JASEM ISSN 1119-8362
All rights reserved

Full-text Available Online at
http:// www.bioline.org.br/ja

J. Appl. Sci. Environ. Mgt. 2005
Vol. 9 (1) 103 - 106

Remediation of Crude Oil Contaminated Soil by Enhanced Natural Attenuation Technique

[1*]EBUEHI, O A T; [1]ABIBO, I B; [2]SHEKWOLO, P D; [2]SIGISMUND, K I; [1]ADOKI, A; [2]OKORO, I C

[1]Department of Biochemistry, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria. (Email: ebuehi@yahoo.com)

[2]Health Safety and Environment Department, Shell Petroleum Development Corporation, Port Harcourt, Nigeria.

ABSTRACT: The concentrations of nitrogen, phosphorus, total hydrocarbon utilizing bacteria (THUB), total heterotrophic bacteria (THB) and total petroleum hydrocarbon were determined using the remediation by enhanced natural attenuation (RENA) in a crude oil contaminated farmland in Rivers state, Nigeria. A TPH concentration of 1.1004 x10 ^{4}mg/kg of the sandy soil was achieved after spiking and tilling. There was a reduction in the TPH level from 300mg/kg after 8weeks, to 282mg/kg after 10weeks.No significant reduction in the TPH level was observed after the 10th week. The nitrogen and phosphorus levels of the sandy soil were 24.6 and 22.8mg/kg respectively. This suggests that the nitrogen and phosphorus levels could no longer support biodegradation at the residual TPH levels of 282mg/kg and 22.8mg/kg after spiking and tilling respectively, which further reduced to 0.12mg/kg and 1.7mg/kg respectively after 10 weeks. The total hydrocarbon utilizing bacteria (THUB) increased from 3.0 x 10 ^{4}cfu/g to 8.55x10 ^{4}cfu/g and finally reduced to 5.38 x10 ^{4}cfu/g, while the total heterotrophic bacteria (THB) reduced from 1.22 x10 ^{8} cfu/g to 5.98 x 10 ^{5}cfu/g. Data of the study indicate that remediation enhanced natural attenuation technique could be employed to remediate a farm settlement contaminated by crude oil. @JASEM

Oil exploration and production activities have significant environmental consequences that occur. The search for oil in Nigeria begun in 1937 (Awobajo, 1981, Ifeadi and Nwankwo, 1980), with increasing production of crude oil and discovery of major oil reserves, more effort was added to exploit this resource. Operations include oil exploration, oil drilling, oil production, oil transportation, oil processing and oil storage (Bossert and Bartha, 1984, Odeyemi and Ogunseitan, 1985). Oil spillages still occur through tanker accidents, well blow out, sabotage and accidental rupture of pipelines, resulting in the release of crude and refined oil into terrestrial and aquatic environments (Atlas, 1981, Colwell and Walker, 1977). The highest incidence of oil spills occurred in the mangrove swamps zones and near off shores areas of the Niger Delta which was shown in an analysis of oil spillage statistics in Nigeria during the period, 1976 to 1988. (Ifeadi and Nwankwo, 1989, Awobajo, 1981). These areas are the most productive and sensitive areas in the ecosystem.

The oil spillages introduce non-organic, carcinogenic and growth-inhibiting chemicals present in the crude oil and their toxicity to microorganisms and man is well known (Atlas and Bartha, 1973a, 1973b, Odu, 1972, 1978, Okpokwasili and Odokuma, 1990). The natural recovery of crude oil from polluted soils is slow, communities affected by such cases are denied of their agricultural lands, a long time, so remediation was brought about (Gradi 1985, Alexander 1978). Remediation has been defined as the management of a contaminant at a site so as to prevent, reduce or mitigate damage to human health, or the environment, which can also lead to quick recovery of the affected lands (Doelman, 1994). Physical, biological and chemical processes are employed for remediation.

Remediation by enhanced natural attenuation (RENA) is a land farming treatment technology for intervention in petroleum hydrocarbon contaminated soils in the Niger Delta regions (Awobajo 1981, Ifeadi and Nwankwo 1980, Odeyemi and Ogunseitan, 1985, Odu, 1972). RENA is a full-scale bioremediation technology in which contaminated soils, sediments and sludge's, are periodically turned over or tilled into the soil to aerate the waste. Soil conditions are often controlled to increase the rate of contaminant degradation (Odu, 1978, Gradi, 1985). Bioremediation process enhances the indigenous bacteria via the addition of oxygen and nutrients to degrade petroleum hydrocarbon to carbon dioxide and water. The actual mechanism involved, which is mediated by microbes is known as biodegradation (Atlas 1981, Colwell and Walker, 1977).

However, it is imperative to study RENA preliminary process of a farm settlement contaminated with crude oil, in Rivers State in the Niger Delta Area of Nigeria. Therefore, we report in this study, using RENA process spanning 10 weeks, to ascertain the total petroleum hydrocarbon, nitrogen and phosphorus levels, total hydrocarbon utilizing bacteria and total heterotrophic bacteria in a contaminated farm settlement at Rumuekpe in Rivers State, Nigeria.

MATERIALS AND METHODS

A farmland settlement contaminated with crude oil located in Rumuekpe, Rivers State, Nigeria, was used

*Corresponding author: Email: ebuehi@yahoo.com

for the study. The test soil obtained was sandy soil. The preliminary process of bioremediation took a period of 10weeks. The bioremediation process comprises field experiment and laboratory simulation, with some physiochemical and microbial analyses. Soil sample was taken from a depth of 0.30metres. The concentration of total petroleum hydrocarbon (TPH) nitrogen and phosphorus were determined, while the total heterotrophic bacteria (THB) and total hydrocarbon utilizing bacteria (THUB) were committed. These physiochemical parameters were monitored once every two weeks for a period of 10weeks.

Remediation by Enhanced Natural Attenuation (RENA) analysis: The following RENA techniques were employed to treat the contaminated farmland.

Spiking of Test Soils: The soils were spiked with water uniformly to soften the soil and to allow the water penetrate the soil matrix.

Initial Tilling: The soils were tilled in a week after they were spiked, that is mixing the soil and breaking the lumps. This was done using shovel, composite samples were collected and sent to the laboratory for physiochemical and microbial evaluation.

Secondary Tilling: The soils were tilled and homogenized a week after the initial tilling. The lumps were broken to very fine particles with a shovel and a rake. The essence of the tilling and homogenization was to uniformly distribute the petroleum contaminants and break up the soil lumps to fine particles thereby increasing the surface area. The composite samples were taken for analysis.

Windrow Construction: Windrows/ridges were constructed after the secondary tilling of the test site. The ridges measured about 2feet high and 4feet wide. The windrows are made to achieve better aeration and optimize the efficiency of the attenuated processes in action, which exposes the microorganisms to oxygen, and aids in the biodegradation process of the petroleum hydrocarbon. Soil samples were taken for analysis

Breaking down of Windrows: The windrows were broken down after standing for between 3 and 4 weeks, after construction. Soil samples were taken for analysis.

Addition of Water: Water was added to the sandy soil to enhance the biodegradation of the petroleum hydrocarbons by the microorganisms when it penetrates the soil.

Addition of Fertilizer: Fertilizer application was done manually by sprinkling the fertilizer over the contaminated area. The process enhances the biodegradation of the petroleum hydrocarbon

Soil Sampling and Analysis: The topsoil samples of the farmland were taken at intervals of two weeks from 0.3metres deep. They were taken using an auger machine into polypropylene bags, free from hydrocarbon contamination. This process is called augering. The soil samples were taken for immediate physico-chemical and microbial analysis.

Physico-Chemical and Microbial Analysis: Total phosphorous, total nitrogen and total petroleum hydrocarbon contents in the soil samples were determined by the method of Association of Analytical Chemists (1990).

Microbial Analysis

*Total Heterotrophic Bacteria (THB) Count***:** The total heterotrophic bacterial count was performed on nutrient agar (Oxoid), using the spread place method (Gradi, 1985). Total viable counts of culturable aerobic heterotrophic bacteria were obtained by preparing serial dilutions of gram wet saline (0.89% w/v NaCl) and surface plating on to sterile nutrient agar in triplicate. Culture plates were incubated at room temperature ($28 \pm 2^{O}C$ for 48h. Plates yielding counts of 30 – 300 colonies were chosen and the counts obtained were multiplied by the dilution factor to obtain the number of bacteria per gramme of soil

Total hydrocarbon Utilizating Bacteria (THUB) Count: Vapour-phase transfer method was adopted to estimate the population of THUB. A modified mineral salt medium of Mills *et al* (1978), was inoculated with suspension of test soil sample. Mixing 1g of wet soil with 10ml of sterile saline made suspension. Sterile filter paper (Whatman No. 1) saturated with crude oil was placed on the inside cover of each petri-dish kept in an inverted position. These filter papers supplied the hydrocarbons by vapour phase transfer to inverted inoculums. Plates were counted after incubation at room temperature for 7days. The percentage of hydrocarbon utilizers within the heterotrophic bacteria population was determined.

RESULTS AND DISCUSSION

The farmland used for the present study was mildly contaminated containing 1.10×10^4, 24.6 and 22.8 mg/kg of total petroleum hydrocarbon, nitrogen and phosphorus contents respectively. The total heterotrophic bacteria and hydrocarbon utilizing bacteria counts were 1.22×10^8 and 3.0×10^4 cful/g of respectively. The results of the physico-chemical

characteristics if the farmlands are presented in Table 1. However, the values should be better percentiles exponential about ten weeks of remediation by natural attenuation of the farmland the level of TPH, nitrogen and phosphorus were 300, 0.12 and 1.7mg/kg while the THUB and THB counts were 5.38 x10 ^{4} and 6.25 x10 ^{5} cfu/g. The degradation trend of TPH concentration, which was achieved after spiking and homogenization after the first week of the bioremediation of the sandy soil, was 1.1004 x10 ^{4}mg/kg, and by 6.25 x 10^{5} the third week, the TPH concentration had significantly ($p<0.01$) dropped by 62.7%, to 4105mg/kg. The TPH concentration dropped to 2534mg/kg about three weeks after application of the various RENA techniques, which cumulatively resulted in 77% loss in concentration.

Table 1: Physicochemical characteristics of the contaminated farmland

S/No	Sampling duration	TPH (mg/kg)	Nitrogen (mg/kg)	Phosphorus (mg/kg)	THUB (cfu/g)	THB (cfu/g)
1.	1	11.4x10 ^{3}	24.6	22.8	3.0 x 10 ^{4}	1220 x10 ^{5}
2.	3	4.105x 10 ^{3}	19.2	22.1	5.2 x 10 ^{4}	550 x 10 ^{5}
3	5	2.534 x 10 ^{3}	0.28	3.1	6.84x10 ^{4}	5.7 x 10 ^{5}
4	7	1.364 x10 ^{3}	0.16	1.6	8.55x10 ^{4}	5.98 x 10 ^{5}
5	9	0.3x10 ^{3}	0.12	1.7	5.38 x10 ^{4}	6.25 x 10 ^{5}

Table 2: Microbial Population changes during Biodegradation of Crude Oil in farmland

S/No	Sampling period (wk)	TPH (mg/kg)	THUB (cfu/g)	THB (cfu/g)	THUB/THB (%)
1.	1	11.4x10 ^{3}	3.0 x 10 ^{4}	1220 x10 ^{5}	0.025
2	3	4.105x 10 ^{3}	5.2 x 10 ^{4}	550 x 10 ^{5}	0.095
3	5	2.534 x 10 ^{3}	6.84x10 ^{4}	5.7 x 10 ^{5}	12
4	7	1.364 x10 ^{3}	8.55x10 ^{4}	5.98 x 10 ^{5}	14.3
5	9	0.3x10 ^{3}	5.38 x10 ^{4}	6.25 x 10 ^{5}	8.6

After 8weeks of the remediation process, the TPH level reduced to 300mg/kg and then further dropped to 282mg/kg the following week respectively. The nitrogen and phosphorus concentrations significantly ($p<0.01$) decreased as the remediation process progressed. Their levels were significant ($p<0.01$) different within the first two weeks after the initial and secondary tilling of the soils. This suggests that microbes degrading the hydrocarbons within the first two weeks do not produce any significant results. However, four weeks after bioremediation process, the nitrogen and phosphorus contents decreased drastically from 24.6mg/kg and 22.8mg/kg to 0.28mg/kg and 3.1mg/kg respectively, which represent a 98.9% and 86.4% reduction in their concentrations respectively. But after 8th week, there was a significant reduction in the nitrogen and phosphorus contents to 0.12mg/kg and 1.7mg/kg respectively. There was a significant ($p<0.01$) decrease in the populations of total heterotrophic bacteria (THB), after 2weeks of tilling from 1.22 x10 ^{8} to 5.5 x 10 ^{7}cfu/g, while those total hydrocarbon utilizing bacteria (THUB) increased from 3.0 x10 ^{4} to 5.2 x10 ^{4}cfu/g. The THB population significantly decreased from 1.22 x10 ^{8} to 5.7 x10 ^{5}cfu/g, showing a 12% of the THB population, while the THUB increased from 3.0 x10 ^{4} to 6.84 x10 ^{4}cfu/g. This is indicative of the increased biodegradation by the THUB.

The THUB continued its upward rise to 14.3% of the THB population, while TPH concentration was reduced to 1364mg/kg, showing progressive trend in the degradation. After 8weeks of the remediation process, the THUB count reduced both in number and proportion to the increase in the THB population. The THUB population finally reduced to 5.38 x10 ^{4}cfu/g, representing 8.6% of an increasing THB population of 6.25 x10 ^{5}cfu/g. This shows an ALARP (As Low As Reasonably Practicable) condition for TPH which explains that TPH has been reduced to a level where if bioremediation proceeds it becomes economical. This study has revealed that the process of bioremediation of a contaminated farm soil is very effective.

The RENA technique is a very effective way of carrying out bioremediation, which helps soils, contaminated with crude oil reach the ALARP condition. The TPH degradation was successful since there was significant reduction in TPH population during the bioremediation process, which showed little or no contamination. The THUB increased until there was no more contamination before a reduction, showing that the hydrocarbon-utilizing bacteria had now migrated to other soil locations since it feeds on petroleum hydrocarbon. However, the nitrogen and phosphate reduction as TPH reduced indicate that the nitrogen and phosphorus concentration can be used as fertilizers to the microbes that degrade the petroleum hydrocarbons. The microbes make use nitrate and phosphate in the degradation process. The

acceleration of growth of THUB during the bioremediation process is indicative of the ability of indigenous microorganisms to adapt to the presence of the contaminants and bring about their transformation to reduce levels of contamination in the soil. The reduction in the concentration of petroleum hydrocarbon contaminant from a crude oil polluted farm site depends on the interplay of biotic and abiotic factors, the manipulation of these factors during remediation by enhanced natural attenuation process can bring about a marked reduction in the concentration of the contaminant. However, despite the optimization of these factors, a point is reached were no significant breakdown or reduction in the concentration of the contaminant can be achieved economically and sustain ably and this point is referred to as ALARP level.

In conclusion, indigenous soil microorganisms and other weathering mechanisms to a concentration that no longer can decrease degrade petroleum hydrocarbons. The microbial enhanced reductions of contaminants to a very low contaminant level are limited by the availability of hydrocarbons and inorganic nutrients such as nitrate and phosphates to the microorganisms.

*Acknowledgement***:** The authors are grateful to Shell Petroleum Development Company, Port Harcourt, for the provision of technical and laboratory facilities used for the study.

REFERENCES

Alexander, M (1978) An introduction to soil microbiology, 2nd ed., John Wiley & Sons/ New York p223-330

Association of Analytical Chemists (AOAC, 1990). Official Methods of Analysis, 14th Ed. Horowits W(ed) Washington DC pg 140-400

Atlas, R.M (1981) Microbial degradation of Petroleum hydrocarbons. An environmental perspective. Microbiol. Reviews 45, 180-209

Atlas, R.M; Bartha, R (1973a) Abundance, Distribution and Oil Degradation Potential of Microorganisms in Raitan Bay. Environ. Pollution 4:291-300

Atlas, R.M; Bartha, R (1973b) Degradation and Mineralization of petroleum in seawater. Limitations by Nitrogen and phosphorus. Biotechnology and Bioengineering 14, 309-317.

Awobajo, S.A (1981) An analysis of spill incidents in Nigeria (1976-1980). In: Proc., Seminar on Petroleum Industry and the Nigerian Environment, NNPC/FMOW and Housing PT, Warri.

Bossert I; Bartha,R (1984): The fate of Petroleum in soil Ecosystems. In. Petroleum microbiology Atlas R.M. ed. Macmillan New York, p435-437

Colwell, R.R; Walker, J.D (1977). Ecological Aspects of Microbial Degradation of Petroleum in the Marine environment CRC Critical Reviews in Microbiology 5(4), 423-445

Doelman, P, (1994) European Perspectives of field research on bioremediation. Special attention to the Netherlands. Invited paper for the 5th International Congress of soil science, Acapulco, Mexico, P15.

Gradi, P.C (1985) Biodegradation. Its Management and Microbiology Basis. Biotechnology and Bio-Engineering 27:660-674.

Ifeadi, C.N; Nwankwo, J. (1980): Oil Spill incidents in Nigeria Petroleum Industry. A Critical Analysis. Napetcor. 8:11-45

Odeyemi, O; Ogunseitan, O.A (1985) Petroleum Oil Industry and its Pollution Potential in Nigeria. Oil and Petroleum Pollution 2:223-229.

Odu, C.T.I (1972); Microbiology of Soils Contaminated with petroleum. Hydrocarbon extent of contamination and some soil and microbial properties affected after contamination. Journal of the Institute of Petroleum (London) 58:201-208.

Odu, C.T.I (1978); The effects of nutrients application and aeration on oil degradation in soil. Environmental Pollution 15:235-240

Okpokwasili, G.C; Odokuma, L.O (1990). Effect of salinity on biodegradation of Oil Spills dispersants. Waste Management 10:141-146.