Bioreactor-Based Bioremediation of Degreaser-Contaminated Soil: A Study on Microbial Synergy and Nutrient Amendments

Abstract

Aim: The aim of this study is to enhance the bioremediation of degreasers used in upstream sectors of Nigeria Petroleum Industry using Bacteria (*Pseudomonas* and *Bacillus* species) grown in Bioreactor.

Study Design: This study employs experimental designs, Randomized Block Design Treatment set up, Statistical analysis of data and Interpretation.

Place and Duration of Study: The area used for this study is within the Rivers State University Demonstration farmland in Nkpolu-Oroworukwo, Mile 3 Diobu, Port Harcourt, Rivers State. The piece of land is situated at Longitude $4^0$48'18.50''N and Latitude $6^0$58'39.12''E measuring 5.486m x 5.1816m with a total area of 28.4283m^2. The study was for a period of six months.

Methodology: The soil was treated for bioremediation as described by Williams & Inweregbu (2019). In this method, nine (9) setups in triplicate each were made. This bioremediation set up was monitored using microbiological (Total Heterotrophic Bacteria, Fungi, Degreaser Utilizing Bacteria (DUB) and Degreaser Utilizing Fungi (DUF) and physicochemical (total hydrocarbon content (THC), nitrogen, potassium, phosphorus, temperature and pH) parameters from day 1 to 56. This was done at 14 days' interval. Eighty milliliters (80ml) of sterilized water was added to the set up three times weekly and agitated for proper aeration and adequate distribution of microorganisms.

Results: The baseline microbiological analyses revealed that Total Heterotrophic Bacteria count (THBC) ranged from 5.4±0.57 x 10^6 to 6.3±1.06 x 10^6 CFU/g, Degreaser Utilizing Bacteria (DUB) 2.1±0.07 x 10^3 to 4.1±0.64 x 10^3 CFU/g, Fungal count (FC) 1.6±0.85 x 10^3 to 3.33±0.64 x 10^3 CFU/g, and Degreaser Utilizing Fungal count (DUF) 1.6±0.21 x 10^3 to 2.5±0.35 x 10^3 SFU/g. The bacterial isolates identified were *Bacillus, Pseudomonas, Aeromonas, Escherichia* and *Micrococcus* species while fungal isolates identified were *Aspergillus flavus, Aspergillus niger, Penicillium* sp and *Alternaria* sp, *Candida* sp and *Fusarium* sp. The results of the physicochemical parameters monitored showed that mean pH value ranged between 5.0 to 7.38, Temperature was between 25 and 30^{o}C, Nitrogen concentration ranged from 3.0 to 9.9 mg/kg, Phosphorus (0.05 to 0.58 mg/kg), Potassium ranged from 0.06 to 3.4 mg/kg and the Total Hydrocarbon Content was between 53.1 and 3983mg/kg. Bioremediation efficiency was estimated from percentage (%) reduction of Total Hydrocarbon Content (THC) from day 1 to the residual hydrocarbon on day 56 of bioaugmented/ bio stimulated plots with the control. Results revealed amount of remediated hydrocarbon and % Bioremediation efficiency after 56 days in the different treatment plots (initial THC value of 3984 mg/kg) in a decreasing order as follows: CS+FPE (76.6%) > CS+Pse+Bac+FPE (74.6%) > CS+Pse (74.4%) > CS+Bac+FPE (73.8%) > CS+Pse+Bac (73.1%) > CS (71.5%)> CS+Pse+FPE (70.6%) > CS+Bac (68.8%) > CTRL 1 –US (66.4%). where bio stimulating agent, FPE (fish pond effluent), CS (Contaminated soil) and bio-augmenting microorganisms, Pse and Bac (*Pseudomonas* and *Bacillus* spp.)

Conclusion: The study showed that bioremediation of degreaser contaminated soils with combination of organic nutrients is a better degradative option. Therefore, amendment with organic nutrients like fish pond effluent is recommended for degreaser contaminated soils due to their high nutrient content as substrates for biostimulation of indigenous and augmenting

biodegrading microbes. Natural bioremediation of degreaser pollutants by activation of naturally occurring microorganisms will be cost effective in cleaning up and protecting the environment from the hazardous effects of hydrocarbons on our agricultural ecosystem.

Keywords: Bioremediation, Degreaser, Bio augmenting, Bio stimulating Agents, Bioreactor.

1. INTRODUCTION

The environment is in danger of ecological damage due to the current rapid population growth, urbanization, and industrialization (Cherniwchan, 2012). Stated differently, the environment is under threat because of the ongoing damage caused by human activities (Goudie, 2013). There is a significant concern about the widespread pollution and contamination of natural elements, including aquatic and terrestrial ecosystems, caused by industrial production, excessive petroleum and its derivative use, polyethene, pesticides, and organic herbicides, which are primarily used to prevent fungal, insect, and weed attacks (Mrozik *et al.*, 2010; Tyagi *et al.*, 2010; Federici *et al.*, 2012; Schultz-Jansen *et al.*, 2016). Gas stations, oil refineries, petrochemical and pharmaceutical companies, seeping water from agricultural lands treated with pesticides and herbicides are the main sources of these pollutants in the soil and water resources. Long-term persistence in the environment is a common characteristic of most of these pollutants (Srirangan *et al.*, 2012).

The most dangerous category of toxic pollutants are the toxic and hazardous chemicals that are released yearly by industries. Industrial facilities all over the world release 310 kg of toxic chemicals into the air, land, and water every second. This is equivalent to about 10 million tonnes annually (Tiefenbacher & Hagelman, 2013). Lubricant oil, which is often used to run various engines and machinery is the next most dangerous material. These oils are hazardous, a build-up from the auto-mechanic workshops could cause major environmental issues in the future (Abdulsalam & Omale, 2009). Nrior and Odokuma (2015) stated that degreasers are chemical compounds that are used to remove water-insoluble materials from vehicle engine parts, including grease, oil, and paint.

According to Kumar *et al.*, (2011) and Sharma (2012), bioremediation is a naturally occurring process that uses microorganisms, plants, and/or their derivatives (enzymes or spent biomass) to break down or modify environmental contaminants while these organisms go about their daily lives. Compared to physicochemical methods, bioremediation is thought to be a more affordable, adaptable, effective, and environmentally friendly method of handling environmental pollutants (Kumar *et al.*, 2011; Sharma 2012; Jeon & Madsen, 2013). It is recognized that using carefully thought-out microbial bioreactors is an effective approach to guarantee that microbial processes and growth take place in a regulated setting that offers the required ideal conditions (Jeon & Madsen, 2013; Quintero *et al.*, 2007; USEPA, 2003). Numerous research works discuss the process of microbial remediation in bioreactors that are designed in various ways, including suspended carrier, slurry and fixed bed, membrane, and fluidized bed reactors. These bioreactors can operate in batch, continuous, or fed-batch modes (Quintero et al., 2007; USEPA, 2003; Pino-Herrera et al., 2017; Gargouri *et al.*, 2011).

They provide a controlled environment where important process parameters can be adjusted to optimize the microbial bioremediation process, microbial bioreactors are a highly attractive options for remediation. Another benefit is the bioreactor's design can be tailored to the reactor's intended use or application, both in terms of size and configuration (Pino-Herrera *et al.*, 2017; Chikere *et al.*, 2012; Azubuike *et al.*, 2016). Bioremediation in bioreactors, however, is costlier when carried out ex-situ because it necessitates the relocation of pollutants. This can be done by excavating soils and sediments, transporting the contaminated media, and possibly

containing or handling it under controlled conditions (Quintero et al., 2007; USEPA, 2003; Pino-Herrera *et al.*, 2017; Chikere *et al.*, 2012; Azubuike *et al.,* 2016). The possibility exists that the contamination may spread to other environments. According to Chikere *et al.* (2012) and Azubuike *et al*. (2016), additional pretreatment of contaminated media, such as crushing and drying, may be necessary, increasing the cost of the process.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The area used for this study is within the Rivers State University Demonstration farmland in Nkpolu-Oroworukwo, Mile 3 Diobu, Port Harcourt, Rivers State. The piece of land is situated at Longitude $4^0$48'18.50''N and Latitude $6^0$58'39.12''E measuring 5.486m x 5.1816m with a total area of 28.4283m^2. At the Rivers State University Demonstration Farm in Port Harcourt, Rivers State, soil samples were randomly taken using a hand-held boring metal soil auger at a depth range of 0 to 15 cm.

2.2 Isolation of the Test Organisms

The bio-augmenting organisms (*Bacillus* and *Pseudomonas* species) were isolated from the soil using standard microbiological methods (spread plate method) as described by Prescott *et al*. (2005). One gram of soil sample was aseptically transferred into 9ml of sterile normal saline and subjected to tenfold serial dilution. Then an aliquot (0.1 ml) of the sample was inoculated into properly dried nutrient agar plates (15 ml in each plate) in duplicate, spread evenly using flamed bent rod and incubated at 37°C for 24 hours, after incubation, the bacterial colonies that grew on the plates were sub-cultured unto fresh nutrient agar plates using the streak plate technique to obtain pure culture of the bacterial isolates as adopted by Williams and Inweregbu (2019).

2.3 Characterization and Identification of Test Organisms

Bacterial isolates were characterized on the basis of their colonial morphology, microscopic and biochemical characteristics (Table 1) and by making reference to the identification manual by Cheesebrough (2000).

2.4 Preparation of Broth Culture and Standardization of Inoculum

Five colonies from the pure culture of each isolate were inoculated into nutrient broth in 500 ml conical flask separately, and incubated at 37°C for 18 to 24 hours. After incubation, an aliquot of 0.1 ml was inoculated on a pre dried nutrient agar to determine the total viable counts of the broth culture. Turbidity of the bacterial suspension (i.e. overnight nutrient broth (250 ml) with population density) was adjusted to match that of 0.5 McFarland Standard (30 to 300 colonies mostly 200 colonies) by making a dilution of 1:100 in sterile nutrient broth (Cheesebrough,2000).

2.5 Bioremediation Set-up

The soil was treated for bioremediation as described by (Williams & Inweregbu, 2019). In this method, nine (9) setups in triplicate each were made. Each basin contained;

1. 2500g of soil which served as control 1(triplicates)
2. 2500g of the soil + 250ml of Degreaser which served as control 2 (triplicate)
3. 2500g of the soil + 250ml of Degreaser + 75ml of *Pseudomonas* sp. broth. (triplicate)

4. 2500g of the soil + 250ml of Degreaser + 75ml of *Bacillus* sp. broth (triplicate)
5. 2500g of the soil + 250ml of Degreaser + 75ml of fish pond effluent (triplicate)
6. 2500g of the soil + 250ml of Degreaser + 35ml of *Pseudomonas* sp + 35ml of *Bacillus* sp. broth (triplicate)
7. 2500g of the soil + 250ml of Degreaser + 35ml of *Pseudomonas* sp + 35ml of fish pond effluent broth (triplicate)
8. 2500g of the soil + 250ml of Degreaser + 35ml of *Bacillus* sp. + 35ml of fish pond effluent broth (triplicate)
9. 2500g of the soil + 250ml of Degreaser + 25ml of *Pseudomonas* sp + 25ml of *Bacillus* sp. + 25ml fish pond effluent broth (triplicate)

2.6 Construction of Bioreactor

A bioreactor was constructed in order to obtain the right cultivation of the two organisms, *Pseudomonas* (Pse) and *Bacillus* (Bac), which were used to carry out degradation of the contaminant (degreaser) under controlled conditions. Seventeen (17) litres of broth were prepared and poured into two containers labelled Pse and Bac and *Pseudomonas* and *Bacillus* were inoculated in the broth. Continuous stirring was carried out every 1hr for 5days. Buffer was added to both organisms to give the required pH needed.

2.7 Monitoring of the Bioremediation Potential of the Microorganisms

This bioremediation set up was monitored for selected microbiological (Total Heterotrophic Bacteria, Fungi, Degreaser Utilizing Bacteria (DUB), Degreaser Utilizing Fungi (DUF) and physicochemical (total hydrocarbon content (THC), nitrogen, potassium, phosphorus, temperature and pH) parameters from day 1 to 56 at 14 days' interval. Eighty milliliters (80ml) of sterilized water was added to the set up three times weekly and agitated for proper aeration and adequate distribution of microorganisms.

2.8 Physiochemical Parameters

Using the procedures outlined by APHA (2012), the following physicochemical parameters were examined: pH, temperature, phosphorus, nitrogen, potassium and total hydrocarbon content.

2.9 Microbiological Parameters

2.9.1 Total Heterotrophic Bacteria (THB)

Total heterotrophic bacteria were enumerated using spread plate method. An aliquot (0.1ml) from 10^{-5} dilution (Dilution (10^{-5}) was found appropriate after dilution range finding test) from each set-up and aseptically transferred unto properly dried nutrient agar plates in duplicate, spread evenly using flamed bent glass rod and incubated at 37°C for 24 hours as described by Prescott *et al*. (2005). After incubation, the bacterial colonies that grew on the plates were counted and the average taken. Total Heterotrophic Bacterial (THB) Counts were then taken and expressed as colony forming unit per milliliter using the equation below as adopted by Nrior and Kpormon (2018).

$$\text{THB (cfu/ml)} = \frac{\text{Number of Colonies}}{\text{Dilution } (10^{-4}) \times \text{Volume plated (0.1 ml)}}$$

2.9.2 Fungi (F)

The fungi in each setup were enumerated using spread plate method. An aliquot (0.1 ml) of the dilution of 10^{-2} dilution was aseptically transferred unto properly dried Sabouraud Dextrose Agar plates containing antibiotic (250 Tetracycline) to inhibit bacterial growth, in duplicate, spread evenly using bent glass rod and incubate at 35°C for 3 days (This incubator temperature when using Sabouraud Dextrose Agar gives optimal clear growth in 3 days but ambient temperature of 28±0.2°C in Southern Nigeria stays for 5 days for optimal growth). Fungal colonies that grew on the plate were counted and expressed as colony forming unit per milliliter using the below equation: Dilution (10^{-2}) was used as appropriate after dilution range finding test.

$$\text{THF (cfu/ml)} = \frac{\text{Number of colony}}{\text{Dilution } (10^{-2}) \times \text{Volume plated (0.1 ml)}}$$

2.9.3 Hydrocarbon Utilizing Bacteria and fungi (HUB and HUF)

An aliquot of 0.1 ml from 10^{-2} dilution of the respective set-ups were inoculated into Mineral salt agar which was formulated as adopted by Nrior and Odokuma (2015) for isolation of both hydrocarbon utilizing bacteria and fungi, in duplicate using spread plate techniques. Sterile filter papers placed in the cover of the Petri dishes were saturated with 1 ml of Degreaser. The plates were then incubated inverted at 28°C for 5-7 days. The filter paper saturated with Degreaser served as a sole source of carbon. Colonies formed in the respective plates were counted and the mean values were recorded and expressed as cfu/g. The mineral salt agar used for enumeration of hydrocarbon utilizing bacteria was amended with fungizone while for hydrocarbon utilizing fungi, the medium was amended with 250 µg of tetracycline to inhibit the growth of hydrocarbon utilizing bacteria (Nrior and Odokuma, 2015).

3. RESULTS AND DISCUSSION

The baseline physicochemical and microbiological properties of the soil before the application of various bioremediation treatments are shown in Table 1. The parameters determined were pH, temperature, potassium, phosphorus, nitrogen, and total hydrocarbon content (THC). The microbiological properties determined were Total Heterotrophic Bacteria (THB), Degreaser Utilizing Bacteria (DUB) and Degreaser Utilizing Fungi (DUF). The baseline results revealed pH value of 5.8 for uncontaminated soil and 7.2 for contaminated, temperature was observed to be 27.8^{O}C for uncontaminated while the contaminated was 28.5 ^{O}C, Nitrogen was 13.3 mg/kg for uncontaminated whereas the contaminated soil was 9.6 mg/kg, potassium was 3.41 mg/kg and 2.38, phosphorus was 0.65 mg/kg and 0.46 mg/kg for uncontaminated and contaminated, respectively and THC for uncontaminated was 754 mg/kg and contaminated value of 3984 mg/kg, respectively.

The baseline analysis for microbiological parameters for THB, HUB, F, HUF for uncontaminated and contaminated showed the average counts of 6.3±1.06cfu/g and 5.4±0.57cfu/g for THB, 3.33±0.64cfu/g and 1.6±0.85cfu/g for F, 4.1±0.64cfu/g and 2.1±0.07cfu/g for HUB, 2.5±0.35cfu/g and 1.6±0.21cfu/g for HUF, respectively.

Table 1: Baseline Physicochemical Parameters of Experimental Soil

Parameters	Uncontaminated Soil	Contaminated Soil
Temperature (^{0}C)	27.8	28.5
pH	5.8	7.2
Nitrogen (mg/kg)	13.3	9.6
Phosphorus (mg/kg)	0.65	0.46
Potassium (mg/kg)	3.41	2.38
Total Hydrocarbon Content (THC) (mg/kg)	754	3984

Table 2: Mean Changes deviation showing Temperature (^{0}C) during Bioremediation of Degreaser Contaminated Soil

	Day				
Treatment	**1**	**14**	**28**	**42**	**56**
US	27.8±0.03 ab	29.2±0.07 c	28.4±0.07 b	26.5±0.07 a	2.5±0.07 a
CS	27.5±0.00a	29.2±0.07 c	28.5±0.07 bc	26.6±0.07 b	26±0.07 a
CS+BAC	27.9±0.07 b	28.6±0.01 ab	27.6±0.07 a	26.8±0.07 cd	26±0.07 a
CS+BAC+FPE	27.6±0.07 ab	28.5±0.07 a	28.5±0.07 bc	26.8±0.07 cd	26±0.01 a
CS+BAC+PSE+FPE	27.6±0.07 ab	28.5±0.07 a	28.7±0.07 c	26.9±0.07 d	26±0.07 a
CS+FPE	27.8±0.00 ab	28.7±0.01 b	28.4±0.7 b	26.7±0.7 bcd	26±0.7 a
CS+PSE	27.6±0.07 ab	28.7±0.07 b	30.8±0.07 d	26.8±0.07 cd	26±0.02 a
CS+PSE+BAC	27.6±0.07 ab	30.0±0.07 d	28.3±0.03 b	26.5±0.01 ab	26±0.01 a
CS+PSE+FPE	27.6±0.02 ab	28.6±0.07 ab	28.3±0.07 b	26.9±0.07 d	26±0.07 a

*Means with similar superscripts down the group showed no significant difference (P>0.05)
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

Table 3: Mean Changes deviation showing pH during Bioremediation of Degreaser Contaminated Soil

	DAYS				
Treatment	**1**	**14**	**28**	**42**	**56**
US	6.7±0.01 [a]	7.26±0.01 [g]	6.43±0.01 [a]	5.58±0.01 [a]	5.0±0.01
CS	5.0±0.00 [a]	7.08±0.01 [d]	6.76±0.0 [b]	5.97±0.01 [c]	5.88±0.01 [b]
CS+BAC	5.2±0.01 [a]	7.21±0.01 [f]	7.15±0.01 [ef]	6.87±0.01 [g]	4.41±0.01 [a]
CS+BAC+FPE	5.2±0.02 [a]	7.38±0.01 [h]	6.93±0.01 [c]	6.71±0.01 [f]	7.0±0.00 [i]
CS+BAC+PSE+FPE	5.2±0.01 [a]	7.18±0.01 [e]	7.04±0.01 [de]	6.55±0.01 [e]	6.81±0.01 [f]
CS+FPE	5.2±0.01 [a]	7.06±0.01 [c]	7.2±0.14 [f]	6.46±0.01 [d]	6.94±0.01 [g]
CS+PSE	5.2±0.01 [a]	6.8±0.01 [a]	6.88±0.0 [bc]	5.88±0.01 [b]	6.1±0.01 [d]
CS+PSE+BAC	5.2±0.01 [a]	6.98±0.01 [b]	7.15±0.01 [ef]	6.51±0.01 [de]	6.97±0.01 [h]
CS+PSE+FPE	5.2±0.01 [a]	7.21±0.01 [f]	7.15±0.01 [ef]	6.85±0.01 [g]	6.68±0.01 [e]

*Means with similar superscripts down the group showed no significant difference (P>0.05)
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

Table 4: Mean Changes deviation showing Nitrogen (Mg/Kg) during Bioremediation of Degreaser Contaminated Soil

	DAYS				
Treatment	**1**	**14**	**28**	**42**	**56**
US	9.1±0.00 [a]	8.8±0.00 [h]	8.6±0.00 [h]	7.9±0.00 [h]	7.47±0.00 [g]
CS	9.1±0.00 [a]	9.9±0.00 [i]	9.87±0.00	8.3±0.00 [i]	8.07±0.00 [h]
CS+BAC	9.1±0.00 [a]	6.6±0.00 [b]	6.6±0.00 [b]	5.7±0.00 [e]	4.07±0.00 [c]
CS+BAC+FPE	9.1±0.00 [a]	8.7±0.00 [g]	7.5±0.00 [d]	5.1±0.00 [c]	4.54±0.00 [f]
CS+BAC+PSE+FPE	9.1±0.00 [a]	8.5±0.00 [f]	7.7±0.00 [e]	4.87±0.00 [b]	3.0±0.00 [a]
CS+FPE	9.1±0.00 [a]	5.1±0.00 [a]	5.3±0.00 [a]	4.5±0.00 [a]	3.60±0.00 [b]
CS+PSE	9.1±0.00 [a]	7.6±0.00 [c]	8.3±0.00 [g]	6.1±0.00 [f]	4.26±0.00 [d]
CS+PSE+BAC	9.1±0.00 [a]	8.2±0.00 [e]	8.0±0.00 [f]	5.5±0.00 [d]	4.47±0.00 [e]
CS+PSE+FPE	9.1±0.00 [a]	8.0±0.00 [d]	7.4±0.00 [c]	6.2±0.00 [g]	4.54±0.00 [f]

*Means with similar superscripts down the group showed no significant difference (P>0.05)
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

Table 5: Mean Changes deviation showing Phosphorus (Mg/Kg) during Bioremediation of Degreaser Contaminated Soil

	DAYS				
Treatment	**1**	**14**	**28**	**42**	**56**
US	0.54±0.00 [a]	0.21±0.00 [c]	0.19±0.00 [c]	0.07±0.00 [a]	0.06±0.00 [b]
CS	0.58±0.00 [b]	0.24±0.01 [e]	0.23±0.00 [e]	0.08±0.01 [a]	0.08±0.01 [cd]
CS+BAC	0.58±0.00 [b]	0.22±0.01 [d]	0.17±0.00 [b]	0.08±0.00 [a]	0.06±0.00 [b]
CS+BAC+FPE	0.58±0.00 [b]	0.23±0.00 [de]	0.25±0.00 [f]	0.09±0.00 [a]	0.08±0.00 [e]
CS+BAC+PSE+FPE	0.58±0.00 [b]	0.13±0.00 [a]	0.17±0.01 [b]	0.15±0.07 [b]	0.05±0.01 [a]
CS+FPE	0.58±0.00 [b]	0.25±0.00 [f]	0.23±0.01 [e]	0.09±0.00 [ab]	0.08±0.00 [de]
CS+PSE	0.58±0.00 [b]	0.21±0.00 [c]	0.21±0.00 [d]	0.08±0.00 [a]	0.05±0.00 [a]
CS+PSE+BAC	0.58±0.00 [b]	0.18±0.00 [b]	0.13±0.00 [a]	0.11±0.00 [ab]	0.08±0.00 [e]
CS+PSE+FPE	0.58±0.00 [b]	0.23±0.00 [de]	0.19±0.00 [c]	0.12±0.00 [ab]	0.07±0.00 [bc]

*Means with similar superscripts down the group showed no significant difference (P>0.05)
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

Table 6: Mean Changes deviation showing Potassium (Mg/Kg) during Bioremediation of Degreaser Contaminated Soil

	DAY				
Treatment	**1**	**14**	**28**	**42**	**56**
US	3.4±0.00 [a]	2.8±0.00 [e]	2.4±0.00 [c]	2.3±0.00 [f]	1.4±0.00 [f]
CS	3.4±0.00 [a]	2.5±0.07 [c]	2.6±0.00 [e]	2.6±0.00 [g]	1.6±0.00 [g]
CS+BAC	3.4±0.00 [a]	2.2±0.00 [b]	2.3±0.00 [b]	1.1±0.07 [b]	0.6±0.00 [a]
CS+BAC+FPE	3.4±0.00 [a]	2.5±0.07 [c]	2.3±0.07 [b]	0.96±0.00 [a]	0.07±0.00 [b]
CS+BAC+PSE+FPE	3.4±0.00 [a]	2.1±0.00 [a]	2.0±0.00 [a]	1.6±0.00 [d]	1.4±0.00 [f]
CS+FPE	3.4±0.00 [a]	2.7±0.01 [de]	2.5±0.01 [d]	1.8±0.00 [e]	1.7±0.00 [h]
CS+PSE	3.4±0.00 [a]	2.6±0.01 [d]	2.9±0.00 [f]	1.1±0.00 [b]	1.1±0.01 [d]

CS+PSE+BAC	3.4±0.00 [a]	2.5±0.07 [c]	2.6±0.00 [e]	1.2±0.00 [c]	1.2±0.00 [e]
CS+PSE+FPE	3.4±0.00 [a]	2.1±0.07 [a]	2.7±0.00 [e]	1.7±0.07 [d]	1.1±0.00 [c]

*Means with similar superscripts down the group showed no significant difference (P>0.05)
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

Table 7: Mean Changes deviation showing THC (Mg/Kg) during Bioremediation of Degreaser Contaminated Soil

	DAY				
Treatment	**1**	**14**	**28**	**42**	**56**
US	754±0.07[a]	347.50±0.07 [a]	287±0.07 [a]	272±0.07 [a]	253±0.28 [a]
CS	3984±0.07 [b]	3460±0.07 [d]	2924±0.07 [f]	2800±0.07 [i]	1134±0.07 [g]
CS+BAC	3984±0.14 [b]	3434±0.07 [c]	2844±0.07 [d]	2564±0.07 [c]	1244±0.07 [i]
CS+BAC+FPE	3983±0.21 [b]	3570±0.07 [g]	2910±0.07 [e]	2660±0.07 [f]	1044±0.07 [e]
CS+BAC+PSE+FPE	3984±0.14 [b]	3571±0.14 [g]	2034±0.07 [b]	2020±0.07 [b]	1014±0.07 [c]
CS+FPE	3983±0.21 [b]	3460±0.07 [d]	2974±0.07 [h]	2744±0.07 [h]	934±0.07 [b]
CS+PSE	3984±0.07 [b]	3504±0.07 [e]	2940±0.07 [g]	2730±0.07 [g]	1020±0.07 [d]
CS+PSE+BAC	3984±0.07 [b]	3521±0.14 [f]	2990±0.07 [i]	2640±0.07 [e]	1070±0.07 [f]
CS+PSE+FPE	3983±0.28 [b]	3424±0.07 [b]	2800±0.07 [c]	2620±0.07 [d]	1170±0.07 [h]

*Means with similar superscripts down the group showed no significant difference (P>0.05)
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

Table 8: Biochemical Identification of Bacterial Isolates

Isolate codes	Gram Rxn	Cell Shape	Catalase	Citrate	Oxidase	Indole	Motilit	Lactose	Glucose	Mannit	Sucrose	STH	MR	VP	Elevation	Margin	Shape	Texture	Colour	Size (mm)	Isolated Organism
1	+ve	Rods	+	+	+	-	+	-	AG	AG	AG	+	-	-	Flat	Serrated	Round	Dry	Milk	5	*Bacillus sp*
2	-ve	Rods	+	+	+	-	+	-	-	-	-	+	-	+	Raised	Entire	Round	Moist	Green	3	*Pseudomonas sp*
3	+ve	Rods	+	+	+	-	-	-	AG	A	AG	+	+	-	Flat	Serrated	Round	Dry	Creamy	3	*Bacillus sp*
4	+ve	Rods	+	+	+	-	+	A	AG	-	-	+	+	-	Raised	Entire	Round	Dry	Creamy	5	*Bacillus sp*
5	-ve	Rods	+	+	+	-	+	-	AG	-	-	+	-	-	Raised	Entire	Round	Moist	Creamy	3	*Aeromonas sp*
6	+ve	Rods	+	+	-	-	-	-	A	A	A	-	+	-	Raised	Entire	Round	Moist	Whitish	4	*Bacillus sp*
7	-ve	Rods	+	-	-	+	+	AG	AG	AG	AG	+	+	-	Raised	Entire	Round	Moist	Milky	3	*Escherichia coli*
8	+ve	Cocci	+	-	-	-	-	AG	AG	AG	A	-	+	+	Raised	Entire	Round	Moist	Creamy	2	*Micrococcus sp*

9	-ve	Rods	+	+	+	-	+	-	-	-	-	+	-	+	Raised	Entire	Round	Moist	Milky	3	*Pseudomonas sp*
10	+ve	Rods	+	-	+	-	+	-	A	A	A	+	-	-	Flat	Undulate	Round	Moist	Whitish	3	*Bacillus sp*

Key: +ve = positive, -ve = negative, AG = acid and gas, A= Acid, VP = voges Proskauer, MR = methyl red, G = gas, SH = starch hydrolysis

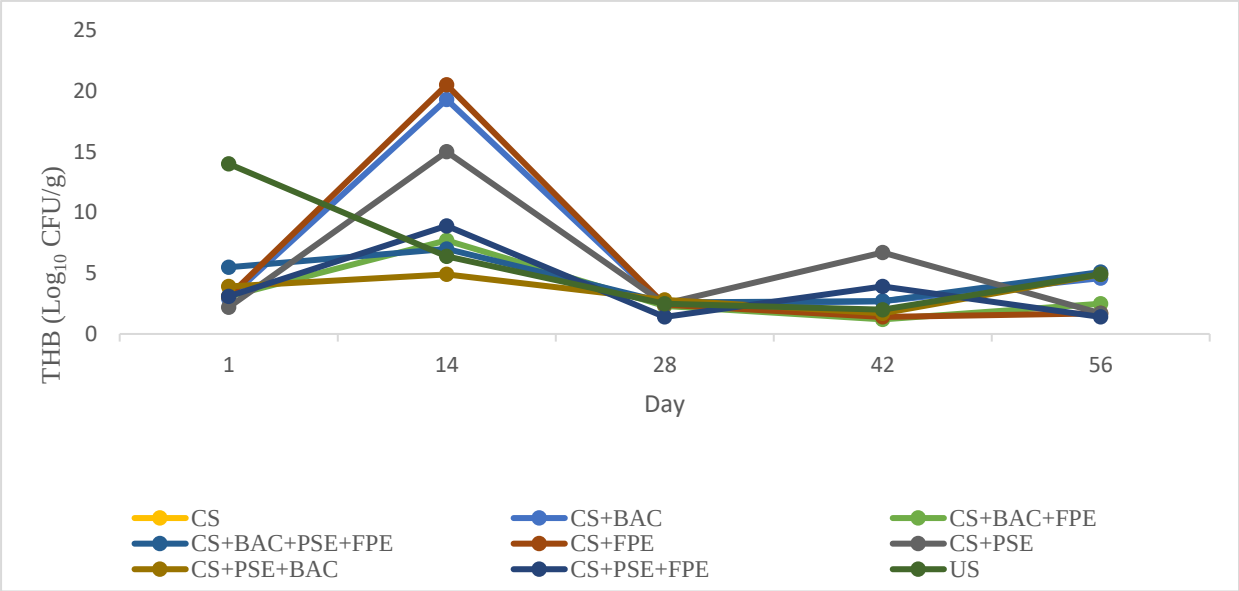

Fig. 1: Changes in Total heterotrophic Bacterial (THB) count During Bioremediation of Degreaser Contaminated Soil]
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

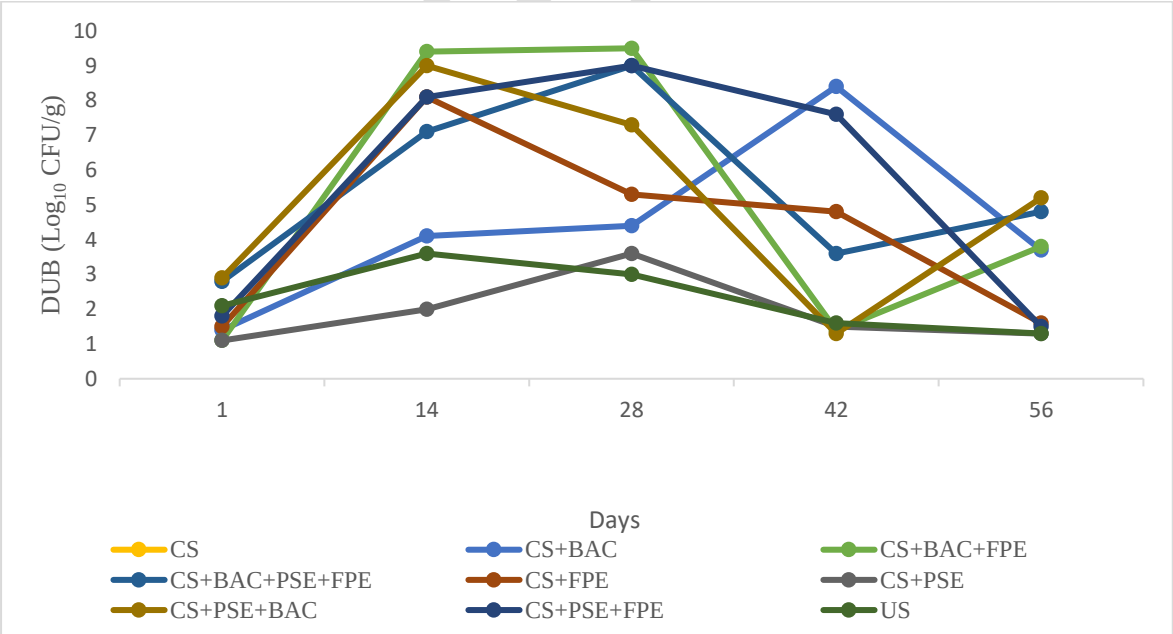

Fig. 2: Changes in Degreaser utilizing Bacterial (DUB) count (CFU/g) During Bioremediation of Degreaser Contaminated Soil
KEY: US – Unpolluted Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

Table 9: Changes in Total Hydrocarbon Content (THC) (Mg/Kg) In Soil during Bioremediation Of degreaser Contaminated Soil augmented with Microbes

Set-up code	Day 1	Day 14	Day 28	Day 42	Day 56	Amount Remediated	% Remediated
US	754	347.50	287	272	253	501	66.4
CS	3984	3460	2924	2800	1134	2850	71.5
CS+Pse	3984	3504	2940	2730	1020	2964	74.4
CS+Bac	3984	3434	2844	2564	1244	2740	68.8
CS+FPE	3984	3460	2974	2744	934	3050	76.6
CS+Pse+Bac	3984	3521	2990	2640	1070	2914	73.1
CS+Pse+FPE	3984	3424	2800	2620	1170	2814	70.6
CS+Bac+FPE	3984	3570	2910	2660	1044	2940	73.8
CS+Pse+Bac+FPE	3984	3571	2034	2020	1014	2970	74.6

KEY: US – Uncontaminated Soil, CS-Contaminated soil, BAC – *Bacillus*, PSE – *Pseudomonas*, FPE – Fish Pond Effluent

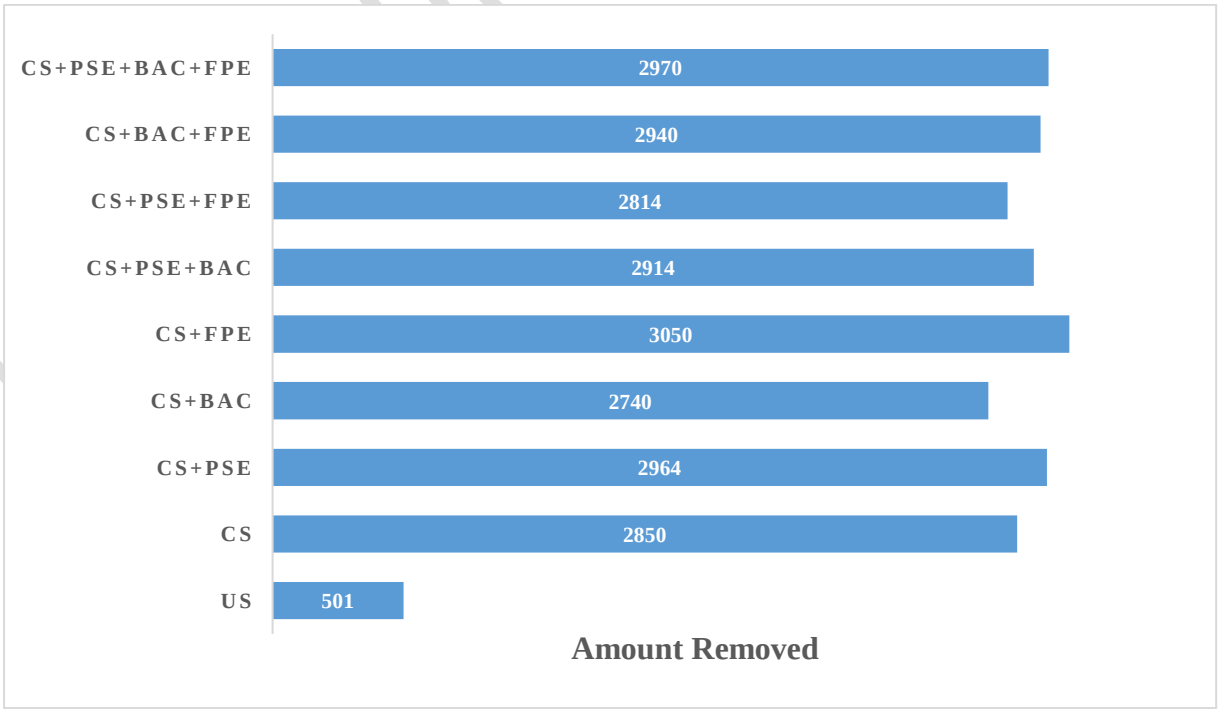

Fig. 3: Amount of THC (mg/kg) Removed during bioremediation of degreaser polluted soil

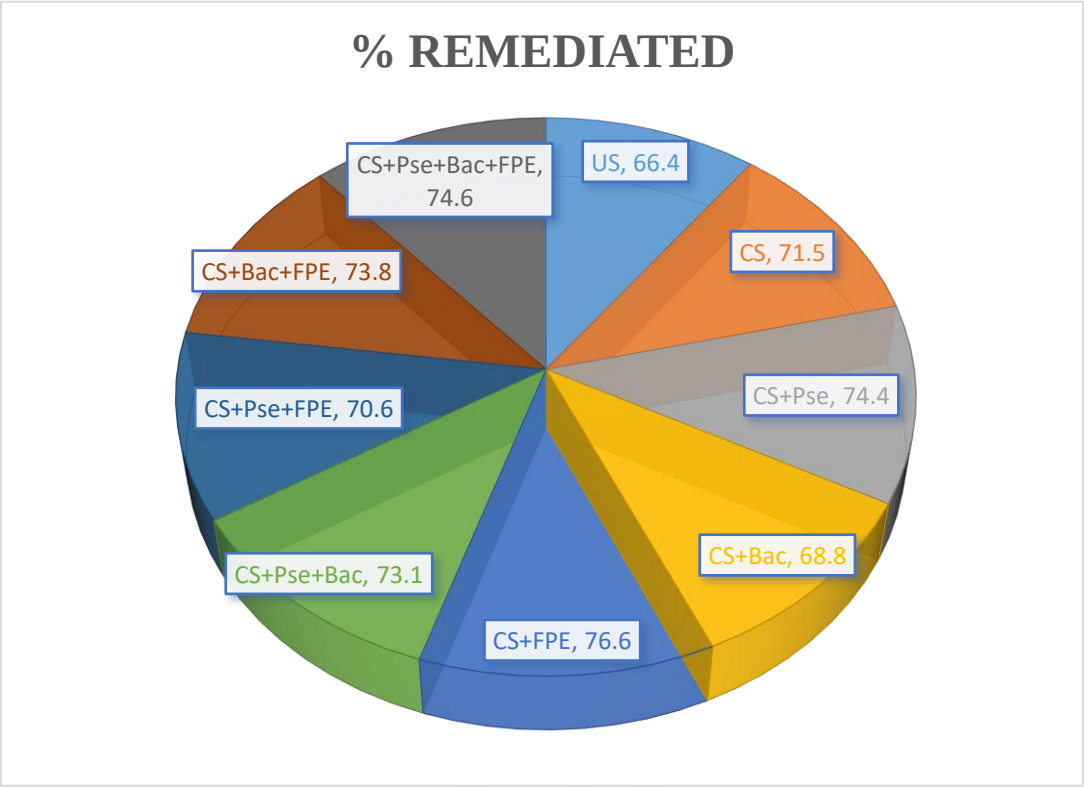

Fig. 4: Percentage THC (mg/kg) Remediated during bioremediation of degreaser contaminated soil

The results of the microbial counts obtained during bioremediation of degreasers are as follows: total heterotrophic bacterial (THB) count are presented in Figure 1 and Degreaser utilizing bacterial (DUB) count are presented in Figure 3.
The Total heterotrophic bacterial (THB) revealed counts from the lowest to highest during monitoring for each set up and days as follows: Day42 CS+BAC+PSE (1.2), day56 CS+PSE+FPE (1.4), day56 CS+PSE (1.7), day1 CS+FPE (3.1), day1 CS+BAC+PSE+FPE (5.5), day1 US (14.0), day14 CS+BAC (19.3) (Figure 1).
Degreaser utilizing bacterial (DUB) counts revealed were as follows: day1 CS+BAC+FPE (1.1), day56 CS+PSE (1.5), day14 US (3.6), day42 CS+PSE (3.6), day14 CS+FPE (8.1), day42 CS+BAC (8.4), day14 CS+BAC+PSE+FPE (7.1), day28 CS+PSE+BAC (7.3), day42 CS+BAC (8.4), day14 CS+BAC+FPE (9.4) (Figure 3).

The mean changes of the physiochemical parameters of the polluted soil with the different treatment during the bioremediation study are shown in Tables 2 – 7.
pH values obtained from the various treatments during bioremediation are shown in Table 2. The highest values for each treatment during monitoring were as follows: day 14 US (7.26), day14 CS (7.08), day28 CS+PSE (6.88), day14 CS+BAC (7.21), day28 CS+FPE (7.2), day14 CS+PSE+BAC (7.15), day14 CS+PSE+FPE (7.21), day56 CS+BAC+FPE (7.0), day28 CS+PSE+BAC+FPE (7.18).
Temperature values obtained during the study were relatively same between treatments. The highest values for eat treatment during monitoring were as follows: day14 US (29.2), day14 CS (29.2), day28 CS+PSE (30.0), day1 CS+BAC (27.9), day14 CS+FPE

(28.7), day14 CS+PSE+BAC (30.0), day28 CS+PSE+FPE (28.3), day28 CS+BAC+FPE (28.5), day28 CS+PSE+BAC+FPE (28.7), Table 3.

Nitrogen concentration during monitoring of the polluted soil is shown in Table 4. day1 US (9.1), day14 CS (9.9), day1 CS+PSE (9.1), day1 CS+BAC (6.6), day28 CS+FPE (5.3), day14 CS+PSE+BAC (8.2), day28 CS+PSE+FPE (7.4), day42 CS+BAC+FPE (5.5), day14 CS+PSE+BAC+FPE (8.5).
Potassium concentration during monitoring of the polluted soil is shown in Table 5. day56 US (1.4), day14 CS (2.5), day28 CS+PSE (2.9), day1 CS+BAC (3.4), day14 CS+FPE (2.7), day28 CS+PSE+BAC (2.6), day28 CS+PSE+FPE (2.7), day28 CS+BAC+FPE (2.3), day1 CS+PSE+BAC+FPE (3.4).
Phosphorous concentration during monitoring of the polluted soil is shown in Table 6. day1 US (0.54), day14 CS (0.24), day28 CS+PSE (0.21, day14 CS+BAC (0.22), day14 CS+FPE (0.25), day14 CS+PSE+BAC (0.18), day14 CS+PSE+FPE (0.23), day28 CS+BAC+FPE (0.25), day1 CS+PSE+BAC+FPE (0.58).

Total hydrocarbon content (THC) during monitoring of the polluted soil is shown in Table 7. The amount of degreaser remediated and the percentage remediated after 56days of monitoring the various treatment are given in their increasing order as; US (501mg/kg;66.4), CS+BAC (2740 mg/kg; 68.8%), CS (2850 mg/kg; 71.5%), CS+PSE+BAC (29.14 mg/kg; 73.1%), CS+PSE+FPE (2814 mg/kg; 70.6%), CS+BAC+FPE (2940 mg/kg; 73.8%), CS+PSE (2964 mg/kg; 74.4), CS+PSE+BAC+FPE (2970 mg/kg; 74.6%), CS+FPE (3050 mg/kg; 76.6%).

Results of the microbial population of the degreaser uncontaminated and contaminated soil showed that total heterotrophic bacterial count had the highest count when compared to the rest of the parameters. This was followed by the degreaser utilizing bacterial counts. This agreed with the study of Williams and Hakam (2016) who recorded an increase in the THB counts. The increase in the counts may be as a result of the abundance nature of bacteria in soil and their ability to utilize hydrocarbon substances as carbon source (Williams & Amaechi 2017).
The bacterial isolates from the experimental soil used in this study were characterized based on their microscopic, biochemical, morphological properties and they belong to the genera: *Bacillus, Pseudomonas, Aeromonas*, *Escherichia* and *Micrococcus* species. The result is in correlation with the work reported by Williams and Akemi (2020) that isolated *Pseudomonas, Klebsiella, Bacillus, Micrococcus,* and *Proteus* species. The result of this study showed that these microorganisms could be used in bioremediation of degreaser contaminated soil.

The main difference of degreaser biodegradation in soil amended with fish pond waste and unamended soil treatment occurred between 14 and 28 days, where bio stimulation resulted in significant increase of oil biodegradation. The addition of nutrients stimulates the degradative capabilities of the indigenous microorganisms, thus, allowing the microorganisms to break down the organic pollutants at a faster rate (Ausma *et al.*, 2002).
The samples amended with Fish Pond effluent degraded the degreaser contaminated soil more than the unamended (Nnemelu *et al.*,2024). This may possibly be due to a higher nutrient level present in Fish pond effluent. Amendment with organic nutrients like goat manure, fish wastes and pigeon droppings are recommended for contaminated soils due to their high nutrient content as substrates for bio stimulation of indigenous

and augmenting biodegrading microbes (Williams *et al*.,2024). Research has shown that organic waste harbors hydrocarbon utilizing bacteria (Agarry *et al*, 2012). The biodegradation recorded in the unamended soil sample could be due to non-biological factors such as evaporation, photo-degradation volatilization, adsorption, abiotic factors (temperature and pH) (Odokuma and Williams, 2012, Onuoha 2013). Reduction of petroleum hydrocarbon in unamended sample has also been reported by previous studies (Idowu & Ijah, 2017). The results of the total hydrocarbon content (THC- mg/kg) of the bioremediation set up suggested that there was a reduction on the 56th day of monitoring.

Conclusion

Bioremediation could be a feasible and efficient response to soil contamination with petroleum hydrocarbons. The selection of proper microbial strains is the key step to a successful bioaugmentation. For a pollutant to be eliminated, it is very important to select microbial inoculants isolated from contaminated sites.

The study showed that bioreactor was effective in multiplying the microbial isolates used in the various treatment plots which in turn enhanced the degradation of the contaminated soil. More so, reduction of degreaser in the contaminated soil indicates the presence of degreaser degrading microorganisms; and the rate of degreaser biodegradation in soil could be enhanced by amendment with fish pond effluent singly and the consortium of bio augmenting organisms (*Pseudomonas* and *Bacillus*).

Biodegradation of degreaser was enhanced positively by the amendment of organic wastes (fish pond effluent). From the study, it was observed that fish pond effluent has high nitrogen and phosphorus content which are known as most important nutrients needed by degreaser utilizing bacteria to carry out effective and efficient activities of biodegradation of xenobiotics in the soil environment.

The study showed that for bioremediation of degreaser polluted soils, combination with organic nutrients is a better degradative option. Therefore, amendment with organic nutrients like fish pond effluent is recommended for degreaser polluted soils due to their high nutrient content as substrates for bio stimulation of indigenous and augmenting biodegrading microbes. Natural bioremediation of degreaser pollutants by activation of naturally occurring microorganisms will be cost effective in cleaning up and protect the environment from hazardous effects of hydrocarbon on the agricultural ecosystem.

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