Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (11): 856-860, 2007
ISSN 1816-4935

Comparative Bioremediation Enhancement Procedures on Kerosine Polluted Ultisol from a Niger Delta Region, Southern Nigeria

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Abstract: A study on the effect of bioremediation treatments on the reclamation of kerosene spills on various soil samples was conducted around Calabar Petroleum Depot within the Niger Delta Region of Nigeria. Soil samples subjected to different levels of kerosene concentrations ranging between 5 mL and 50 mL/100 g (v/w) of soil were analysed and a total of 4 bacterial isolates *Bacillus subtilis*, *Pseudomonas acidovorans*, *Serratia marcescens* and *Micrococcus* sp. identified in decreasing order of kerosene utilizing potentials. The mean hydrocarbon utilizing bacteria counts increased significantly ($p<0.05$) at higher incubation periods ranging from 22.0×10^3 to 59.1×10^3 CFU g^{-1} though no significant difference was observed ($p>0.05$) with depth of polluted soil. A combination of all treatments (tilling, fertilizing with NPK and microbial seeding with *Bacillus subtilis*) significantly increased the amount of reclaimed kerosene during the 8th week to 69.9, 58.2, 35.7 and 20.0% using 5, 10, 20 and 50 mL/100 g (v/w)of polluted soil samples, respectively. The amount of reclaimed kerosene differed significantly ($p<0.05$) with the various levels of kerosene pollution. These results are highly recommendable in the context of other oil cleansing procedures currently in use.

Key words: Bioremediation enhancement, kerosene-polluted ultisol, tilling, fertilizing, microbial seeding, Southern Nigeria

INTRODUCTION

Bioremediation as presently defined, involves the use of naturally occurring micro-organisms to treat specific chemical pollution associated with the environment (Alexander, 1977; Pelczar *et al*., 2002). The importance of micro-organisms in decomposing natural organic residues in soil sediments and aquatic systems has long been recognized (Zobell, 1971; Focht, 1987; Morgan and Watkinson, 1989). The microbial biodegradation of crude oil and other aliphatic and aromatic hydrocarbons carried out by both autochthonous and allochthonous species bring about the biotransformation which reduces the complex mixture of noxious materials by breaking intermolecular bonds to simple nutrients in soil and aquatic ecosystems (Bucker *et al*., 1999; Burland and Edwards, 1999). Under certain optimal conditions, bacterial cell can assimilate an amount of nutrient equivalent to their own weight in few seconds (Raymond *et al*., 1987). It is therefore, not surprising that bioremediation has over the years continued to show promise as an alternative to conventional environmental cleaning techniques (Odu, 1987; Admon *et al*., 2001; Itah and Essien, 2005). Such traditional methods often used in reclaiming polluted soils include, excavation, use of absorbent material, sinking and burning all having undesirable ecological implications. Harnessing the natural potentials of micro-organisms to accomplish the degradation and detoxication of hazardous contaminants for the protection of health and the environment is not only simple and effective but also consistent with the philosophical thrust of Amendments and Reauthorization Act of 1986 (SARA) which forms the goal of on-site bioremediation

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of contaminated soil (Sloan, 1987; Artiola and Warrick, 2004). Since bioremediation relies on the hydrocarbon degradation capabilities of micro-organisms in contact with the oil pollutant, concerted efforts by environmental scientists have shifted to the isolation of organisms with potentials for degradation and how these potentials can be enhanced (Chikere and Okpokwasili, 2003).

Generally, sources of kerosene pollution include accidental spills, pipe leakage or vandalization, deliberate disposal of oily wastes, corrosion of pipes, kerosene seeps and other operational deficiencies. Apart from the aesthetic and economic damage caused by kerosene spills, plant, vertebrates, invertebrates and micro-organisms in both the terrestrial and aquatic environments are adversely affected (Atlas and Bertha, 1975; Toogood, 1977; Odu, 1987; Guzman *et al*., 2004). In Nigeria, the Niger Delta Region constantly experiences high levels of crude oil refining activities and transportation to other regions of the country. Studies on bioremediation of coastal environments have been restricted to crude oil contamination with respect to individual treatment with little emphasis on the effects of combined treatment processes on microbial activities (Chikere and Okpokwasili, 2003; Chukwara *et al*., 2005; Itah and Essien, 2005; Asitok and Antai, 2006; Ekpo and Ekpo, 2006). Kerosene serves as a major source of energy to all sectors of the society for cooking and lighting, hence spills arising from tank overflow, bunkering, poor vending facilities etc are inevitable. It is also logical that a study of bioremediation of kerosene polluted soils would substantially provide the necessary guide for studying bioremediation effects on other forms of pollutants arising from crude oil refining. This is exactly what the present investigation seeks to achieve.

MATERIALS AND METHODS

Collection of Kerosene and Soil Samples

Kerosene samples were obtained from service Depot in Calabar with 100 mL collected in each of 200 mL sterile glass bottles and stored at room temperature (28±2°C). Top soil samples were collected from areas within 100 m from the depot and 1 kg quantities stored in each of 32 wooden boxes measuring 0.5 m^3. Each was subjected to kerosene contamination concentrations of 5 mL/100 g (v/w), 10 mL/100 g (v/w), 20 mL/100 g (v/w) and 50 mL/100 g (v/w) and analysed successively at durations of 0, 2, 4, 6 and 8 weeks.

Enumeration of Total Heterotrophic Bacterial (THB) Counts and Hydrocarbon Utilizing Bacterial (HUB) Population

Isolates of THB were obtained through serial dilution of polluted soils obtained from 3 depth ranges (0-0.05, 0.06-0.15 and 0.16-0.5 m) and pour plating on Nutrient Agar (Difco Lab) as described by Odu (1987). Discrete colonies of different morphological types were counted, subcultured to obtain pure cultures and stored at 8°C for further microbiological investigations. In determining the kerosene utilizing potentials, isolates on Nutrient agar slants were transferred to 0.9 mL of Mineral Salt Broth (MSB) containing filter-sterilized kerosene. Each suspension was incubated at room temperature (28±2°C) and observed for degree of growth using gravimetric methods as described by Morgan and Watkinson (1989). Growth patterns of kerosene utilizers were described as Heavy (++++), moderately heavy (+++), moderate (++) or scanty (+).

Determination of Reclaimed Kerosene

The effect of remediation procedures on the kerosene reclamation process was investigated as described by Finn (1983). Polluted soil samples representing all levels of kerosene concentrations were subjected to 3 remediation treatments comprising tilling only using a sterile metallic spatula every 3 days (T), tilling and augmentation with 100 g of NPKfertilizer per kilogram of soil samples (T+F) and tilling with fertilizer before seeding with 0.1 mL (6.2×10^6 CFU mL^{-1}) of 24 h nutrient broth culture of *Bacillus subtilis*. Control samples at all levels of kerosene concentration were left untreated. The amount of kerosene reclaimed at a given time was assayed using 5% carbon tetrachloride solution.

Results obtained were analysed statistically using the student t test and Analysis of variance (ANOVA).

RESULTS

A total of 4 isolates namely *Bacillus subtilis*, *Pseudomonas acidovorans*, *Serratia marcescens* and *Micrococcus* sp. were identified as kerosene utilizing organisms from the polluted soil samples as shown in Table 1. *Bacillus subtilis* was predominant followed closely by *Pesudomonas acidovorans* with moderately heavy growth while *Serratia marcescens* and *Micrococcus* sp. showed moderate and scanty growths, respectively. Initially, the upper half of each box (0-0.06 m) had lower microbial counts than the lower half (0.06-0.5 m) (Table 2), however, large increases of THB and HUB were observed after the 4th week with no significant difference at $p>0.05$. Highest percentages of kerosene reclamation were obtained during the 8th week with 5, 10, 20 and 50 mL/100 g (v/w) having 69.9, 58.2, 35.7 and 20.0%, respectively when all remediation treatments were applied (Table 3). Though periodic extraction and measurement of residual kerosene revealed that it could be degraded without a remediation treatment technology, a significant difference was observed at $p<0.05$ with higher values recorded when remediation treatments are applied. Application of remediation technology therefore significantly enhance bioremediation rate by 1:5 as observed in this research.

Table 1: Isolates of kerosene utilizing bacteria from contaminated soil samples

Organism (Isolates)	Growth potential
Pseudomonas acidovorans	+++
Micrococcus	+
Bacillus subtilis	++++
Serratia marcescens	++

++++: Heavy growth; +++: Moderately heavy growth; ++: Moderate growth; +: Scanty growth

Table 2: Variation of bacterial populations with depth and duration of polluted soil ($\times 10^3$ CFU g^{-1})

	Duration of contact with pollutant (weeks)				
Level of sampling (m)	0	2	4	6	8
THB					
0-0.5	55	35.0	54.9	68.8	62.5
0-0.15	45	48.0	63.9	73.1	68.0
HUB					
0-0.06	22	28.4	41.9	51.8	48.2
0.06-0.16	20	36.8	55.0	59.1	53.0

THB: Total Heterotrophic Bacteria Count. HUB: Hydrocarbon Utilizing Bacteria count

Table 3: Effect of remediation treatments on reclamation of kerosene from soil samples

		Amount of kerosene (%)			
Kerosene concentration in soil samples	Remediation treatments	2 weeks	4 weeks	6 weeks	8 weeks
5 mL/100 g (v/w)	C	21.9	24.5	27.1	28.0
	T	21.7	45.5	64.7	64.9
	T + F	22.5	48.3	66.1	68.5
	T + F + M	21.3	49.1	69.9	69.9
10 mL/100 g (v/w)	C	10.5	15.6	18.3	19.0
	T	10.3	33.8	46.8	47.3
	T + F	9.8	35.2	50.3	51.5
	T + F + M	10.0	40.1	55.7	58.2
20 mL/100 g (v/w)	C	8.3	11.5	15.0	16.2
	T	8.0	18.3	27.6	28.0
	T + F	8.2	21.8	30.2	32.2
	T + F + M	7.3	23.2	34.9	35.7
50 mL/100 g (v/w)	C	4.3	6.9	8.5	9.2
	T	4.0	10.6	13.7	13.8
	T + F	4.2	12.8	15.9	16.5
	T + F + M	4.1	16.0	19.5	20.0

C = Control, T = Tilling, T+F = Tilling and Fertilizing, T+F+M = Tilling, Fertilizing and Microbial seeding

DISCUSSION

The present study has considerably appraised the bioremediation of kerosene polluted soil by indigenous microbial population. Species of *Bacillus* and *Pseudomonas* were better degraders of kerosene than those of *Micrococcus* and *Serratia*. The ability of these species to efficiently utilize petroleum hydrocarbons from crude oil polluted soils was also confirmed by Asitok and Antai (2006) who observed high Optical Density (OD) of 1.976 and 2.785 for *Pseudomonas* and *Bacillus*, respectively. This potential is attributed to their abilities to produce biosurfactants (Banat, 1995; Asitok and Antai, 2006) and the possession of appropriate enzyme system for crude oil utilization. Initially, the total bacterial count decreased substantially from the surface to the bottom, an observation suggestive of the reliance on oxygen for the break down process (Atlas and Odu, 1981; Raymond *et al*., 1987). However, sequel to the stabilization of the pollutants in the medium, a sudden increase in the microbial population of the bottom soil was recorded, due partly to accumulation of kerosene by gravity, weathering activities of the box which pushes the micro-organisms downwards and the moisture content. Similar studies had also recorded significant increases in microbial population due to downward migration of oil (Bartha and Bonnet, 1984; Chaudling and Cortez, 1988; Song and Bartha, 1990). Previous investigations following the oil well blow out in the Gulf of Mexico also confirmed these results. (Atlas and Odu, 1989; Sloan, 1987). Remediation treatment involving tiling, fertilizing and microbial seeding provided adequate aeration, increased nitrogen and phosphorus content and improved the quality of the hydrocarbon utilizing microbial population respectively. The role of oxygen as a necessary requirement for biodegradation of crude oil had also been reported by Chukwura *et al*. (2005) working on crude oil polluted Escravos River in Delta State, Nigeria. Also, the importance of nutrient supplementation in the enhancement of biodegradation potentials of microorganisms was confirmed by Chikere and Okpokwasili (2003) studying on petrochemicals from Port Harcourt Refinery Nigeria. Highest potentials for crude oil utilization in the Niger Delta Region of Nigeria have been attributed to *Bacillus subtilis* (Antai, 1990; Asitok and Antai, 2006; Ekpo and Ekpo, 2006). These findings support the suitability of the bacterial species for bioaugmentation during bioremediation enhancement processes. Compared to unremediated soil (control) and individual treatments, a combination of these remediation processes significantly increased the rate of degradation of kerosene in the soil. According to studies on the bioremediation potentials on terrestrial fuel spills (Lehtomaki, 1975; Bartha and Bonnet, 1984; Antai, 1990), bioremediation produces the desired infilteration, amplifies microbial food available and dilutes their metabolic end products.

Specifically therefore, the degree of degradation of kerosene contaminants depends to some extent on soil depth, period of incubation, degree of pollution and remediation treatments. Results from this study are relevant in explaining the following phenomena; that changes in the dynamic equilibrium state of soil environment leads invariably to a corresponding change in the microbial composition and whereas species with inherent kerosene hydrocarbon assimilatory potential are enriched by the contaminants, the less adapted species among the total heterotrophic populations are gradually being eliminated resulting in qualitative shift in species composition. The basis for applying appropriate bioremediation treatments to other oil spills in the environment is drawn from these conclusions.

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