 g of air-dried samples were weighed into a 50 mL beakers. Distilled water (20 mL) was added and allowed to stand for 30 min and stirred occasionally with a glass rod. The electrode of the pH meter was inserted into the partly settled suspension and the pH measured.

RESULTS

In 10% crude oil polluted soil total bacterial count was observed to be high in sample A_1 with 24.3 to 26.7×10^8 CFU g^{-1} between day 0 and 5 and at the end of biodegradation had 9.8×10^8 CFU g^{-1}. While sample A_2 had total bacterial count of 21.4 to 23.9×10^8 CFU g^{-1} from day 0 to 5 and 8.7×10^8 CFU g^{-1} at day 20 as shown in Table 2. While in 5% crude oil polluted soil total bacterial count was high in B_1 with 25 to 28.1×10^8 CFU g^{-1} from day 0 to 5 and 10.6×10^8 CFU g^{-1} at day 20

Table 2: Total bacterial count ($\times 10^8$ CFU g^{-1}) during biodegradation of 10% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Mean±SD
A_1	24.3	26.7	19.9	14.0	9.8	18.94±6.29
A_2	21.4	23.9	18.7	12.9	8.7	17.12±5.58
A_3	19.6	21.8	16.9	11.8	8.0	15.62±5.07
A_4	16.4	17.9	15.0	11.0	7.8	13.62±3.71
A_5	12.3	13.4	11.1	9.9	6.9	10.92±2.24
A_6	9.5	10.1	9.1	8.9	7.0	8.92±1.04

Table 3: Total bacterial count ($\times 10^8$ CFU g^{-1}) during biodegradation of 5% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Mean±SD
B_1	25.0	28.1	21.7	15.9	10.6	20.26±6.30
B_2	20.9	24.4	20.6	13.7	9.5	17.82±5.42
B_3	18.0	21.1	17.8	13.0	7.8	15.54±4.66
B_4	16.9	20.1	15.7	12.8	5.9	14.28±4.80
B_5	12.1	13.3	10.9	9.2	4.7	10.04±2.99
B_6	10.3	10.9	8.9	7.7	6.8	8.92±1.54

Table 4: Hydrocarbon utilizing bacterial count ($\times 10^7$ CFU g^{-1}) during biodegradation of 10% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Mean±SD
A_1	10.9	11.3	10.1	8.7	5.1	9.22±2.24
A_2	10.1	10.9	9.8	7.6	5.0	8.68±2.14
A_3	9.9	10.1	9.4	7.1	4.8	8.26±2.03
A_4	8.7	9.2	8.9	6.8	4.3	7.58±1.84
A_5	8.0	8.3	7.8	6.5	4.0	6.92±1.58
A_6	7.8	8.0	7.6	7.0	5.6	7.2±0.880

Table 5: Hydrocarbon utilizing bacterial count ($\times 10^7$ CFU g^{-1}) during biodegradation of 5% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Mean±SD
B_1	10.1	10.9	8.7	6.9	5.7	8.46±1.94
B_2	9.8	10.1	8.5	7.0	5.3	8.14±1.79
B_3	9.7	10.0	8.0	6.8	4.9	7.88±1.89
B_4	8.6	9.3	7.7	6.5	4.3	7.28±1.76
B_5	8.2	8.6	7.3	6.3	4.0	6.88±1.64
B_6	7.3	7.9	7.2	7.0	5.3	6.95±0.98

as shown in Table 3. Total hydrocarbon utilizing bacterial count was slightly high in A_1 having 10.9-11.3$\times 10^7$ CFU g^{-1} from day 0 to 5 and 5.1$\times 10^7$ CFU g^{-1} at day 20 as shown in Table 4. While B_1 had 10.1 to 10.9$\times 10^7$ CFU g^{-1} from day 0 to 5 and 5.7$\times 10^7$ CFU g^{-1} at day 20 as shown in Table 5. pH of the samples ranged between 7.2 and 7.6 for day 0 and 5 and 6.7 to 6.8 at day 20 for all samples (Table 6, 7). High reduction in Total Petroleum Hydrocarbon (TPH) was observed in sample B_1, A_1 and B_2 with 97, 96 and 96%, respectively as shown in Table 8 and 9.

Table 6: pH levels during biodegradation of 10% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Mean±SD
A_1	7.4	7.6	7.3	7.1	6.8	7.24±0.27
A_2	7.3	7.6	7.3	7.2	6.7	7.22±0.29
A_3	7.3	7.5	7.2	7.1	6.8	7.18±0.23
A_4	7.1	7.3	7.1	7.1	6.7	7.06±0.20
A_5	7.2	7.4	7.2	7.1	6.7	7.12±0.23
A_6	7.3	7.4	7.3	7.2	6.8	7.2±0.210

Table 7: pH levels during biodegradation of 5% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Mean±SD
B_1	7.3	7.6	7.4	7.2	6.7	7.24±0.30
B_2	7.2	7.4	7.2	7.1	6.7	7.12±0.23
B_3	7.2	7.3	7.1	6.9	6.6	7.02±0.25
B_4	7.3	7.4	7.2	7.1	6.7	7.14±0.24
B_5	7.2	7.3	7.2	6.9	6.7	7.06±0.23
B_6	7.3	7.4	7.3	7.1	6.5	7.12±0.33

Table 8: Total petroleum hydrocarbon levels (mg 100 g^{-1}) during biodegradation of 10% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Reduction(%)
A_1	710	306	130	79	32	96
A_2	708	322	141	87	40	94
A_3	709	347	149	91	51	93
A_4	707	352	157	97	59	92
A_5	709	359	163	109	78	89
A_6	708	667	631	507	489	31

Table 9: Total petroleum hydrocarbon levels (mg 100 g^{-1}) during biodegradation of 5% crude oil sludge polluted soil

	Weeks of biodegradation					
Samples	0	5	10	15	20	Reduction(%)
B_1	511	209	97	43	18	97
B_2	509	229	110	57	21	96
B_3	507	237	115	72	29	94
B_4	510	242	121	89	53	90
B_5	508	250	129	98	61	88
B_6	509	452	421	378	301	41

Data: Mean of results in triplicate

DISCUSSION

Tremendous increases in total bacterial counts were observed at week 5 and a decrease was observed as the biodegradation proceeded. The increase in microbial count was most likely observed because of favorable conditions created by the addition of bulking agent (municipal solid waste),

moistening and aeration as reported by Wolter *et al.* (1997). The decrease in microbial count from week 10 of biodegradation was likely due to nutrient reduction, changes in the physicochemical properties of the soil as a result of microbial metabolism. pH values recorded during the period of degradation ranged between 6.5 and 7.1 (Table 6, 7) and are known to be favorable to bacterial growth including hydrocarbon utilizing bacteria (Al-Qahtani, 2011). Total Petroleum Hydrocarbon analysis of samples at intervals indicates a reduction in Total Petroleum Hydrocarbon (TPH) with time of biodegradation as shown in Table 8 and 9. Very high reduction in the Total Petroleum Hydrocarbon (TPH) was recorded in all the samples with 88-97% TPH reduction except the controls (A_6 and B_6) which had 31 and 41% TPH reduction, respectively (Table 8, 9). This could be implicated with the role played by municipal waste as organic supplement, enabling microorganisms to thrive efficiently for the biodegradation process. Organic materials facilitate sludge degradation; this has been reported (El-Nawawi *et al.*, 1992; Hiebert *et al.*, 1994). The degradation rate increased gradually over the 20 weeks period, this has been reported (John, 2007). The addition of municipal waste to the polluted soil samples created a favorable condition for the microbial degradation of the oily sludge. The significant reduction in the Total Petroleum Hydrocarbon (TPH) is of great importance as this indicates the enhancing role played by municipal solid waste in the biodegradation of soil polluted with crude oil sludge.

CONCLUSION

Biodegradation of crude oil sludge with municipal solid waste as bulking agent is a cost effective and economical approach to crude oil sludge biodegradation. The significant reductions in the Total Petroleum Hydrocarbon of the samples indicate an effective biodegradation of crude oil sludge with municipal waste as bulking agent. Total Petroleum Hydrocarbon reduction could be enhanced by large volume of bulking agents. Therefore, this research has revealed that the addition of solid municipal waste to crude oil sludge polluted soils as bulking agent could facilitate petroleum hydrocarbon breakdown by microorganisms. The rate of petroleum hydrocarbon breakdown by microorganisms is proportional to the volume of bulking agent (solid municipal waste) added to the soil.

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