Bioremediation of Soil Contaminated with Spent Motor Oil

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Abstract - The contamination of soil and water by used and unused motor oil has continued to pose serious problems in form of economic loss, loss of water lives, ailing soil fertility amongst several others. Bioremediation is a low-cost alternative green technology that can remediate such polluted soil. This work studies the bioremediation of spent oil-contaminated soil in anaerobic batch reactors at room temperature with cow dung, horse dung, camel dung and donkey dung as organic stimulants over a period of 35 days. The study adopted bioremediation indicators such as oil and grease content, total petroleum hydrocarbon (TPH) and total heterotrophic bacterial counts. Results obtained showed increase in the bacterial population with decrease in crude contents of the polluted soils with time. The treatment options 3 and 4 (addition of cow dung and horse dung) gave the best performance in reduction of oil and grease content by 40 and 60% respectively, and also TPH a treatment H gave the lowest residual percentage of TPH as 0.414%. The study suggested the ability of the organic wastes used to be utilized in enhancing the activities of the microbial hydrocarbon-degrading bacteria during bioremediation of crude petroleum-oil polluted soil.

Indexed Terms : Bioremediation, Contamination, Motor-oil, Soil

I. INTRODUCTION

Crude oil is the largest and most commonly used source of energy in the world, major portions of it are used as transportations fuel such as kerosene, gasoline and diesel. The operations of extraction and drilling of this fossil energy resource, however, pose a serious threat to the environment (Hamoudi-belarbi *et al.*, 2018). Crude oil contains a wide range of compounds having environmental, medical, cytotoxic, mutagenic, and carcinogenic effects (Ramirez *et al.*, 2017). Reducing the petroleum hydrocarbon compounds in a polluted environment becomes a significant challenge for oil companies that are forced to conduct an adequate and effective treatment of these pollutant emissions. Thermal treatment, soil washing, soil vapor extraction, solidification, and stabilization are physical and chemical techniques used to treat petroleum hydrocarbon-polluted soil (Dadrasnia *et al.*, 2015). However, they are often expensive, ineffective, and rarely neutral.

Bioremediation is a process used for the treatment of contaminated media by altering environmental conditions in order to stimulate the growth of the microorganisms that naturally degrade the targeted pollutants (Bioremediation, 2019). This process stands out to be the most effective, non-invasive, the least expensive and at the same the most eco-friendly technique (Abdulsalam *et al.*, 2012). Bioremediation of hydrocarbons in polluted soils is a promising treatment technique, based on the principle of complete mineralization or transformation of petroleum products into less toxic forms by different groups of microorganisms (Hamoudi-belarbi *et al.*, 2018). Bioremediation conserves the soil's texture and essential characteristics. Moreover, physical and chemical properties of the soil, such as pH, water-holding capacity, aeration, and ion exchange capacity can be improved through bioremediation (Hamoudi-belarbi *et al.*, 2018)

Techniques in bioremediation accelerate the naturally occurring biodegradation of hydrocarbons by optimizing the conditions of this process through aeration, addition of nutrients, controlling pH, moisture content, and temperature. Physical and chemical technologies, such as dispersion, dilution, sorption, volatilization, abiotic transformations though important, have their limitations. These limitations include; expensive to implement at full scale, they are not environmentally friendly, their technologies are complex and they lead to destruction of soil texture and characteristics (Abdulsalam *et al.*, 2012). Furthermore, the physicochemical technologies do not always result in complete neutralization of pollutants. Due to limitations of the physicochemical technologies stated above, researchers have reported that bioremediation technologies are alternatives and/or supplements to these technologies. This is

because bioremediation of hydrocarbon-contaminated soils has been established as an efficient, economic, versatile, and environmentally sound treatment (Abdulsalam *et al.*, 2011). The most widely used bioremediation procedure is bio-stimulation of the indigenous microorganisms by addition of nutrients, as input of large quantities of carbon sources (i.e. contamination) tends to result in rapid depletion of the available pools of major inorganic nutrients, such as Nitrogen and Phosphorous (Margesin, 2007). The use of compost in bioremediation treatment is a form of bio-stimulating indigenous microorganisms to carry out contaminants clean up in contaminated soil. Compost bioremediation has received little attention despite its application in the treatment of soils contaminated with organic compounds for many years. It is an established fact that composts have been reported to have potential for remediation of heavily contaminated sites. In an attempt to further enhance this remediation technology, cow dung, camel dung, donkey dung and horse dung will be applied to the contaminated soil for treatment process.

Bioremediation attempts to accelerate the biodegradation rates through the optimization of limiting environmental conditions (Margesin, 2007). These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population and the environmental factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients (Vidali, 2001)

II. METHODS

2.1.1 Sample collection

Surface soil samples contaminated naturally with spent motor oil were collected from an Auto-Mechanic Workshop situated along Nassarawa gate in Bauchi, Bauchi state Nigeria from ground surface to a depth of 15 mm, in black polythene bags and transported to Chemical Reaction Laboratory of the Abubakar Tafawa Balewa University Bauchi where the experiment was carried out.

2.1.2 Sample preparation

Large debris and unwanted particles of the collected sample were removed. The sample was air-dried in a clean well-ventilated laboratory. The soil sample was pulverized and passed through a 2 mm pore size sieve. The soil sample was thoroughly mixed to ensure proper mixing of the contaminant thereby achieving homogeneity. The prepared soil sample was kept at room temperature for later use. The organic stimulants were sun-dried and sieved by passing it through another 2 mm sieve to achieve uniform particle size and kept in a neat polythene bag at room temperature for use.

2.2 Physicochemical analysis of contaminated soil sample and organic wastes

Portion of the prepared contaminated soil was collected and analyzed to determine total petroleum hydrocarbon (TPH), pH, moisture content, organic matter, organic carbon, nitrogen, phosphorous, and potassium prior to amendment. The organic wastes as an amendment material were also subjected to pH, moisture content, organic matter, organic carbon, nitrogen, phosphorous tests. The methods described below were employed in the analysis of the afore-mentioned physicochemical parameters.

2.2.1 Determination of Oil and Grease Content (Spectrophotometry Method)

Moist samples were collected aseptically from each bioreactor and air-dried for 48 h. 5 g of each air-dried sample were extracted by vigorous hand shaking for 3 min with 20 mL of toluene in a separation funnel. The mixtures were allowed to settle and the extracts were decanted into a volumetric flask and plug. The above procedure was repeated twice using 20 mL of toluene each time (Vu-Duc *et al*., 2002). The total extracts were combined and diluted in the ratio 1:3 (extract: toluene), and then the absorbance of each sample was quantified using a CE 1020 (1000 Series) UV Spectrophotometer at 400 nm. Oil and Grease contents were extrapolated from a standard curve of absorbance (A400 nm) against concentration. Values of concentration obtained were multiplied by the dilution factor (DF) to give the actual concentration.

2.2.2 Determination of Organic Carbon in Samples (Walkley black procedure)

0.5 g of sample was sieved through 0.5 mm sieve and weighed into a 250 ml volumetric flask, 10 mL of 1M $K_2Cr_2O_7$ solution accurately into each flask and swirled gently to disperse the sample. As well, 20 mL of concentrated H_2SO_4 was carefully added. The solution was shaken gently and allowed to cool at room temperature. 100 mL of distilled water was added after standing for 30 minutes. In addition, a blank solution was prepared but this time without soil sample to standardize the dichromate. 3 – 4 drops of indicator were added and titrated with 0.5M ferrous sulphate solution on a white background. As the endpoint is approached, the solution takes on a greenish; cast and then changes to dark green. At this point, ferrous sulphate was added drop by drop until the color changes sharply from blue to red (maroon color) in reflected light against a white background. Samples concentrations were calculated using the following relations:

$$\%\ Organic\ Carbon = \frac{(Blank - Sample\ TV) \times 0.003 \times f)}{weight\ of\ sample} \times 100$$

Where correction factor, f = 1.33

2.2.3 Determination of total heterotrophic bacteria in the contaminated soil and organic stimulants

Microbial population of contaminated soil sample and organic stimulants were conducted prior to the study, using the plate count method. Total viable count of heterotrophic bacterial population was determined by isolating using the pour plate technique and growth on plate count agar (PCA). Serial dilutions of 10^{-1} to 10^{-10} were prepared aseptically by diluting 1g of the contaminated soil sample into 10 ml of sterile distilled water and mixed using the pulsifier. 1 ml aliquots from each of the dilution were inoculated into or on sterile petri dishes with already prepared PCA. The plates were then incubated at a temperature of 28±2°C for 24 hours after which identification of the individual colonies was carried out. after incubation and recorded as total viable counts using the colony counter. Results were recorded as colony forming unit cfu per gram of soil.

2.2.4 Determination of pH of soil sample and organic stimulants

A gram of dried contaminated soil sample was measured and transferred into a beaker; 20 mL of distilled water was added as a suspension medium. The suspension was thoroughly stirred for 30 minutes, after which the calibrated pH meter was dipped into the beaker containing the suspension and the pH value was recorded. This was triplicated and the average pH values recorded. The pH of the organic stimulants was equally determined by the procedural method described above.

2.2.5 Determination of Nitrogen in contaminated soil sample and compost

Digestion:

1g of air dry soil sample was weighed into 500 ml long-necked Kjeldahl flask and 10 ml distilled water was added to moisten the sample. 1 spatula-full of Kjeldahl catalyst (mixture of 1part selenium + 10 parts $CUSO_4$ + 100 parts Na_2SO_4), followed by 20 ml concentration of H_2SO_4 was added to the mixture in the Kjeldahl flask. The mixture was then left to digest until the solution appeared clear and colourless. The flask was allowed to cool, and the fluid decanted into a 100 ml volumetric flask and made up to the mark with distilled water.

Distillation

By means of a pipette, an aliquot of 10 ml fluid from the digested sample was transferred into Kjeldahl distillation flask. 90 ml of distilled water was added to make it up to 100 ml in the distillation flask. 20 ml of 40% NaOH was added to the content of the distillation flask. Distillate was collected over 10 ml of 4% boric acid and 3 drops of mixed indicator in a 200 ml conical flask was collected as shown in appendix 4. The presence of nitrogen gives a light blue color.

Titration

The collected distillate was titrated (about 100 ml) with 0.1M HCl until the blue color slightly changes to grey and then suddenly flashes to pink. A blank determination was first carried out without a sample.

Calculation:

Weight of sample used, considering the dilution and the aliquot taken for distillation

$$1g * 10ml/100ml = 0.1\ g$$

$$\% \text{ Nitrogen } = 14 * (A - B) * N * 100/1000 * 0.1$$

III. 3.3 Experimental Set-up

Bioremediation of soil contaminated with spent motor oil was conducted in an aerobic fixed bed bioreactor. The study was carried out using six treatments of varying compositions as described in Table 1 below.

Table 1. Experimental Design

Treatment	Composition
1	Sample + Camel Dung
2	Sample + Donkey Dung
3	Sample + Cow Dung
4	Sample + Horse Dung
5	Sample + Heat Treatment at 121^{o}C
6	Sample

3.3.1 Process description

These biodegradation investigations were carried out on six aerobic fixed bed bioreactors designated as TR 1-6 which were connected in parallel with 700g of contaminated soil; this included, where appropriate, the various additives at room temperatures. The additives so included were inoculation of 2133 cfu/g CS of consortium of Pseudomonas aeruginosa and Bacillus subtilis, heat sterilization at 12°C or 30.42 g of NPK 20:10:10 and 5.6 g of KH_2PO_4 to give carbon, nitrogen and phosphorus molar ratio of 100:10:1. The bioreactors were completely closed in order to avoid CO_2 leakage to the surrounding before passing into the CO_2 traps. The absorber (AB) before the bioreactors contained a solution of 60 % (w/v) NaOH used to absorb the CO_2 from the atmosphere and the humidifying unit (HD) to moisten the air before entering the bioreactors. The absorbers, after the bioreactors, contained 10 M solution of NaOH each meant to absorb the CO_2 generated from the bioremediation processes. The moisture content in all the treatments was set at 20 % (w/w) at the initiation of bioremediation. The airflow rate was maintained in all cases at an average rate of 10 L/hr using a flow meter for 14 hrs daily over the bioremediation period.

The experimental setup is presented as plate 1 and 2 showing the front and side.

This process was assessed by measuring the oil and grease, the total heterotrophic bacteria counts (THBC) and physicochemical properties of soil and organic wastes.

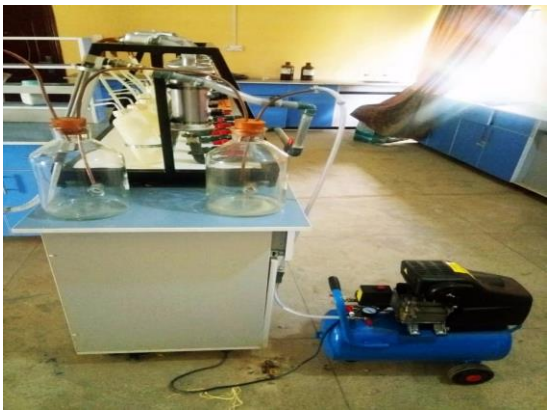

Plate 1: Side view of Experimental Rig

Plate 2: Front view of Experimental Rig

IV. RESULTS AND DISCUSSIONS

4.1 Physicochemical Properties of Soil and Organic Wastes

Physicochemical properties of the soil and those of the nutrient supplements used in the study were analyzed and are presented in Table 2 below. The test soil under investigation is contaminated with hydrocarbon contaminants to some degree, having a mean total petroleum hydrocarbon of 36.80 % with oil and grease content of 600mg which was above the safe limit of 500 mg/kg set by the Nigeria Ministry of Environment

(Abdulsalam *et al.,* 2011), hence the need for the remediation of the polluted soil. The pH of the soil sample used for the research was basic (8.1) which was within the acceptable limit (5.5 – 8.5) for effective bioremediation as reported by Vidali (2001). The Nitrogen content 1.4% of the polluted soil was low, hence the need for amendment with organic stimulants (Cow, Camel, Donkey and Horse dung whose nitrogen contents were found to be 16.8%, 30.8%, 61.6%, and 14% respectively).

Table 1: Physicochemical Properties of Contaminated Soil and Organic Stimulants

Parameter	Soil	Cow dung	Camel dung	Donkey dung	Horse dung
Nitrogen (%)	1.40	16.80	30.80	61.60	14.00
Carbon (%)	36.8	35.63	39.50	35.20	39.50
Phosphorous (ppm)	1416.22	1201.81	1913.5	2054.05	3210.81
Oil and Grease (g)	0.6	-	-	-	-

4.2 Total heterotrophic bacterial count (THBC)

Table 3 shows the total heterotrophic bacterial counts for the different reactors before and after the bioremediation period. Similar trends were observed for all the bioreactors as the total heterotrophic bacteria were all increasing. This demonstrates the ability of utilizing used motor oil as the energy source.

The heterotrophic bacterial count after 35 days were higher in the amended samples than that of the control samples, this conflicts with the findings of Abioye *et al.,* (2010) who stated that organic amendments have compositions that may stimulate the growth of microbiota in the soil. This might be as a result of the presence of appreciable quantities of nitrogen and phosphorus in the organic stimulants used here, which are necessary nutrients for bacterial biodegradative activities. The reason for higher counts of bacteria in amended soil could also be due to the fact that the organic stimulants may have served as bulking agent which helped to loosen the compactness of the soil making sufficient aeration available for the indigenous bacteria present in the soil, thereby enhancing their metabolic activities in the contaminated soil.

Table 2: Total Heterotrophic bacterial count (THBC)

THBC (cfu/g)		
TR	Initial	After
1	1.7215E+12	2.13E+12
2	1.92E+12	3.335E+12
3	1.83E+12	5.04E+12
4	1.665E+12	4.13E+12
5	4.175E+12	3.315E+12
6	4.175E+12	4.705E+12

4.3 Total Petroleum Hydrocarbon (TPH) removal

The Total Petroleum Hydrocarbon for all the treatment options (TR1 to TR6) was determined before and after the bioremediation process, as shown in Table 4. These results showed a marked significant decrease in the TPH content reduction of the amended samples relative to the non-amended samples. The additional phosphorus and nitrogen contained in the supplements waste stimulated microbial growth and led to synthesized enzymes required to degrade petroleum hydrocarbon compounds (Vidali, 2001b). This also makes them capable of releasing linoleic acid, which can increase the bioavailability of poorly soluble petroleum(Yi and Crowley, 2007). The low percentage of crude oil degraded in the unamended samples showed the possibility of natural degradation which occurs rather slowly. This varied with the work of Akpe *et al.,* (2013) whose non-amended samples performed extremely well paralleling the amended samples in percentage crude oil degraded. This study also revealed that higher concentration of the pollutant (crude oil) in the soil reduced the rate of biodegradation because such high concentration could pose serious challenge to the metabolic activities of soil microorganisms.

Out of the six treatment options investigated, treatment option 3 (TR3) and treatment option 4 (TR4) resulted in maximum TPH removal. Treatment option 5 (TR5), in which the indigenous microorganisms were reduced by heat sterilization gave the least TPH removal. This observation is in line with previous work that the appreciable degradation obtained for

TR5 could be linked to the residual bacteria that resisted heat sterilization action. In addition, since all the treatments were humidified and aerated at equal rates, this residual bacterium would proliferate in the vessel and hence, degrade the pollutant (Abdulsalam *et al.*, 2012).

Table 3: Total Petroleum Hydrocarbon (TPH) Removal

Total Petroleum Hydrocarbon		
TR	Initial (%)	After (%)
1	39.50	2.664
2	35.20	3.996
3	35.63	0.414
4	39.50	2.997
5	36.80	36.63
6	36.80	35.29

V.

VI. 4.4 Oil and grease biodegradation

The Oil and grease content is an important index in measuring the extent of bioremediation in spent oil since the total petroleum hydrocarbon is low due to the increase in C-H bond in the oil (Abdulsalam *et al.*, 2011)

The oil and grease content of all treatments reduced after degradation which goes in line with all surveyed scenarios. However, most treatment studied here recorded lower percentage reduction compared to the norm obtained using organic waste (30-75 %) as reported by (Chaineau *et al.*, 2002). The highest oil and grease content recorded by the horse dung amended sample indicated the efficacy of horse dung in the bioremediation process.

Table 4: Oil and Grease Content

Oil and Grease			
TR	Initial (g)	After (g)	Removal Efficiency(%)
1	0.6	0.5	17
2	0.6	0.5	17
3	0.5	0.3	40
4	0.5	0.2	60
5	0.6	0.5	17
6	0.6	0.6	0

VII. CONCLUSION AND RECOMMENDATION

From the results obtained in this study, it showed that application of organic stimulants such as cow dung, camel dung, donkey dung and horse dung significantly improved the rate of petroleum hydrocarbon bioremediation of contaminated soil. The result shows a decrease in oil and grease content as exhibited by treatment option 1, 2, 3, 4,5. The treatment options 3 and 4 gave highest reduction in oil and grease content of 40 and 60% respectively. Hence, the treatment option 3 and 4 (cow dung and horse dung respectively) are the most recommended procedures for soil contaminated with used motor oil, giving it a high rate of bioremediation efficiency of contaminant. Furthermore, all the treatments except 5 and 6 gave appreciable reduction in total petroleum hydrocarbon with the highest reduction been treatment 3.

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