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RESEARCH ARTICLE

EX-SITU BIOREMEDIATION OF CRUDE OIL POLLUTED SOIL USING ALGAL SPECIES, (*Aphanocapsa elachista*)

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ABSTRACT

An investigation into the *ex-situ* bioremediation of crude oil polluted soil using algal species was carried out. The bioremediation was initiated by spilling crude oil on both sterilized soil (SS) and unsterilized soil (UNS) and inoculating with crude oil degrading micro-algal species of *Aphanocapsa elachista*. The total heterotrophic count was carried out in both the SS and UNS by viable count. Aliphatic hydrocarbon in the remediated soil was determined by gas chromatography Hewlett-Packard HP 6890 model equipped with FID at initial temperature of 65^0C and final temperature of 300^0C. The heterotrophic count in the SS was initially 1.90 x 10^4 cfu/ml and biweekly measurement for six weeks gave 3.51 x 10^4, 4.26 x 10^4 and 3.01 x 10^4 cfu/ml respectively. The UNS sample gave initial count of 1.92 x 10^4 cfu/ml and the biweekly measurement for 6 weeks gave 2.69 x 10^4, 2.83 x 10^4 and 2.24 x 10^4 cfu/ml respectively. The gas chromatographic analysis showed a decrease in total hydrocarbon from 863.68402 mg/ml on zero day to 596.76653 mg/ml in the week six sample. Analysis of the sterilized soil showed a decrease of physicochemical parameters after bioremediation.The research concludes that *Aphanocapsa elachista* algal species had the potentials for ex-situ bioremediation of crude oil polluted soils.

Key words: algae, physical parameters, bioremediation, , *Aphanocapsa elachista*, heterotrophic count, chromatographic analysis, soil.

INTRODUCTION

Crude oil pollution of soil and ground water are amongst the most evident negative impact of the petroleum industry to the environment. Pollution of soil is the condition arising from man's activities that render the soil less suitable for use. The cause of crude oil pollution can be from oil well drilling, transportation, storage, refining, and marketing (Mentzer and Ebere, 1996). The need to cost effectively remediate contaminated soil has led to the development of a wide range of physical, chemical and biological cleanup techniques. Bioremediation is a good option for the remediation of polluted environment because of its versatility, eco-friendliness, public acceptance, etc. (Grant and Long, 1981: Coombs, 1986; Mentzer and Ebere, 1996). Soil is a dynamic medium, which is polluted by a multitude of living organisms most of which has the ability to degrade hydrocarbons. Crude oil polluted soil can be naturally remediated and the process is known as natural attenuation. Algae are a diverse group of eukaryotic organisms that contain chlorophyll and carry out photosynthesis. They are mainly aquatic organisms, although some are found in soil or vegetation where there is enough moisture (David *et al*., 2003). Some species of micro-algae have the ability to degrade hydrocarbons, example is, *Onchromonas danica* that can degrade polycyclic aromatic hydrocarbons. Crude oil is a dark viscous liquid and a mixture of organic compounds. It varies in composition and color, which depends on the area from which it is obtained (Bajah and Godman, 1975). Since hydrocarbons are natural products, it should be no surprise to find hydrocarbon degrading microorganisms in many environments irrespective of whether they have been contaminated or not (Sayles,1993). This research aimed at determining the *ex-situ* bioremediation of polluted soil using *Aphanocapsa elachista* algal species.

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MATERIALS AND METHODS

Collection of sample: The micro-algal water sample was collected from a river at the Shell Petroleum Development Company (SPDC) drilling site at Mahu, Ohaji Egbema, Imo state. Serial dilutions of the sample was made up to 10^{-9}. The method of Ramond *et al*. (1976) was employed in the soil sample collection from a depth of 30cm in a site measuring 2m x 1. 5m.

Sterilization of Materials and Medium

Soil extract medium (with the following composition: water 900ml, soil extract solution 100ml; KN03; 200mg, MgS04 7H20; 20mg, Agar (optional); 10g, Vitamin B12 (Cyanocobalamin); 2ml, streptomycin 0.1g and nystatin;

0.1g) was appropriately prepared and aseptically dispensed into pre-sterilized Petri-dishes. Glasswares and other heat stable materials were sterilized as described by Cheesbrough, (2005) and Cruickshank *et al*. (1980). The enumeration of the alga was done by inoculating in duplicates the soil extract medium in Petri dishes using 0.1ml of a 10^{-3} and 10^{-4} dilutions of the water sample, then incubated at 27^0c for seven days on a regularly surface sterilized table facing the light source for illumination. Several plating and streaking were carried out before a pure culture was obtained. Identification of the algal species was according to John *et al*. (2002) guide. The vapour phase transfer technique (Amanchukwu and Okpokwasili, 1989) was employed and then incubated at 27^0c for seven days in the dark.

Physicochemical parameters

Physicochemical parameters of both sterile and unsterile loamy soil samples (100g each) were analysed before and after the bioremediation. Method of analysis employed APHA AWWA and APCF (1985) and Nwachukwu and Ugoji (1995). Soil sterilization was done in hot air oven at a temperature of 150^0C for 1hour

Experimental Design and Bioremediation

Both the SS and UNS samples were simulated with crude oil to effect pollution by adding 10ml of crude oil to 50g fractions of soil in a biodegradation bottle. The samples were thoroughly mixed. The soil-crude oil mixtures were then mixed with 100ml of soil extract broth making the soil into a paste. The controls (SS and UNS) had only crude oil but no algal species. The Bioremediation was applied by pippetting 25ml of the algal species into the soil- crude oil mixture. These samples were inoculated at room temperature and incubated in the dark for six weeks. The total heterotrophic algal count was carried out at day zero after serial dilutions using 1ml of sample taken from each of the experimental setup and plating from 10-3 and 10-4 on soil extract medium in duplicates, and then incubated at room temperature on a surface sterilized table facing a light source for seven days. The colonies were counted and this procedure was repeated at biweekly interval for six weeks. Gas chromatographic analysis of the total petroleum hydrocarbon (TPH) in the SS sample was determined as described by Chikere and Okpokwasili (2003) at day zero and after six weeks using a GC (Hewlett-Packard HP 6890 model equipped with FID at initial temperature of 65^0C and final temperature of 300^0C).

RESULTS

Figure 1 is the total algal heterotrophic count in the UNS and SS soil samples polluted with crude oil. The initial algal population was 1.92 x 10^4 cfu/ml in both soil samples. At week 4, algal population in the UNS polluted soil increased to 2. 83 x 10^4 cfu/ml and 4.26 x 10^4 cfu/ml in the SS. At week 6, there was a decrease of algal population in both soil samples. The decrease in the number of algal colonies after week four in both the UNS and SS show that the nutrient content of the sample had decreased. Table 1 shows a decrease in the nutrient content of the soil after the period of treatment and this suggests that the alga made use of available nutrients in the soil during the period of bioremediation. Tables 2 and 3 are the day zero and week six report of the gas chromatograms (GC) of the TPH in the SS samples. The aliphatic hydrocarbon range of nC8-nC36 characteristic of Nigerian crude oil was observed. The results showed a decrease of TPH after six weeks of bioremediation. At week 6, the total aliphatic hydrocarbon dropped from 863.68mg/L to 596.77mg/L. There is a correlation between Figure 1 and Table 3 suggesting that the increase in algal population was as a result of the algal utilization of the crude oil.

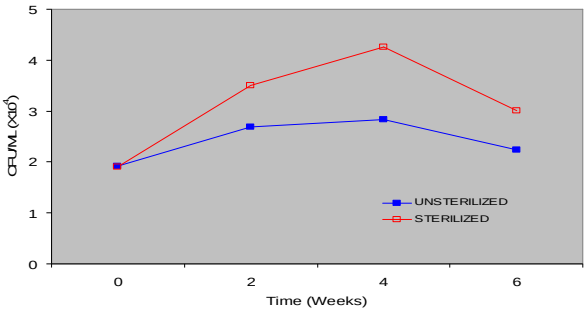

Figure 1. Total algal heterotrophic count in the unsterilized and sterilized soil samples polluted with crude oil

Table 1 The result of soil analysis before and after simulated pollution

Parameters	Pre-pollution	Post-pollution
pH(H20)	6.84	6.94
pH(KCI)	5.65	5.70
% Organic matter	2.03	0.92
% Organic Carbon	1.17	0.50
Phosphorus (mg/kg)	26.40	19.60
%Nitrogen	0.112	0.013
Exchange acidity (mol/kg)	0.40	0.96
Calcium (mol/kg)	3.20	1.60
Magnesium (mol/kg)	1.60	1.80
Potassium	0.225	0.09
Sodium (mol/kg)	0-061	0.052
%Base saturation	92.71	7.259
NO3 Nitrogen	0.028	0.09
%NO3 Nitrogen	0.014	Trace
% Sand	82.40	82.40
% Silt	6.80	5.80
% Clay	10.80	7.80
Texture	Loamy sand	Loamy sand

Table 2. Determination of Total Petroleum Hydrocarbon at Day Zero

External Standard Report

Sorted By : Signal
Calib. Data Modified : Tuesday, December 06, 2005 11:37:39 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FIDI A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/l]	Grp	Name
4.010		-	-	-		C6
4.765		-	-	-		C7
8.675	VV T	28.97681	5.34567	110.24136		C8
9.895		-	-	-		C9
11.957		-	-	-		C10
12.734	BV	11.73456	13.25347	129.34250		C11
14.645	VV	9.975641	12.24153	143.87321		C12
15.743	VV	27.14257	6.15249	126.23837		C14
16.634	VV	4.65741	21.26921	101.55112		C16
18.142	VV	5.26457	11.29465	49.67561		C18
19.284	VV	7.25349	2.84657	5.03576		C20
22.342	VV	48.37586	2.99253	7.12478		C24
24.142	VV	50.26475	2.14253	21.35433		C28
25.263	VV	18.26354	4.29578	79.12485		C32
30.274	PB	6.85746	17.45765	90.12213		C36
39.290		-	-	-		C40
Totals :				863.68402		

Table 3. Determination of Total Petroleum Hydrocarbon after Six Weeks

Ret time [min]	Type	Area [pAs]	Amt/Area	Amount	Grp	Name [mg/l]
4.010		-	-	-		C6
4.765		-	-	-		C7
5.650	VV T	25.63564	3.32285	85.18341		C8
6.720		-	-	-		C9
7.685		-	-	-		C10
8.781	BV	9.75873	10.16853	99.23190		C11
9.591	VV	8.83972	12.65475	111.86441		C12
11.355	VV	24.16555	4.06485	98.22944		C14
13.621	VV	2.90157	19.91028	57.77113		C16
15.185	VV	3.13373	8..22870	25.78657		C18
16.529	VV	6..90039	0.00000	0.00000		C20
19.037	VV	44.29306	0.00000	0.00000		C24
20.876	VV	45.50386	1.37559	6.25944		C28
23.350	VV	15.49377	3.22965	50.03945		C32
27.632	PB.	4.789377	13.02788	62.40079		C36
34.453		4.78979	-	-		C40
Totals				596.76653		

DISCUSSION

The increase in the number of algal colonies suggests successful adaptation and the utilisation of the crude oil by *Aphanocapsa elachista* for carbon and energy sources. This agrees with Ogbulie and Ifeanyi (2006) who established that *Microcystis flo-aquae* and *Chaetophora tuberculosa* micro-algae grew on mineral salt medium using crude oil as the only source of carbon and energy. This agrees with Al-Hassan *et al*. (1992) who observed that extensive mats of blue green algae appeared on the soil layers when crude oil polluted environments were left alone. The subsequent decrease as a result of reduction in soil nutrients was in line with Pfaender and Bartholomeur (1982) who reported that a reduction in substrate in environment leads to slower growth. The decrease is also supported by the report of Sayles *et al*. (1993) who reported about reduction of microorganisms in an environment as a result of competition. The reduction of TPH corroborates with the report of Walker *et al*. (1975) who made the first report of the degradation of oil by an algal species Prototheca zopfii. The reduction in the hydrocarbon also agrees with Semple and Cain (1996). Similarly, Ifeanyi and Ogbulie (2008) reported a reduction of TPH in a hydrocarbon challenge test carried out using *Microcystis flo-aquae*. Algae were previously not considered capable of degrading petroleum hydrocarbon. This research in corroboration with earlier researches concludes that Aphanocapsa elachista algal species had the potentials for ex-situ bioremediation of crude oil polluted soils.

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