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Biodegradation of crude oil in soil amended with groundnut shell

O.P.Abioye*, U.J.J.Ijah

Department of Microbiology, Federal University of Technology, P M.B. 65 Minna, (NIGERIA)

E-mail: bisyem2603@yahoo.com

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ABSTRACT

The use of groundnut shells in reclaiming crude oil spilled soil was studied. The groundnut shell was found to contain nitrogen (1.20%), phosphorus (18.50 ppm) and hydrocarbon degrading bacteria. The results of bioremediation studies revealed that the counts of crude oil degrading bacteria (CDB) in oil polluted soil amended with groundnut shells was about 100% higher than that of unamended polluted soil. The crude oil degrading microorganisms identified were species of *Micrococcus, Bacillus, Acinetobacter, Penicillium, Aspergillus and Fusarium.* The rate of crude oil loss (biodegradation) in soil was higher in oil polluted soil amended with groundnut shell (60.90%) than that of unamended soil (44.20%). The rate of biodegradation of crude oil in the amended soil was significantly different (P >0.05) than the unamended soil. The results of this study indicate that groundnut shell can be useful in reclaiming oil polluted soil.

KEYWORDS

Microbial breakdown;
Crude oil;
Polluted soil;
Amended.

INTRODUCTION

Oil spill both on land and in water have been a problem since the discovery of oil as source of fuel. They can have devastating effects on the biota of an environment. Oil spills and wastes discharge into the sea from refineries, factories or shipping contains poisonous compounds that are potential danger to plants and animals. The poisons can pass through the food chain of an area and may eventually be eaten by humans[1]. Petroleum hydrocarbons released into the environment can pose risk to ecosystems and human health. Some compounds in petroleum products are known to be mutagenic and carcinogenic[2].

Microbial degradation of crude oil as a means of clearing oil spills in the natural environment is a slow process and therefore, stimulated biodegradation through microbial seeding, application of fertilizer, tilling and liming (if the soil is acidic) or a combination of all these methods may be the answer[3,4]. Various materials have been used to rehabilitate oil-polluted environment. These include chicken droppings, and periwinkle shell[5,6]. Positive effects of nitrogen amendment on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated[7,8].

Agricultural wastes such as groundnut shell are abundant in the Nigerian environment and constitute waste disposal problem. They are cheaper to obtain

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than inorganic fertilizer; therefore it may be necessary to utilize groundnut shell to enhance biodegradation of oil spilled soils.

The aim of this study was to determine the potential of groundnut shells in enhancing microbial activities for petroleum degradation in soil.

MATERIALS AND METHODS

Collection of samples

Ubefan crude oil (Nigerian light crude oil) was collected from Kaduna Refining and Petrochemical Company, Nigeria. The oil was collected in sterile bottle and transported to the laboratory for use. The soil sample used for bioremediation study was collected from the farm of the school of Agriculture and Agricultural Technology (with no history of petroleum pollution), Federal University of Technology, Minna. The groundnut shells were collected from Bosso Town, Minna, Niger State, Nigeria. The groundnut shells were grinded into fine power with Nulux mills (Model RPM SR 400-061, Bombay, India) to pass through a sieve of 2mm mesh size.

Biostimulation studies

Two kilogrammes (2kg) of soil sample each was introduced into 3 different plastic containers (PC) labeled A to C. (PC) A and B was treated with 10% (w/v) of crude oil each. While (PC) labeled C had no crude oil and served as a control. (PC) A was treated with 400g of grinded groundnut shells. 200ml of sterile distilled water was introduced into each of the PC and the contents were thoroughly mixed and incubated at room temperature (28 ± 2^0C), the experiment was set up in triplicates. Periodic sampling of soil from each PC was carried out every seven days for the period of 28 days. The samples were analyzed for residual total petroleum hydrocarbon (TPH), changes in pH, moisture contents as well as total aerobic heterotrophic bacterial, crude oil utilizing bacterial and fungal counts.

Biodegradation of crude oil in soil

The amount of crude oil degraded in each soil sample was determined by the weight loss method of Bossert and Bartha[9]. This was done by suspending 10g of soil in 25ml of diethyl ether in a 50ml conical flask. This conical flask was shaken vigorously at 200 rpm for 1hour on an orbital shaker to extract the oil. The solvent - oil mixture was transferred slowly into a beaker. This was repeated until all the oil was extracted from the soil. The diethyl ether oil mixture in the beaker was filtered using Whatman No. 1 filter paper into a beaker of known weight. The diethyl ether was allowed to evaporate completely at room temperature (28 ± 2^0) in a fume chamber. The new weight of the beaker containing residual oil was taken and the percentage biodegradation of crude oil was calculated using the formula of Bento *et al*.[10].

Microbial counts and isolation

Ten grams (10g) each of samples were suspended in 90ml of sterile water and serially diluted. The diluted samples were plated on nutrient agar (NA), oil agar (OA) (1.8g K_2HP0_4, 4.0g NH_4Cl, 0.2g $MgS0_4.7H_20$, 1.2g KH_2P0_4, 0.01g $Fes0_4$. $7H_20$, 0.1g NaCl, 20g agar, 1% crude oil in 1000ml distilled water, pH7.4), and Potato dextrose agar (PDA) for the enumeration of total aerobic heterotrophic bacteria (AHB), crude oil utilizing bacteria (CUB) and fungi respectively. The NA and OA were incubated at 37^0c for 48 hours while PDA was incubated at room temperature (28 ± 2^0c) for 72 hours. Colonies which developed on the plates were counted and recorded as colony forming units per gramme of sample ($CFUg^{-1}$). The bacterial isolates were characterized after Gram staining and biochemical tests by comparing their characteristics with those of known taxa as outlined in Bergey's manual of systematic bacteriology[11]. The fungi isolates were characterized based on macroscopic and microscopic examination[5] and identified using the scheme of Alexopoulos and Mims[12].

Determination of pH of samples

The pH of the samples was determined using pH meter (crison micro pH 2000 model). Five grammes (5g) of the samples were suspended in 25ml of distilled water and mixed well. The pH meter was standardized at pH 7.0 using phosphate buffer solution after which the pH of the samples were determined in triplicates.

Utilization of crude oil by microbial isolates

The ability of the microbial isolates to grow on and utilize crude oil as a source of carbon and energy was determined by the method of Okpokwasili and

Okorie[13]. For bacterial isolates 0.1ml of 24 hours old nutrient broth grown culture was inoculated into each test tube containing 10ml of sterile mineral salts medium of Zajic and Supplisson[14] and 1 percent (v/v) crude oil. Control tubes containing 10ml of mineral salts medium plus 1 percent (v/v) of crude oil, but with no added organisms was also set up.

Fungal ability to grow on crude oil was tested by inoculating 0.1ml of fungal spores into 10ml of Bushnell and Hass[15] medium contained in test tubes. Control experiment was as well set up for fungi. Tubes inoculated with bacterial isolates were incubated at 30 ± 2^0c for 16 days while those of fungal isolates were incubated at room temperature (28 ± 2^0c) for 21 days under a stationary condition. At the end of incubation period, the growth of inocula was determined by visual observation of oil medium turbidity and each tube assigned (+) to (+++) depending upon the intensity of the growth.

RESULTS

The results (TABLE 1) show the microbiological and physiochemical properties of groundnut shells and soil used for bioremediation studies. The counts of aerobic heterotrophic bacteria (AHB) ranges from $4.2x10^4$ to $3.5x10^5$cfug^{-1}, counts of crude oil utilizing bacterial (CUB) ranges from $2.0x10^1$ to $3.0x10^2$cfug^{-1} while that of fungal counts ranges from $7.0x10^1$ to $1.5x10^3$cfug^{-1} for both groundnut shell and soil sample used for bioremediation. The physicochemical properties of groundnut shells and soil show variation in their values.

TABLE 1 : Microbiological and physicochemical properties of groundnut shells and soil used for bioremediation

Parameter	Value[a]	
	Groundnut shell	Soil
pH	6.47 ± 0.3	6.99 ± 0.3
Nitrogen (%)	1.20 ± 0.2	0.06 ± 0.01
Phosphorus (ppm)	18.50± 0.4	22.00 ± 1.0
Moisture (%)	1.20 ± 0.1	3.00 ± 0.2
Total aerobic bacteria	$4.2x10^4$ CFUg^{-1}	$3.5x10^5$ CFUg^{-1}
Crude oil utilizing bacteria	$2.0x\ 10^1$ CFUg^{-1}	$3.0x10^3$ CFUg^{-1}
Total fungi	$7.0x10^1$ CFUg^{-1}	$1.5x10^3$ CFUg^{-1}

[a] = Mean of triplicates

TABLE 2 shows the pH of polluted soil amended with groundnut shells. The pH of unamended polluted soil ranges from 7.37 to 8.21 that of polluted soil amended with groundnut shells ranges from 7.50 to 7.78 while that of unpolluted soil ranges from 7.4 to 7.73 within the 28days of the study.

TABLE 2 : pH of oil polluted soil amended with groundnut shells

Sampling Period (days)	pH Values[a]		
	A	B	C
0	7.37 ± 0.5	7.50 ± 0.4	7.14 ± 0.6
7	7.93 ± 0.1	7.54 ± 0.3	7.63 ± 0.5
14	8.07 ± 0.4	7.57 ± 0.5	7.73 ± 0.7
21	8.10 ± 1.0	7.78 ± 0.6	7.31 ± 0.8
28	8.21 ± 0.3	7.57 ± 0.5	7.29 ± 0.6

[a] = Mean of triplicates; A = soil + crude oil; B = soil + crude oil + groundnut shells; C = soil only

TABLE 3 shows the counts of aerobic heterotrophic bacteria (AHB) in polluted soil amended with groundnut shell. The counts of AHB in unamended polluted soil ranges from $2.0x10^6$ to $10.0x10^6$cfug^{-1} that of oil polluted soil amended with groundnut shells ranges from $12.0x10^6$ to $30.0x10^6$cfug^{-1} while that of unpolluted soil ranges from 25.0×10^6 cfug^{-1} to 40.0×10^6cfug^{-1}.

TABLE 4 shows the counts of crude oil utilizing

TABLE 3 : Counts of aerobic heterotrophic bacteria (AHB) in oil polluted soil amended with groundnut shells.

Incubation Period (days)	Bacteria counts (10^6 CFUg^{-1})		
	A	B	C
0	3.0	12.0	30.0
7	6.0	12.0	25.0
14	2.0	15.0	40.0
21	10.0	30.0	30.0
28	8.0	28.0	35.0

A = soil + crude oil; B = soil + crude oil + groundnut shells; C = soil only

TABLE 4 : Counts of crude oil utilizing bacteria (CUB) in oil polluted soil amended with groundnut shells.

Incubation Period (days)	Crude oil utilizing bacterial counts (10^5 CFUg^{-1})		
	A	B	C
0	2.5	2.0	3.0
7	2.0	4.0	2.2
14	1.0	6.0	2.0
21	4.0	8.3	2.0
28	3.5	12.0	2.5

A = soil + crude oil; B = soil + crude oil + groundnut shell; C = soil only

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bacteria (CUB) in polluted soil amended with groundnut shells. The counts of CUB in unamended polluted soil ranges from $1.0x10^5$ to $4.0x10^5$ $cfug^{-1}$ that of polluted soil amended with groundnut shells ranges from $2.0x10^5$ to $12.0x10^5$$cfug^{-1}$ while that of unpolluted and unamended soil ranges from $2.0x10^5$ to $3.0x10^5$ $cfug^{-1}$.

TABLE 5 also revealed the counts of total fungi in polluted soil amended with groundnut shells. The counts of total fungi in unamended polluted soil ranges from $6.0x10^1$ to $12.0x10^1$$cfug^{-1}$ that of soil amended with groundnut shells ranges from $7.0x10^1$ to $15.0x10^1$$cfug^{-1}$ while that of unpolluted soil ranges from $8.0x10^1$ to $18.0x10^1$$cfug^{-1}$.

TABLE 6 shows the rate of biodegradation of crude oil in soil amended with groundnut shells. The percentage rate of crude oil degradation in unamended soil ranges from 10.0 - 44.2 % degradation, while that of soil amended with groundnut shells ranged from 8.50 - 60.90

TABLE 7 shows the result of the potential of the microbial isolates to utilized crude oil as a source of carbon and energy. *Bacillus subtilis* and *Micrococcus sp*. utilized the crude oil at a higher rate compared to other bacterial isolates. Similarly *Pencillium sp*. utilized the crude oil at a considerably higher rate than other fungal isolates.

TABLE 5 : Counts of total fungi oil polluted soil amended with groundnut shells.

Incubation period (days)	Fungal counts (10^1 $CFUg^{-1}$)		
	A	B	C
0	12.0	7.0	15.0
7	8.0	9.0	13.0
14	6.0	8.5	16.0
21	10.0	12.0	18.0
28	6.0	15.0	8.0

A = soil + crude oil; B = soil + crude oil + groundnut shells; C = soil only

TABLE 6 : Rate of biodegradation of crude oil ion soil amended with groundnut shells

Incubation period (days)	Weight loss of crude oil (%)[a]	
	A	B
7	10.0 ± 0.5	8.50 ± 0.6
14	20.0 ± 1.2	34.80 ± 1.2
21	28.0 ± 1.0	52.20 ± 0.7
28	44.20 ± 1.4	60.90 ± 0.9

[a]Mean of Triplicates; A = soil + Crude oil; B = soil + crude oil + groundnut shells

DISCUSSION

Groundnuts shells and soil used for bioremediation in this study harbor different types of bacteria and fungi. The crude oil utilizing bacterial identified from this waste and soil were species of *Micrococcus, Bacillus, Pseudomonas* and *Acinetobacter.* These organisms have been implicated in crude oil biodegradation by several investigators[5,16-19]. However B*acillus* species was the predominant bacterial species isolated. This may be due to the fact that bacillus form spores which help the organism to survive harsh environmental conditions.

TABLE 7 : Utilization of crude oil by microbial isolates

Microbial isolates	Growth in crude oil medium
Micrococcus sp.	+++
Bacillus sp.	++
Pseudomonas sp.	++
Bacillus subtilis	+++
Bacillus megaterium	++
Acinetobacter sp.	++
Bacillus firmus	-
Aspergillus niger	+
Penicillium sp.	+++
Aspergillus flavus	++
Aspergillus sp.	+
Rhizopus stolonifer	-
Fusarium sp.	+
Saccharomyces sp.	+

+++: Heavy growth; ++: Moderate growth; +: Minimal growth; -: No growth.

The bacteria were able to utilize crude oil as a sole source of carbon and energy. *Bacillus* sp. and *Micrococcus* sp. degraded the crude oil at considerably high rates probably due to the fact that the organism has efficient degradative enzyme systems. The study also identified the following fungi species in the groundnut shells *Pencillium, Aspergillus, Fusarium* and *Mucor.* The fungi are similar to those isolated from chicken dropping and which had the ability to degrade crude oil[5].

The groundnut shells used in this study contained appreciable quantities of nitrogen and phosphorus. This might be due to the fact that it is a leguminous plant. These nutrients are necessary for microbial degradation of oil in the environment. Bioremediation studies

revealed that the rates of oil breakdown in the soil increased with time in soil amended with groundnut shell compared to that of unamended soil. This may be due to the nutrient (nitrogen and phosphorus) present in the groundnut shells which might have been easily released into the soil for use by the oil degrading microorganisms. It is also possible that crude oil degrading bacteria inherent in groundnut shells contributed to the biodegradation process.

The various bacterial isolates degraded the crude oil at varying degrees. It is clear from the results obtained that groundnuts shell is a good material for reclaiming crude oil polluted soil. Its use in reclaiming oil polluted soil might also to some extent solve the problem of solid waste disposal in the Nigerian environments.

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