BIOSTIMULATION SUPPLEMENTED WITH PHYTOREMEDIATION IN THE RECLAMATION OF A PETROLEUM CONTAMINATED SOIL

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Abstract

Biostimulation, supplemented with phytoremediation was employed in a study aimed at evaluating the effect of both treatments on the reclamation of a petroleum-contaminated soil. Petroleum contamination of soil was simulated under controlled field conditions, biostimulation of indigenous microbes through the addition of N-P-K fertiliser and tillage was then utilised for remedial treatment.

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Keywords: Biostimulation, ecological rehabilitation, elephant grass, petroleum contamination, phytoremediation, total hydrocarbon content.

INTRODUCTION

Petroleum contamination of soils has received significant attention over the last decades. Remediation of contaminated sites can be achieved through physical (e.g. disposal in landfill, incineration), chemical (use of chemical oxidants) and biological processes. Biological treatment, commonly referred as bioremediation, involves the breakdown of contaminants

into non-toxic forms through the activities of microorganisms (Riser-Roberts 1998). Several studies have highlighted the effectiveness of different bioremediation techniques in achieving the mineralisation of most petroleum hydrocarbons in contaminated soil (Brar et al. 2006; Mohan et al. 2006; Peng et al. 2008). During an oil spill, a large influx of petroleum hydrocarbons results in an environment where biodegradation of the carbon compounds is limited by nutrient availability. Consequently, biostimulation of indigenous microbes through the addition of nutrients, coupled with frequent tilling has gained wide acceptance in biological cleanup of contaminated land. A number of studies have documented positive effects of biostimulation in the attenuation of total petroleum hydrocarbons (Rosenberg et al. 1998; Rhykerd et al. 1999; Sarkar et al. 2005; Kogbara 2008). In some cases, hydrocarbon contamination might be very toxic leading to inhibition of the biodegradative capacity of indigenous microbes, hence for effective in-situ biodegradation, bioaugmentation might be necessary. Bioaugmentation involves the introduction of microorganisms that have been cultured to degrade various chains of hydrocarbons into a contaminated system. The cultures may be derived from the contaminated soil or they may be obtained from a stock of microbes that have been previously proven to degrade hydrocarbons (Sarkar et al. 2005).

It can be deduced from the foregoing that successful field deployment of bioremediation usually involves a combination of techniques to maximise the capabilities of soil microbes. Biostimulation is sometimes combined with phytoremediation – the use of plants and their associated microorganisms for the in situ treatment of contaminated soil and sediment (Alkorta and Garbisu 2001; Reichenauer and Germida 2008). Phytoremediation has the advantage of promoting ecological rehabilitation of contaminated land. Ecological rehabilitation mainly involves revegetation of derelict land, which has been rendered severely infertile by pollution from industrial activities with a view to control pollution, and enhance

long term stability of the soil surface. It is known that contaminated soils reclaimed through bioremediation do not necessarily require intervention for ecosystem restoration; left to natural processes the ecosystem will return to something close to their pre-contaminated condition if populations of the original species still exist nearby. How long this takes depends upon the type of ecosystem and the extent of contamination. Hence, it makes sense to rehabilitate the treated land with species that could tolerate residual contamination and contribute to phytoremediation, thus saving time and costs associated with continuous tillage – a major operation in bioremediation works. An advantage of this is that recalcitrant petroleum molecules that could not be treated with bioremediation may be amenable to phytoremediation leading to quicker ecosystem restoration. To our knowledge, very few studies have investigated supplementing biostimulation with phytoremediation in the cleanup of oil-polluted soils. Biostimulation has been combined with phytoremediation in enhancing oil degradation (Lin and Mendelssohn 1998; Ayotamuno et al. 2006a). However, these were largely cases of phytoremediation where fertilisers were applied to support plant growth, and both techniques were applied concurrently, not one after the other. Ecological rehabilitation with Vetiver grass (*Vetiveria zizanioides*) has been found to enhance the phytoremediation of an oil shale mined land contaminated with heavy metals (Xia 2004).

Compared to many other plants, grasses have characteristics of rapid growth, large biomass, strong resistance, and effective stabilisation to soils and, therefore, usually result in excellent restoration effects in degraded lands, particularly in the tropics and subtropics with high temperature and precipitation (Xia 2004). The potential of a common tropical grass, elephant grass (*Pennisetum purpureum*) to enhance the decontamination of a crude oil polluted soil has been reported (Ayotamuno et al. 2006a). The present study sought to evaluate the effect of utilising biostimulation alongside agro-technical processes like tilling and watering for

decontamination of a petroleum-polluted soil, and thereafter employ elephant grass for revegetation of the soil thus treated. This research is based on the hypothesis that greater reduction in hydrocarbon concentration will be achieved when biostimulation is supplemented with phytoremediation, as opposed to the use of only biostimilation or phytoremediation, or the concurrent use of both techniques. It is thought that the order of applying the techniques is likely to affect the biodegradation potential of soil microbes involved.

The objective of the study was to investigate the effect on contaminant attenuation produced by supplementing biostimulation with the phytoremediation potential of elephant grass used for ecological rehabilitation of a petroleum-contaminated site treated with bioremediation.

MATERIALS AND METHODS

Experimental design

The experimental cells were located in Port Harcourt, Nigeria. The ambient conditions include mean annual rainfall of 2,400 mm and monthly relative humidity of 85%; mean daily minimum and maximum temperature of 23^0C and 31.5^oC respectively.

Four experimental cells composed of mounds of earth, each having an area of 0.17 m^2 and a depth of 0.3 m, were employed. These were located in the open air but shielded from the rain. The cells served to provide controlled conditions for nutrient concentration, watering, tilling, and in particular to prevent excessive run-off of the contaminant. Each treatment option had three replicate cells.

Soil treatment

Each experimental cell was contaminated with 1000 cm^3 (1 litre) of Bonny light crude oil. The cells were left undisturbed for three days to allow for infiltration and percolation of the contaminant. All treatment applications commenced after the three-day period. Fertiliser application, tilling and watering as well as the time points chosen for the treatments were in line with the findings of an earlier study in the same area (Kogbara, 2008) which showed the effectiveness of the levels utilised. Detailed description of the method of treatment used for each cell during the nine-week study period is as follows.

O. Control

Cell O served as the control untreated soil. It was contaminated without any remedial treatment.

A. Biostimulation

The biostimulation option received 200g of 20-10-10 NPK fertiliser, 2 litres of water three times a week, and three times tillage per week. The fertilizer was applied three times during the study period, three days after contamination, and after three and six weeks of remediation.

B. Phytoremediation

200 g of 20-10-10 NPK fertilizer was worked into the soil three days after contamination to facilitate plant growth, thereafter 5 stands of elephant grass was planted on the soil. Watering and fertiliser application were the same as in cell A above (the biostimulation option) but there was no tilling.

C. Biostimulation supplemented with Phytoremediation

The same treatment described in the biostimulation option was applied for a period of six weeks while the remaining three weeks had the same treatment as the phytoremediation option.

Soil sampling

Soil samples were obtained at set periods for analysis. These were collected from different random spots using a hand-dug soil anger and bulked together to form composite samples. Samples for total hydrocarbon content (THC) measurements were placed in glass bottles and sealed with aluminium foil. The samples were immediately transferred to the laboratory for analysis.

Analytical methods

Soil physicochemical parameters such as particle size distribution were conducted before contamination while pH, electrical conductivity (EC), moisture content, total hydrocarbon content (THC), total nitrogen (Total N), organic carbon (Organic C), and bacterial counts were conducted before and after contamination using methods adapted from relevant literature (Page et al. 1982).

Particle size distribution was carried out using the hydrometer method, pH was determined using an EIL model 7020 pH meter by dipping the electrode into a 1:5 soil:water suspension that has been stirred and allowed to equilibrate for about 1 hour. EC was determined from the filtrate obtained from the suspension used for the pH; the oven drying method was used for moisture content determination. In the determination of total hydrocarbon content, toluene was used to extract the hydrocarbon content of the soil, the absorbance of the extract thus

obtained was then determined at 420nm in a Spectronic 20 spectrophotometer. The THC of the soils was then determined from standard curves of known concentrations of petroleum fractions. Organic carbon was determined by the Walkley-Black combustion method, while total nitrogen was determined by the Kjeldahl method. Bacterial counts were determined using plate count agar (oxoid).

RESULTS AND DISCUSSION

Background conditions of the soil before contamination are as shown in Table 1. The particles size distribution analysis showed that the soil texture is silty clay.

Table 1. Essential soil characteristics before crude oil contamination

Percentage (%) by mass				pH	EC	THC	Percentage		THB count
Sand	Silt	Clay	Moisture	1:5	$\mu s/cm$	mg/kg	Organic C	Total N	(x 10^6 cfu/ml)
11.5 ± 0.2	41.0 ± 0.7	47.5 ± 0.4	23 ± 1	5.26 ± 0.12	55.9 ± 15	14.0 ± 5	0.19 ± 0.05	0.11 ± 0.02	5.6 ± 0.01

Results represent mean ± standard deviation of three replicates

After petroleum contamination of the soils, the THC ranged between 30,000 mg/kg and 35,000 mg/kg in the various treatment cells. This relatively high level of contamination led to a decline in bacterial numbers from a background value of 5.6 x 10^6 cfu/ml to an average of about 2.6 x 10^6 cfu/ml across the various treatment cells (Table 2). As expected, there was an increase in soil organic carbon and a decrease in total nitrogen in the aftermath of petroleum contamination.

Table 2. Soil characteristics 3 days after contamination, before remediation

Cell	Moisture content (%)	P^H 1:5	EC $\mu s/cm$	THC mg/kg	Percentage		THB count (x 10^6 cfu/ml)
					Organic C	Total N	
O	25 ± 1	5.01 ± 0.30	55 ± 2	33,940 ± 200	1.89 ± 0.04	0.099 ± 0.01	2.3 ± 0.01
A	22 ± 1	5.21 ± 0.19	94 ± 4	30,560 ± 120	1.10 ± 0.05	0.053 ± 0.03	2.6 ± 0.02
B	24 ± 1	5.13 ± 0.20	99 ± 8	34,960 ± 250	1.90 ± 0.03	0.078 ± 0.01	2.6 ± 0.05
C	20 ± 2	5.10 ± 0.30	95 ± 6	32,100 ± 150	1.85 ± 0.04	0.080 ± 0.01	2.8 ± 0.03

Results represent mean ± standard deviation of three replicates

After four weeks of applying the different treatments there was a marked reduction in THC in all treatment options while the control had very little THC reduction. This was corroborated by a decrease in organic carbon and an increase in total heterotrophic bacterial counts; mineralisation of the hydrocarbon contaminant leads to a decline in organic carbon content while increase in bacterial numbers points to microbial degradation. All treatment options recorded the same trend of denitrification as there was enormous reduction in total nitrogen in the soils (Table 3). A similar observation has been reported in previous related studies (Ayotamuno et al. 2006a; Ayotamuno et al. 2006b; Kogbara 2008). It is likely that loss of nitrogen could have resulted from conversion of nitrate ions to gaseous forms of nitrogen by a series of widely occurring biochemical reduction reactions brought about by denitrifying

bacteria involved in the biodegradation process (Brady and Weil 1999). The other parameters, moisture content, pH and electrical conductivity were fairly stable across the options as there was little response of these parameters to differences in treatment applications; however the EC of the treated soils was higher than that of the control. This is expected as EC is traditionally used as a measure of soil salinity and since the applied fertiliser is a salt the treated options are bound to have higher levels of EC.

Table 3. Soil characteristics 4 weeks after remediation

Cell	Moisture content (%)	P^H 1:5	EC $\mu s/cm$	THC mg/kg	Percentage		THB count (x 10^6 cfu/ml)
					Organic C	Total N	
O	23 ± 1	5.03 ± 0.20	60 ± 3	31,500 ± 200	1.90 ± 0.03	0.098 ± 0.01	2.6 ± 0.02
A	23 ± 1	5.03 ± 0.30	102 ± 6	2200 ± 100	0.31 ± 0.03	0.0065 ± 0.03	3.2 ± 0.01
B	25 ± 1	5.14 ± 0.10	100 ± 9	2480 ± 110	0.11 ± 0.02	0.002 ± 0.02	3.1 ± 0.03
C	23 ± 2	5.22 ± 0.20	90 ± 3	2660 ± 100	0.31 ± 0.03	0.006 ± 0.03	3.3 ± 0.06

Results represent mean ± standard deviation of three replicates

Results obtained after a period of nine weeks by which time the elephant grass has been grown on cell C (biostimulation supplemented with phytoremediation) indicated a similar reduction in hydrocarbon content between cell A (biostimulation only) and cell C. Biostimulation caused a reduction in THC from 30,560 mg/kg to 776 mg/kg, while supplementing biostimulation with phytoremediation brought about an attenuation in THC

from 32,100 mg/kg to 511 mg/kg (see Tables 2 and 4). This implies a 97.5% reduction for option A and 98.4% reduction for option C (Table 5) at the end of nine weeks. There was an anomaly in the THC result recorded in the phytoremediation option (cell B) at the ninth week as it appeared that there was an increase in hydrocarbon concentration (by 5110 mg/kg) in the soil between the fourth and ninth week. Such anomalous behaviour has previously been observed with different explanations provided (Vance 1991; Ayotamuno et al. 2006b; Kogbara 2008). Though the exact mechanism responsible for such behaviour is not clear, it is thought to be linked with anoxic conditions in the soil especially as most cases where it occurred were sites with insufficient oxygen supply. In this case, it was peculiar to the phytoremediation option where there was no tillage to facilitate soil aeration. The accumulation of anaerobic metabolites produced and excreted by microorganisms during degradation of the substrate is likely to be responsible for the increase in THC. Furthermore, the THC results of cell B corroborates the findings of Ayotamuno et al. (2006b) and Kogbara (2008) where such anomalous increase in THC was associated with the use of phytoremediation alone but biostimulation with frequent tilling for soil aeration was found to compass continuous contaminant attenuation. This study has shown that a way to overcome such anomaly with phytoremediation is to use biostimulation with tilling for some time and then replace with phytoremediation at a later stage.

Comparing THC reduction across the treatments, it can be seen from Table 5 that after four weeks of remediation the percentage THC loss was similar across the various treated cells with the cells A and B having about 1% hydrocarbon loss ahead of cell C. At this stage, cells A and C were receiving the same kind of treatment so the difference between both cells is not due to treatment application. However, at the ninth week though the percentage THC reduction was quite similar, cell C produced approximately 1% greater loss of the

contaminant than the biostimulation option. By this time, the grasses have been grown on the soil for a period of three weeks. As opposed to what was observed in the phytoremediation option, there was reduced sign of leaf burn in cell C, which indicates that the contaminant level was more tolerable to the plants at this stage hence they could be used for rehabilitation of the degraded land while at the same time contributing to mineralisation of hydrocarbons left after bioremediation treatment.

Throughout the experiment, increase in microbial numbers did not strictly correspond to percentage hydrocarbon loss in the various treatments, but generally, there was a good correlation between hydrocarbon degradation and bacterial counts. It is clear that due to tilling and watering of the soils certain amounts of contaminant loss are due to abiotic processes such as sorption and volatilisation; however, this study sought to compare the result of reducing the effort on bioremediation by introducing phytoremediation at an advanced stage of bioremediation treatment hence did not focus on isolating abiotic losses. Moreover, at the ninth week, reduction in THC obtained in cells where bioremediation has been supplemented with phytoremediation at the sixth week (hence tilling was discontinued) was slightly higher than those on which tilling was carried out throughout the study period (Table 5). If much of the losses had been abiotic, greater contaminant loss would have occurred at this stage with tilling than without since volatile organic compounds are more likely to be lost with tilling than with phytovolatilisation – the use of plants to transfer volatile petroleum compounds to the atmosphere. Furthermore, plant root zone has certain associated microbes, which enriches soil microbial flora, thus greater biodegradation is more likely to result when plants capable of phytoremediation are introduced at an advanced stage of bioremediation since conditions then would be more tolerant for their survival.

In the light of the above discussion, we do not feign ignorance of the argument that the reduction in THC seen in cell C (bioremediation supplemented with phytoremediation) might have occurred without elephant grass planting especially as there was no treatment having biostimulation for six weeks with tillage followed by three weeks without tillage. This study sought to compare the effect of continuous tillage (frequent tilling) which is a major characteristic of contaminated soils undergoing bioremediation (with biostimulation) with replacing tillage at a later stage with phytoremediation hence the afore-mentioned treatment was not considered in the experimental design. Moreover, experience has shown that decontamination of contaminated soils is more likely with tilling than without, hence tilling was continued throughout the experiment in cell A (biostimulation with tilling throughout the experiment).

Furthermore, although the THC of the cells was not analysed at week 6 when the grasses were planted, and there is the likelihood for the contaminant attenuation seen between weeks 4 and 9 to have occurred by week 6 through the effect of biostimulation with tilling alone, a little consideration would show that this was not the case. It is thought that if the contaminant attenuation seen between weeks 4 and 9 had occurred by the time the grasses were introduced at week 6, continuous tilling of cell A (biostimulation option) for a further three weeks would have led to cell A having greater percentage THC reduction than cell C (on which the grasses were grown). As mentioned earlier, evidence from previous studies have shown that decontamination is more likely with tilling than without, hence cell A would naturally have performed better than cell C at week 9 if the decontamination seen had already occurred at week 6. However, the results of the study demonstrate the utility of introducing phytoremediation at a later stage of bioremediation with biostimulation especially as cell C performed slightly better than cell A.

Table 4. Soil characteristics 9 weeks after remediation

Cell	Moisture content (%)	P^H 1:5	EC $\mu s/cm$	THC mg/kg	Percentage		THB count (x 10^6 cfu/ml)
					Organic C	Total N	
O	21 ± 1	5.01 ± 0.20	70 ± 3	30,600 ± 150	1.94 ± 0.04	0.099 ± 0.01	2.8 ± 0.03
A	23 ± 1	5.05 ± 0.30	110 ± 5	776 ± 60	0.34 ± 0.06	0.0065 ± 0.03	3.5 ± 0.02
B	26 ± 1	5.20 ± 0.10	101 ± 10	7590 ± 100	0.14 ± 0.04	0.002 ± 0.02	3.6 ± 0.07
C	25 ± 2	5.10 ± 0.20	98 ± 8	511 ± 40	0.34 ± 0.06	0.006 ± 0.03	3.8 ± 0.01

Results represent mean ± standard deviation of three replicates

Table 5. Percentage hydrocarbon loss with time

Cell	Description of treatment application	Percentage THC reduction	
		4 weeks	9 weeks
O	Control	7.2	9.8
A	Biostimulation	92.8	97.5
B	Phytoremediation	92.9	78.3
C	Biostimulation supplemented with Phytoremediation	91.7	98.4

CONCLUSION

The findings of this study have shown that introducing phytoremediation at an advanced stage of bioremediation treatment shows great potential as it compares favourably with continuous treatment using biostimulation coupled with frequent tilling. This is likely to save cost and energy associated with tilling operations and also confers the advantage of compassing a quicker ecosystem restoration. This research also highlights the importance of treatability studies prior to the use of phytoremediation in order to forestall the kind of anomalous increase in contamination at a later stage of the treatment as was experienced in this study. To fully exploit the advantage of supplementing biostimulation with phytoremediation, it is recommended that further studies continue along the lines of investigating the potential of using other plants, which might be more suited to a particular environment, or a combination of plants; as well as the appropriate stage of bioremediation treatment at which tillage should exchange for phytoremediation with plants.

NOMENCLATURE: THC-total hydrocarbon content (mg/kg), EC-electrical conductivity (μs/cm), Organic C-organic carbon, Total N-total nitrogen, cfu/ml-colony forming unit per millilitre, THB-total heterotrophic bacteria.

The authors declare that they have no conflict of interest.

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