Chemosphere 162 (2016) 148–156

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Studies on organic and in-organic biostimulants in bioremediation of diesel-contaminated arable soil

Amechi S. Nwankwegu [a,*], Michael U. Orji [a], Chukwudi O. Onwosi [b]

[a] Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria
[b] Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

HIGHLIGHTS

- Compost and N-P-K were compared with natural attenuation in the bioremediation of diesel-polluted soil.
- Use of compost resulted in >90% removal of total petroleum hydrocarbon within two months.
- Experimental data fitted second order adequately.
- The phytotoxicity test indicated that compost was most effective in the reclamation of the polluted soil.

ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form
15 July 2016
Accepted 23 July 2016

Handling Editor: Caroline Gaus

Keywords:
Total petroleum hydrocarbon (TPH)
Natural attenuation
Ecotoxicity
Compost
Fertilizer

ABSTRACT

In this study, use of inorganic fertilizer (N.P.K) was compared with organic manure (compost) in the bioremediation of diesel-polluted agricultural soil over a two-month period. Renewal by enhanced natural attenuation was used as control. The results revealed that total petroleum hydrocarbon removal from polluted soil was 71.40 ± 5.60% and 93.31 ± 3.60% for N.P.K and compost amended options, respectively. The control (natural attenuation) had 57.90 ± 3.98% of total petroleum hydrocarbon removed. Experimental data fitted second order kinetic model adequately for compost amended option. The fertilizer amended option was found to be 1.04 times slower ($k_2 = 4.00 \pm 1.40 \times 10^{-7} gmg^{-1}d^{-1}$, half-life = 28.15 d) than compost amended option ($k_2 = 1.39 \pm 0.54 \times 10^{-5}$ $gmg^{-1}d^{-1}$, half-life = 8.10 d) but 1.21 times (20.6%) faster than the control ($k_2 = 2.57 \pm 0.16 \times 10^{-7}$ $gmg^{-1}d^{-1}$, half-life = 43.81 d). The hydrocarbon utilizers isolated from the diesel contaminated soil were: *Bacillus nealsoni*, *Micrococcus luteus*, *Aspergillus awamori*, and *Fusarium proliferatum*. The phytotoxicity test showed that germination indices for natural attenuation (control), fertilizer (NPK) and compost amended options were 34%, 56%, and 89%, respectively.

1. Introduction

The continuous drive to increase the production, distribution and utilization of petroleum and gas products to meet the energy needs of the population is usually associated with some environmental pollution problems especially on the soil. In Nigeria, over 20 percent of crude oil spills result from vandalism of pipelines while transportation accounts for over 10 percent pollution in both marine and soil ecosystems (Orji et al., 2012). Automobile spent engine oil and exhaust release of gases such as carbon (IV) oxide and carbon (II) oxide are other ways of environmental pollution. Pollution of the soil by hydrocarbon components such as diesel, kerosene, asphaltenes and the recalcitrant polynuclear aromatic hydrocarbon (PAH) has resulted in reduced agricultural productivity through loss of soil quality, leaching and erosion (Anozie and Onwurah, 2001). While, most physicochemical remediation approaches (evaporation, dissolution, use of chemical dispersants/emulsifiers and photooxidation) for these pollutants have been found to be eco-harzardous (Admons et al., 2001), bioremediation has been more promising. Bioremediation relies mainly on the harnessing of metabolic potentials of indigenous microorganisms to degrade contaminants (especially hydrocarbon) into less toxic compounds (Atlas and Bartha, 2001; Frankenberger, 1992). This is possible since these microorganisms are capable of secreting different catabolic enzymes needed for the conversion of hazardous organic contaminants or transforming them to environmentally safe level.

* Corresponding author.
E-mail address: smetchie@yahoo.com (A.S. Nwankwegu).

http://dx.doi.org/10.1016/j.chemosphere.2016.07.074
0045-6535/

However, microorganisms cannot effectively utilize hydrocarbons unless limiting nutrients such as nitrate, phosphate as well as microelements are incorporated into the polluted environment (Seklemova et al., 2001). Different methods have been employed in delivering these limiting nutrients to the polluted environment. These include: inorganic fertilizers, chicken droppings, periwinkle shell, compost materials, liming and land farming (Orji et al., 2012; Ijah and Antai, 2003). Whereas the use of inorganic fertilizer as source of nutrient has been extensively carried out, it is still challenged by the huge cost during remediation, soil and plant toxicities, as well as and the likely chance of eutrophication – especially in aquatic environments.

Meanwhile, it is worthy to note that an effective remediation approach should be environment friendly and affordable. Therefore, the aims of this study were to evaluate the effectiveness of compost, and fertilizer as sources of additional nutrients in the bioremediation of diesel polluted soil. We also compared the removal efficiency of the diesel contaminant among the different treatment options and modeled the degradation rate. The extent of the clean-up was assessed using seed germination indices as monitoring tool.

2. Materials and methods

2.1. Sample collection

Soil sample at the depth of 10 cm (Horizon A) was collected from an arable land of Community Secondary School, Okpuno, Awka South Local Government Area of Anambra State, Nigeria. This is located at latitude 6° 12′25″ N and longitude 70° 4′04″ E. The land had no recorded history of oil/hydrocarbon contamination as maize plants there in were in good condition. The sample was collected in circular plastic can with depth 6.30 cm and uniform diameter of 45 cm and an area of 1590 cm^2 which was sterilized with cotton wool soaked in 70% alcohol (Eziuzor and Okpokwasili, 2009). The collected soil was thoroughly mixed to ensure homogeneity. The soil sample was then transported to the laboratory for bioremediation study.

2.2. Pristine soil contamination

The pristine soil was contaminated with the diesel (grade 2 – D-centane number rating). The experiment was divided into three treatment options as described in Table 1. The diesel oil used in this study was purchased from a local petrol station. The polluted soil at this point was sampled for base lines studies.

2.3. Preparation of amendments

About 2 kg of compost (chiefly made of grass straw biomass) was obtained, prepared; sun dried for 5 d until moisture was completely driven off and then stored until used. Also, Fifty gram of fertilizer (NPK) was purchased from a local market and stored under dried condition until used.

2.4. Bioremediation study

We carried out three treatments options to evaluate the efficiency of diesel oil degradation in the contaminated soils. These include: (a) remediation by enhanced natural attenuation (This option which does not have any nutrient amendment served as the control); (b) biostimulation (adding organic and inorganic nutrients to improve the natural biodegradation rate). In Option one (CO), the biostimulation was achieved with organic nutrient. Herein, the polluted soils in the micrococosm were amended with 80 g of compost manure. In the second biostimulation option (FO), 80 g of inorganic nutrient source (N.P.K fertilizer, 15:15:15) was used for the amendment. Each of the three treatment options was turned weekly to enhance mixing and aeration. The experimental sets were run for period of two months. Soil samples (10 g) were collected at intervals as defined by the experiment for laboratory analysis. The one sampled immediately after various amendments were designated as the zero week. Thereafter, sampling was done on weekly basis for the first month but in the second month at interval of two weeks. The bioremediation of petroleum hydrocarbon under different designs was monitored using parameters as: total culturable heterotrophic bacteria and fungi counts (TCHB and TCHF), total culturable hydrocarbon utilizing bacteria and fungi counts (TCHUB and TCHUF), pH, weekly rate of degradation of the total petroleum hydrocarbon (TPH) and ecotoxicity test.

2.5. Soil analysis

The physicochemical characteristics of the soil such as cation exchange capacity (CEC) (mEq/100 g), total organic carbon (TOC) (%), particle sizes (%) (sand, clay and silt) and pH prior to contamination with the diesel were determined (Table 2). Briefly, the ethylene glycol and pure ethanol method was used in cation exchange capacity determination.

For the detection of soil total organic carbon, standard methods of APHA5310 (2005) was used. Briefly, 5.0 g weight of soil was heated with an excess volume of standard $K_2Cr_2O_7$ in the presence of conc. H_2SO_4. The soil was slowly digested at the low temperature by the heat of dilution of H_2SO_4 and the organic carbon in the soil was thus oxidized to CO_2. The highest temperature attained by the heat of dilution reaction produced with the addition of concentrated H_2SO_4 was approximately 12 °C which is sufficient to oxidize the active forms of the soil organic carbon but not the more inert forms of carbon that may be present. The excess of $K_2Cr_2O_7$ not reduced by organic matter was titrated against a standard solution

Table 2
Baseline physicochemical and microbiological properties of the pristine soil.

Parameters	Value
pH	
Unpolluted soil	7.91 ± 0.30
Fertilizer (NPK)	5.7 ± 0.02
Compost	8.4 ± 0.13
Diesel oil	5.4 ± 0.41
Cation exchange capacity CEC (meq/100 g)	18.10
Particle size, clay and sand (%)	98.20 and 1.80
Total organic carbon TOC (%)	0.83
Total cultural heterotrophic bacteria (TCHB) (log cfu g^{-1})	6.30 ± 0.21
Total culturable heterotrophic fungi (TCHF) (log cfu g^{-1})	5.99 ± 0.12

Table 1
Design of the bioremediation experiment.[a]

Experimental group	Description
CO	800 g of pristine soil + 200 ml of diesel + 80 g of sterile compost manure
FO	800 g of pristine soil + 200 ml of diesel + 80 g of N.P.K fertilizer (15:1515)
NA	800 g of pristine soil + 200 ml of diesel + no compost or fertilizer (control)

[a] Each experimental set was run in three replicates.