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Bioremediation of Soil Microcosms from Auto-Mechanic Workshops

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ABSTRACT: Microbial activities are essential for the restoration of soil contaminated with hydrocarbons, their roles includes biotransformation and mineralization of petroleum products into harmless compounds. In this study eighty six soil samples from fifteen auto-mechanic workshops within Ilorin metropolis were collected. These were analyzed by selective enrichment technique, resulted in the isolation of five bacterial species which are Acinetobacter sp, Flavobacterium sp, Pseudomonas sp., Serratia and Bacillus sp. Bioremediation of the soil microcosms was designed for 2months using mixed culture of the isolates. TVC, Protein, Dehydrogenase and lipase activities were used as bioindicators for the bioremediation processes. Highest TVC, Protein and Dehydrogenase activities were recorded in four week as 163.15µgTPFg^{-1}soil,6.3×10^{7}and5.53mg/g were obtained respectively at pH of 7.17 and gradually declined while lipase activities and percentage of oil was highest in week six as 65.41% and4.72unit/g. These findings have environmental implication towards developing a bioremediation protocol that could be exploited for cleaning oil polluted soil *@JASEM*

***Key words**: Bioremediation, automechanic workshop, Bioindicator, Hydrocarbon, Selective enrichment.*

Oil released in to the environment is a well recognized problem in today's world. Oil spills affect many species of plants and animals in the environment, as well as humans (Pohl *et al*., 2002;Yakubu, 2007)

Spent engine oil is a common and toxic environmental contaminant not naturally found in the environment (Dominguez-Rosado and Pichtel, 2004), large amount of them are liberated into the environment when the motor oil is changed and disposed into the soil which is a common practice by motor mechanics and generator mechanics including small scale engine oil sellers along the road. (Odjegba and Sadiq, 2002;Achuba and Perehemo-Clarke,2008). The oil is also released into the environment from the exhaust system during engine use and due to engine leaks (Anolieto and Edegbai, 200; Osubor and Anoliefo, 2003).

The illegal dumping of used motor oil is an environmental hazard with global ramifications (Blodgett, 2001). The release of oil into the environment causes environmental concern and attracts public attention (Rolling *et. al.*, 2002). Used motor oil contains aromatic hydrocarbons (P A Hs) that could contribute to chronic hazards including mutagenicity and Carcinogenicity (Keith and Telhard, 1979; Hagwell *et al*.,1992; Boochan *et al.,* 2000; Lin and Mandri, 2007).Petroleum products such as engine oil, petrol, diesel and kerosene are used daily in various forms in mechanic workshops. These products tend to harden and change the colour of the soil, which have untold health hazard on technicians and artisans (Udeani *et al*., 2008).

Bioremediation makes use of indigenous oil-consuming microorganisms, called petrophiles by enhancing and fertilizing them in their natural habitats. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a food source (Harder, 2004). Microbial remediation of a hydrocarbon contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil.These microorganisms can degrade a wide range of target constituents present in oily sludge (Barathi and Vasudevan, 2001; Mishra *et al*., 2001; Eriksson *et al*., 1991) there by, can be used in cleaning up contaminated sites (Alexander, 1999; Lin and Mandri, 2007).

Many studies have been conducted to isolate and characterize hydrocarbon degrading bacteria from auto mechanic workshops' soils but little or no work has been done to determine the bioremediation potential of the isolated autochthous bacterial consortium by measuring the percentage oil loss and their enzyme activities as a monitoring tool for bioremediation process which is the major focus of this work.

MATERIALS AND METHODS

Collection of Samples: Fifteen random spots from different auto-mechanic workshops about 15cm apart to a depth of 30cm were augured and bulked together and put in well labeled black polythene bag and transported to the laboratory for analysis.

Isolation of Microorganisms: Selective enrichment technique was used for the isolation of hydrocarioclastic bacteria using Bushnell-Haas medium containing 0.3% yeast extract supplemented with 10% V/V oil substrates. The oil substrates represent equivalent mixture of Gasoline, Engine oil, Diesel oil and spent engine oil (Katarina 2005).

Ten grams of oil contaminated soil was inoculated into the medium and incubated for two weeks. Bacterial species were isolated by spread plate technique using oil aliquots of appropriate dilution unto PCA and incubated for 48 hours. Individual colonies were identified by morphological

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and biochemical techniques using Cowan, 1974; Gerhardt, *et al*., 1981; Holt, *et al*; 1994 and taxonomic scheme of Bergey's Manual of Determinative Bacteriology (1994).

Inoculation of Soil Microcosms: Hundred grams of auto-mechanic workshops soil contained in cotton wool plugged conical flasks (500ml) in triplicates was autoclaved twice at 121^{o}c for 30mins to sterilize it completely. Mineral Salts medium (MSM) Bushnell-Haas Medium supplemented with 0.3% of yeast extract all dissolved completely in soil extract. In this study MSM was used as fertilizer to adjust the moisture content.

A single colony of each of the isolates was inoculated into 10ml of nutrient broth incubated at 30^{o}C overnight. The overnight culture was washed twice and resuspended in MSM until OD $_{600}$ was equivalent to 0.1. A 10% (V/V) of bacterial inoculums (0.1=OD_{600}) equivalent was used to inoculate the soil microcosms and incubated at room temp. Total Viable Count (TVC), Protein, Dehydrogenase, pH and lipase activities of the soil microcosm were determined every week for eight weeks.

Total Viable Count: The mean total aerobic bacteria present in the samples were determining at weekly interval using spread plate method with Plate Count Agar (PCA) medium. A ten fold dilution using phosphate buffer dilution saline and 0.1ml of appropriate dilution was plated in triplicates, incubated for 24-48 hours at room temperature after which the colonies btw 30-300 colonies were counted.

Total Protein Estimation: For the estimation of total protein, lml supernatant without any particle was taken from the soil and 9ml water was added. It was centrifuged at 13000 rpm for 10mins and to the pellet obtained and added l min of 3N NaOH Solution and boiled for 3mins. after cooling at room temp. l min of I M H_3PO_4 solution was added. A 50ml aliquot was taken and mixed with 950µl Coomassie protein assay reagent and incubated at 30^{o}C for 10mins and the optical density was measured at 595min using UV-Visible spectrophotometer. The total protein was estimated using a standard curve prepared with albumin (Bradford, 1976).

Dehydrogenase Activity: To 6g of soil sample, 30mg glucose, Iml of 3% TTC (2,3,5-Triphenyltetrazolium Chloride) solution and 2.5ml pure water were incubated for 24hours at 37^{o}C. The formation of TPF (1,3,5 triphenyformazan) was determined spectrophotometercally at 485nm and result were expressed as µgTPFg^{-1} soil sample (Pepper et al., 2004).

Lipase Activities: Method of Duye *et al*., (1984) as reported by Ugochukwu *et al*., 2008 was adopted with little modification, briefly, one hundred milliliters of phosphate buffer, pH 7.25 was added to 10g of soil and homogenized gently. The soil suspension was filtered using Muslin cloth. The filtrate was centrifuged at maximum speed of 7000g for 10mins to obtain supernatant. A quality of 3ml each of the supernatant was used as source of crude enzyme solution.

Lipase Activity: Was measured by titrating the fatly acid released with 0.1M NaOH using 0.l% alcoholic phenolphthalein as indicator. Fresh palm oil was used as the source of glyceride. The oil (0.5ml) was taken in a glass stopper Erlenmeyer flask and 10ml of acetate buffer (0.107M) and 1ml of hexane were added. The contents were stirred for 5mins, and then 1ml of the enzyme solution was added. The set up was allowed to stand for 20mins. Twenty milliliter of ethyl alcohol was added. The liberated fatty acid was titrated against 0.1ml NaOH. The determination of the blank was carried out in the only difference that the enzymes solution was added after 20mins. The activity was expressed in arbitary units.

*Determination of % of oil loss and pH***:** The oil content was determined by gravimetric method (Ijah and Antai, 2003) after extraction with carbon tetrachloride, values obtained were expressed as % of the amount of oil in the sample. To determine the pH, 10g of the soil sample was mixed with 25ml of sterile water in a beaker stirred and allowed to stand for 30mins; the pH was then taken with the pH meter

RESULTS AND DISCUSSION

Auto mechanic workshop is noted for high indiscriminate dumping of waste engine oil and other refined petroleum products as a result of their activities ranging from servicing, maintenance and repair of auto mobiles. Soil samples connected from difference spot in mechanic workshops characterize by intense oil spillage, blackish and devoid of plant growth. Selective enrichment technique for the isolation of hydrocarbonoclastic bacteria resulted into distinct species of *Acinetobacter* sp, *Pseudomonas* sp, *Flavobacterium* sp, *Bacillus* sp, and *Serratia* sp which were found to be prevalent highest percentage of oil loss and lipase activities were recorded after week six at pH of 7.34 while highest Dehydrogenase activities, total viable count, and protein content were recorded in week 4 at pH 7.17 as shown in Table 1.

The auto-mechanic workshop soil samples collected were oily dark in colour which may be due to long term exposure of the soil to waste engine oil discharged and accumulated. The presence of higher oil degrading bacterial populations in contaminated soils agrees with the results of ;(Michlcewice,1995; Hubert *et al*.,1997; Ndip et al, 2008).In this present

work five general of bacteria isolated were *Acinetobacter* sp, *Pseudomonas* sp, *Flavobacterium* sp, *Serratia* marcescen and *Bacillus* sp. which was blended together to make mixed culture. The mixed culture used in this research work confirmed microbial consortia as better degraders as reported by several authors (Obire, 1988; Amund and Nwakaye, 1993; Facundo *et al*,2001; Kulwadee *et al*.,2001; Ndip *et al*., 2008)

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Table 1: Showing response of bacterial consortium with respect to protein, pH, dehydrogenase and lipase activities in soil microcosms from auto-mechanic workshops within a period of eight weeks

Parameters	1st	2nd	3rd	4th	5th	6th	7th	8th
Oil loss (%)	4.03	20.17	22.14	38.40	45.14	65.41	71.12	40.21
pH	5.82	6.23	6.84	7.17	7.24	7.34	7.47	7.50
DHA ($\mu gTPF^{-1}$ soil)	49.64	87.94	94.49	163.15	152.80	147.20	121.73	100.21
Lipase (Unit/g)	0.63	1.72	1.96	2.76	3.05	4.72	4.93	4.55
TVC (cfu/g)	$4.8x10^3$	$8.8x10^3$	$6 x10^5$	$6.3x10^7$	$3.5x10^7$	$7.3x10^6$	$8.9x10^4$	$2.5x10^4$
Protein (mg/g)	1.57± 0.04	1.73± 0.1	3.12± 0.12	6.53± 0.1	5.21± 0.01	5.01± 0.23	4.02± 0.03	3.98± 0.1

In a mixed culture, some species utilizes intermediates of degradation of the original hydrocarbon produced by other members of the culture leading to a complete degradation of the oil (Atlas, 1981; Facundo *et al*., 2001; Ndip *et al*., 2008). The utilization of the oil resulted in increase in both total viable count, protein and dehydrogenase activities evidenced from the reduction in pH of the soil confirmed by chemical changes of the hydrocarbon substrates which must have caused by microbial enzymes. Atlas and Bartha, 1972; Oboh *et al*., 2006 Alkhavan sepahi *et al*.,2008. Microbial degradation of hydrocarbon, often leads to production of organic acids and other metabolic products. (Nwachukwu and Ugoji, 1995; Okpokwasili and Jane 1995) thus acids probably produced accounted for the reduction in pH levels. The increase in soil dehydrogenase activity may be as a result of increase in total microbial respiratory rate. It reflects the total range of oxidative activity of the isolates and consequently represents a good indicator of microbiological activity in the soil microcosm (Skujins, 1973).

Dehydrogenase was found to be synthesized in proportionate to total viable count and protein content while percentages of oil loss correlate with lipase activities when biodegradation process decline. The indicator of lipase may be attributed to the appearance of products released from oil degradation, which may be substrate for lipase (Margins and Schinner, 2005). Soil lipases can be used in monitoring bioremediation of hydrocarbon (Margesin *et al*., 1999). Dehydrogenase, catalases and urease have been found only to be useful for indicating the onset of the biodegradation process as their activities decline after the rate of biodegradation has decreased (Frankenberger and Johanson, 1982; Janke *et al*., 1992; Vanderwarde *et al*., 1995; Margesin and Schinner 1997; Maila and Cloete, 2005). The enzyme activity is regulated through product inhibition, covalent modification and feedback inhibition (Brock and Madigan, 1991). Though, not all enzymes are synthesized by a cell in the same amounts; some enzymes are present in far greater number than others which accounted for the differences in the synthesize of dehydrogenase and lipase and that the required amount of hydrocarbons in the soil that can induce the necessary enzymes to bring about the metabolisms of specific hydrocarbons not known (Maila and Cloete, 2005).

Conclusion: The findings of this research work has proven that the selected bacterial consortium can be optimized for the cleanup of oil spills and that the enzymes used can serve as bio indicator for monitoring bioremediation of oil spills.

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