Vermi-Assisted Bioremediation of Used Engine Oil Contaminated Soil

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Abstract

Effect of earthworm inoculation on vermi-assisted bioremediation of spent engine oil contaminated soil was investigated. The rate of biodegradation of used engine oil was studied for a period of fourteen weeks under laboratory conditions. After 14 weeks, the control-sample (without earthworm or organic amendment), the bio-stimulated sample (with organic amendment but without worms) and the earthworm-inoculated sample (with organic amendment and earthworms) recorded 10.08%, 24.33% and 91.92% reduction in petroleum hydrocarbon content respectively. The co-application of organic amendment and earthworm is recommended for the acceleration of bioremediation of used engine oil contaminated soil.

Keywords: cassava peels, used engine oil, bacterial flora, kinetics, Eudriluseugeniae.

Introduction

Used engine oil refers to any lubricating oil that has served its purpose in a vehicle and is withdrawn from the meant area of application, being considered unfit for the initial intended purpose. Used motor oil may contain toxic materials that could contribute to chronic hazards including mutagenicity and carcinogenicity (Hagwell et al, 1992).

In some developing countries, used engine oil is discharged into the environment. In Nigeria for instance, about 20 million gallons of waste engine oil are generated annually from mechanic workshops and most of which is discharged into the environment (Faboya, 1997 & Adegoroye, 1997). According to USEPA (1996), a liter of used engine oil is enough to contaminate one million gallons of freshwater. Used engine oil also renders the environment unsightly and constitutes a potential threat to humans, animals, and vegetation (Adelowo et al, 2006). With the ever increasing use of petroleum products, environmental contamination with same becomes of increasing concern.

Bioremediation is the enhancement of live soil organisms such as fungi, bacteria and plant to break down hydrocarbon and organic contaminants. Bioremediation involves the transformation of complex or simple chemical compounds into nonhazardous forms by biological agents. It is a relatively cheap and effective means of cleaning the environment and involves the application of organisms and nutrients (Atlas & Bartha, 1992).

When environmental conditions (pH, toxicity, etc.) are corrected and microbes are acclimated, the bioremediation rate depends on the biodegradation rate of the oil, the supply of oxygen and nutrients, and the mass transfer of contaminants to the right environment (Li et al, 1995 & Koren et al, 2003). This mass transfer rate of oil is in general smaller than either the oxygen transfer rate or the biodegradation rate and therefore is the controlling step of bioremediation of oil-contaminated soil due to the strong hydrophobicity and low water solubility of these compounds. Earthworms have been used in bioremediation studies: Schaefer and Juliane (2007) studied the bioremediation of crude oil contaminated soil using different earthworm species and concluded that earthworms may trigger the degradation process and might therefore, be applied in the remediation of oil contaminated soil with moderate Total Petroleum Hydrocarbon (TPH) values. Schaefer et al. (2005) reported that earthworms can contribute positively to bioremediation of oil-contaminated soil, but that the effect may be species-dependent. Ameh et al. (2011) reported the survival of earthworms in used engine oil (from automobiles) contaminated soil. They reported that used engine oil concentration level tolerable to earthworms was dependent on the source of the used engine oil (the engine type). Ameh et al. (2012) indicated that vermi-assisted bioremediation would be more effective at elevated TPH values provided it be not lethal to the worms.

The epigeic earthworm species, Eudriluseugeniae commonly referred to as the West African night crawler, occurs all over the world but mostly in West African regions (Shagoti, 1985 & Segun, 1998). It is a composting worm largely found in West Africa; from Ghana to Nigeria to West Cameroun and Gabon. Thus, the research studied the effect of earthworm (Eudriluseugeniae) inoculation on the rate of bioremediation of used engine oil contaminated soil, spiked with fermented bitter cassava peels as organic amendment.

Materials and Methods

Procurement and pre-treatment of materials

Soil

Soil sample was collected from Ahmadu Bello University Zaria. The soil was crushed using mortar and sieved using 0.60mm sieve to remove stones and plant residues. The soil was analyzed for carbon, nitrogen and phosphorus contents using Walkley-Black (Walkley & Black, 1934), Kjedahl (AOAC, 1990) and Bray No 1 (Bray and Kurtz, 1945) methods respectively. The moisture content and water holding capacity of the soil was determined as described by Wilke (2005) and Srivastava and Thakur (2006) respectively.

Cassava peels

Cassava tubers (identified as Manihotesculenta, Crantz) were obtained from Wukari Local Government Area of Taraba State and peeled. The cassava peels were then soaked in water for a period of one week so as to eliminate cyanohydrin that may be present. This was followed by decantation of the water and subsequent drying, crushing and sieving (using 0.60mm sieve) of the fermented peels. 50 grams of the sieved fermented cassava peels were then used to analyse for its nitrogen, carbon and phosphorus contents.

Used engine oil

Used engine oil was obtained from a motorcycle mechanic workshop. The choice of motorcycle used engine oil was as a result of the higher earthworm survival rate reported by Ameh et al. (2011). Two drops of the engine oil were added to 20ml of dichloromethane in a sample bottle and analysed using a GCMS machine (GCMS-QP2010 Plus Shimadzu, Japan).

Earthworms

200 earthworms (identified as Eudriluseugeniae, Kinberg), were collected from Samaru village, Zaria. The worms were collected along with the soil in which they were found and kept for 24 hours before use. Prior to introduction into used engine oil contaminated soil, the worms were conditioned: they were washed and kept for 24 hours on moist filter papers so as to void their gut contents.

Preparation of sample

500g of the sieved soil ($\leq$ 0.6mm) was measured and placed into a transparent bowl. 20g of engine oil was added and thoroughly mixed. 20g of the prepared fermented cassava peels was also added and again thoroughly mixed. 150ml of de-ionized water was added to make 60% of the water holding capacity. The content of the bowl was again homogenised manually. 20 adult worms (with clitellum) were added to the sample after which the sample was weighed. The sample (called S1) was covered with a perforated material to allow the inflow of air into the sample but prevent the worms from escaping. The elemental analysis of the soil sample (S1) was determined using EDXRF (MiniPal PW4025, Philips Analytical). A second sample (S2) was prepared with the same content as before but without earthworms. The third sample (S3) called the control was prepared without earthworms and organic amendment but with all other parameters (quantity of soil, used engine oil and water) same as the two previously described samples. All samples were prepared in duplicates and monitored for 14 weeks. All samples were maintained at 60% water holding capacity by weighing every three days to determine the loss in weight and then adding make-up de-ionized water where necessary.

At the start (day zero) and after every 2 weeks, the TPH of each of the three samples was determined using the toluene cold extraction method. The TPH of the soil samples was determined using the toluene cold extraction method (Adesodun and Mbagwu, 2008). 5gm of soil from mechanic workshop was placed in a 200 milliliters beaker into which was added 50 milliliters of toluene. The mixture was manually stirred continuously for 30 minutes, left to stand in a fume cupboard for 2 hours and afterwards filtered using Whatman No 42 filter paper. The residue (soil) was allowed to dry in an oven at 50oC. The TPH was computed as:

$$\text{TPH (g/kg)} = \frac{\text{(initial weight of soil - weight of soil after solvent extraction in g)}}{\text{(initial weight of soil in kg)}}$$

The pH of all samples were determined after preparation. In doing this, 4.0g of soil samples were mixed with 10cm3 of de-ionized water in a beaker and properly stirred. A pH meter was then used to determine the pH.

Microbial count and identification

After the fourteen week treatment period, 10g of the soil sample was dissolved in 90mls of 0.1% peptone water (1:10). Ten folddilution was then prepared using a sterile pipette i.e. 1ml of 104 dilution was aseptically transferred into sterile plate of nutrient agar. With the cold sterile glass rod, the inoculums (0.1ml) was spread on the surface of the culture media (Nutrient agar). The inoculated plates were then incubated at 37^{0}C for 24 hours. The number of bacterial colonies were counted and recorded as colony forming units per ml (cfu/ml). Total colony forming unit per ml was expressed as total number of colonies counted multiplied by the dilution factor and then divided by the volume of the inoculums.

The isolates were gram stained and the gram reaction observed (Talaro & Talaro, 2002). Colonies to be identified were picked from the nutrient agar plates and maintained on slants of the same medium. These were subsequently used for various biochemical tests which include: mortality test, coagulase, catalase, citrate utilization, methyl red vogesproskaner, and indole tests.

Results and Discussion

Physicochemical property of soil and fermented cassava peels

The carbon, nitrogen and phosphorus contents of the soil and fermented cassava peels are presented in Table 1.

Table 1: *Physicochemical properties of soil and manure*

Sample	Nitrogen %	Carbon %	Phosphorus PPM
Soil	2.8	2.3	721.7
Fermented cassava peels	8.23	38.30	390.00

The phosphorus content of the fermented cassava peels (721.7ppm) was much lower than the 3233ppm reported by Aro et al. (2010) for cassava peels but similar to those reported by Aro et al. (2008) for unfermented cassava peels.

GCMS Analysis of used engine oil

Figure 1 presents the GC result of the used engine oil and Table 2 presents the result of MS comparison of the chromatogram.

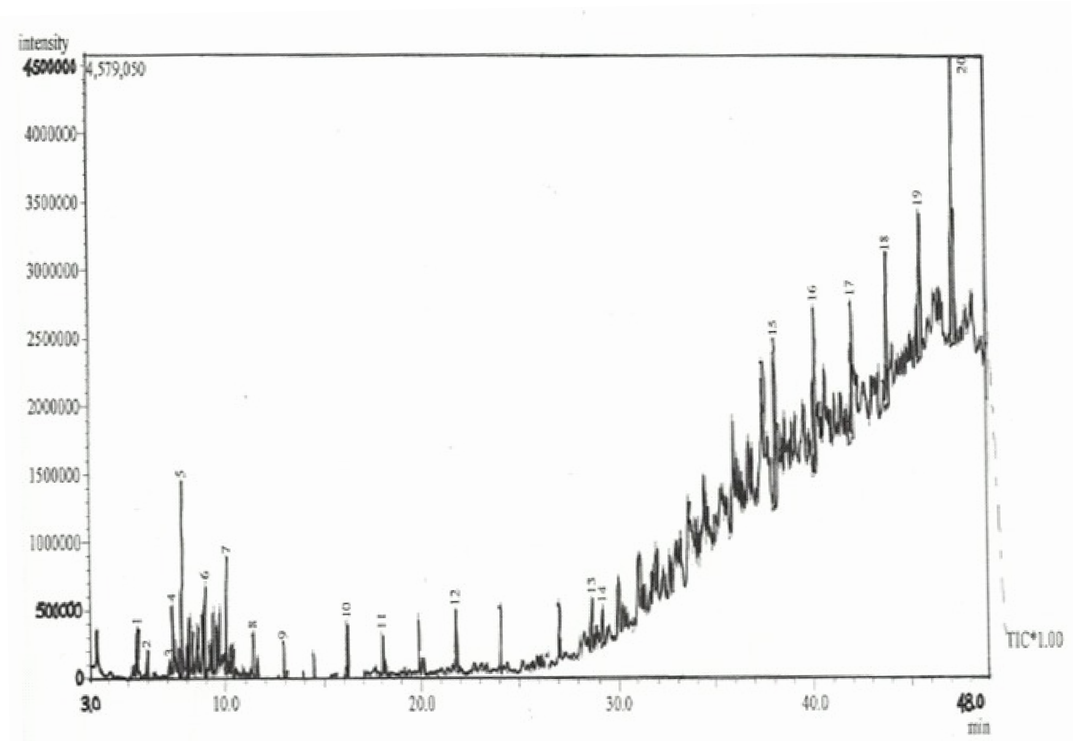

***Figure 1**: GC of used engine oil*

***Table 2**: Composition of used engine oil GCMS analysis*

Components	Composition %
C9 - C19	15.8
C20 - C29	28.2
C30 - C54	56.00

The composition of the used engine oil shown in Table 2 is consistent with Dominguez-Rosado and Pichtel (2003) that used motor oil may contain a broad range of aromatic and aliphatic hydrocarbons with chain lengths ranging from C 15 to C50.

Elemental composition of used engine oil contaminated soil

Table 3 presents the elemental composition of soil before and after contamination with used engine oil. The table shows that the concentration of heavy metal in the soil was significantly affected by the introduction of used engine oil at the contamination level considered (1g oil/25g soil). However comparison of the metal content with the regulatory set limits reported in Ameh et al. (2011) indicates that only chromium was in excess of the limits. Heavy metal content of soil was analysed because it has been reported to be important when considering bioremediation (Vidali, 2001).

Table 3: *Composition of used engine oil contaminated soil (XRF)*

Element	Composition by %	
	Uncontaminated Soil	***Used Engine Contaminated Soil***
Al	0.151	0.022
Si	32.977	30.782
K	3.420	4.482
Ca	0.461	0.479
Ti	0.911	1.198
V	0.041	0.051
Cr	0.043	0.075
Mn	0.060	0.062
Fe	5.202	8.111
Ni	0.009	0.050
Cu	0.010	0.062
Zn	0.004	0.042

pH of bioremediated samples

Table 4: presents the pH values of the samples at the start and finish of the bioremediation process.

Table 3: *pH value of bio remediated sample*

Sample	PH	
	Day 0	***Day 56***
S1	6.8/27°C	6.35/27°C
S2	6.8/27°C	6.80/27°C
S3	7.1/27°C	6.34/27°C

The pH values of all samples in Table 4 fall within the literature range of 6.5 8.0 optimum pHs, for microbial activity (Vidali, 2001).

Progression of TPH with time

Table 5 presents the total petroleum hydrocarbon content of the three samples with time.

Table 5*: Reduction in TPH with time*

Time (weeks)	TPH (g/kg)		
	S1	***S2***	***S3***
0	38.50	38.5	38.5
2	22.00	34.8	35.3
4	10.60	32.4	35.1
6	5.00	30.5	34.9
8	4.90	30.4	34.6
10	2.45	15.05	17.28
12	3.75	30.09	34.61
14	3.11	29.13	34.62

Table 5 shows that the organic amendment treated and earthworm treated sample (S1) recorded a progressive drop in TPH during the 14 weeks study period. The % drop in TPH at week 14 stood at 91.92%. The sample with organic amendment but without earthworms (S2) as well as the sample without worms and without organic amendment (S3) recorded progressive drops in TPH with time. However, for S2 and S3 the rate was much slower as indicated by thedrops of 24.33% and 10.08% respectively. These results show the catalytic effect that earthworms (E. eugeniae) have on the degradation of used engine oil in soil. Earthworm inoculation accelerated PH degradation and may be recommended for use in the ex-situ bioremediation of used engine oil contaminated soil. It may therefore be theorised that the presence of earthworms leads to an increase in the solubilisation of petroleum hydrocarbon, considered to be the rate limiting step by Li et al. (1995). The results of this work however do not show the mechanism of earthworm assisted bioremediation, of which understanding is important for optimization.

Microbial identification

Table 6 presents the result of the biochemical investigations to identify the microorganisms in the earthworm/organic amendment treated contaminated soil (S1)

Table 6: *Identification of microorganism*

	Gram reaction	Shape	Indole	MR	VP	Citrate	Catalase	Coagulase	Organism
1	-	Rod	+	+	-	-	-	-	*E.coli*
2	-	Rod	-	-	-	-	+	-	*Pseudomonads Aerogenosa*
3	+	Rod	-	-	-	-	+	-	*Bacillus Cereus*
4	+	Rod	-	-	-	-	+	+	*Staphylococcus aureus*

The organisms identified E. coli, Bacillus sp, pseudomonas sp and staphylococcus sp. Staphylococcus sp. are known to produce biosurfactants of interest for application in petroleum hydrocarbon contaminated sites. Therefore, observed catalytic effect of Table 5 was probably a combined effect of earthworm action and microbial activities.

Conclusion

In the fourteen-week study period, the percentage soil clean-up of used engine oil contaminated soil in the soil sample treated with organic amendment and earthworm was found to be 91.92%. The contaminated soil sample treated with organic amendment only recorded 24.34% reduction in petroleum hydrocarbon while the control (with neither organic amendment nor earthworm) recorded 10.34% reduction in petroleum hydrocarbon concentration. Hence, with respect to petroleum hydrocarbon degradation, the use of earthworm offers a means of accelerating the bioremediation process.

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