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Biodegradation of Crude Oil in Soil Amended with Melon Shell

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Abstract

The potential of melon shell in speeding up the microbial breakdown of crude oil-spilled soil was examined. The melon shells were found to contain nitrogen (10.25%), phosphorus (17.33ppm) and hydrocarbon degrading bacteria. The results revealed that the counts of crude oil degrading bacteria (CUB) in crude oil polluted soil amended with melon shell were higher than unamended crude oil polluted soil. The rate of crude oil biodegradation in soil was higher in crude oil polluted soil amended with melon shells than unamended soil. The crude oil degrading bacteria identified in soil amended with melon shells are species of Bacillus, Acinetobacter, Micrococcus and Pseudomonas. Amendment of soil with melon shell raised the pH of the soil from 7.67 to 8.03 which implies that melon shells have buffering effect. The results of this study show that melon shells are efficient and can be used in reclaiming crude oil polluted soil.

Keywords*: Microbial breakdown, spilled oil, degrading bacteria, polluted soil, buffering effect, reclaim.*

Introduction

In Nigeria, oil pollution problem have been prevalent since the commencement of oil exploration and development of the petroleum industry (Okoh *et al.* 2001).Different methods have been used in restoring petroleum polluted soil, some of these include the use of oil degrading microorganisms, inorganic fertilizers, chicken droppings, periwinkle shell, liming and tilling (Leahy and Colwell 1990; Ijah *et al.* 2003a; and Ijah *et al.* 2003b).

Biostimulation using inorganic fertilizer has been extensively employed worldwide in reclaiming oil polluted soil (Jobson *et al.* 1974; Dibble and Bartha 1979; and Song *et al.* 1990 and USEPA 1990). However melon shell due to its high nitrogen content can be used to enhance biodegradation of crude oil in soil and at the same time solving the problem of wastes from the environment.

Treatment of oil contaminated soil is necessary to protect water supplies, human health and environmental quality (Chang *et al.* 1996), owing to the fact that plant derive nutrient for their living from soil, animals derive their from plants and human derive theirs from plants and animals living on soil. The objectives of this work were to determine the potential of melon shells in enhancing crude oil degradation in soil and to determine changes in physicochemical properties of soil during bioremediation of oil polluted soil.

Material and Methods

Collection and Processing of Samples

Nigerian light crude oil was collected from the Kaduna Refining and Petrochemical Company, Nigeria. The melon shells (brown and white melon shells) were collected from Paiko town, Niger State, Nigeria. The melon shells were grinded into fine powder with Nulux mills (Model RPM SR 400-061, Bombay, India) to pass through a sieve of 2mm mesh size. The soil sample used was collected from the farm of the School of Agricultural Technology, Federal University of Technology, Minna, Nigeria. The soil was sieved with 2mm mesh size before use.

Bioremediation Studies

Two kilograms of soil sample each was introduced into four different plastic buckets (PB) labeled A to D. The PB A to C were treated with 200ml of crude oil each, while D had no crude oil and serve as control. PB A and B were treated with 400g of grinded white and yellow melon shell respectively, 250ml of distilled water was introduced into each of the PB and the contents were thoroughly mixed and incubated at room temperature. Periodic sampling from each PB was carried out at seven days intervals for 28 days. The samples were analyzed for changes in pH, moisture, biodegradation of crude oil as well as microbial counts.

Biodegradation of Crude Oil

The amount of crude oil degraded in each soil sample was determined by the weight loss method of Bossert and Bartha (1984). This was done by suspending 3g of soil in 10ml of diethyl ether in a universal bottle and shaken vigorously to extract the oil. The solvent-oil mixture was transferred slowly into a beaker of known weight. This was repeated until all the oil was extracted from the soil. The solvent-oil mixture was exposed at room temperature overnight to allow the solvent to evaporate completely. The new weight of the beaker containing the residual oil was taken and the percentage of oil degraded was calculated (Ijah and Ukpe 1992).

pH Determination

pH of the sample was determined using pH meter (Crison micro pH 2000 model) on 1:2.5 (w/v) soil/water mixture.

Microbial Counts and Type

Bacteria and fungi were enumerated by spread plate technique by inoculating 0.1ml serially diluted sample unto nutrient agar (NA) plates and Sabouraud dextrose agar (SDA) plates respectively. The NA plates were incubated at 30^{o}C for 48 hrs. while SDA plates were incubated at room temperature for 72 hrs. The crude oil utilizing bacteria were enumerated on oil agar OA (1.8g K_2HPO_4, 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 1.2g KH_2PO_4, 0.01g $FeSO_4.7H_2O$, 0.1g NaCl, 20g agar, 1% crude oil in 1000ml distilled water, pH 7.4). The oil agar was incubated at 30^{o}C for seven days. The colonies which developed were counted and expressed as colony forming units per gram ($CFUg^{-1}$) of sample.

Isolated bacterial colonies were characterized based on their gram stain reaction and biochemical tests. The organisms were identified to the genus level using the scheme of Cowan (1974). The fungi isolates were characterized based on macroscopic and microscopic examinations (Ijah *et al.* 2003a) and were identified using the scheme of Alexopoulos and Mims (1979).

Determination of Utilization of Crude Oil by Bacterial Isolates

The method of Kokub *et al.* (1989) was used, 5ml of mineral salts medium of Zajic and Supplisson (1972) was dispensed into each universal bottles containing 0.05ml of crude oil and sterilized for 15 minutes at 121^{o}C, and the bottles were allowed to cool and inoculated with 0.1ml of nutrient broth-grown culture of crude oil degrading bacterial isolates. The bottles were incubated at room temperature (28±2^{o}C) for 21 days. The turbidity produced as a result of bacterial growth was monitored visually at the end of incubation period and assigned (+) to (+++) depending on the intensity of the growth.

Results and Discussion

Table 1 show the biological and physiochemical properties of melon shells used for bioremediation studies.

The results from Table 1 above revealed that both melon shells used has appreciable quantities of nitrogen and phosphorus 10.25 – 9.92% and 17.33 – 4.21 ppm, respectively, which are major nutrients required by bacteria to degrade organic substances from the environment (Dibble and Bartha 1979). The results of bioremediation studies with crude oil polluted soil amended with melon shells shows

that the counts of aerobic heterotrophic bacteria in unpolluted soil ranged from 2.0 x 10^7 CFU/g to 3.5 x 10^7 CFU /g of soil, that of polluted but unamended soil ranged from 1.0 x 10^7 CFU/g 1.5 x 10^7 CFU /g of soil. The counts in oil-polluted soil amended with brown melon shell ranged from 1.2 x 10^7 CFU /g to 4.2 x 10^7 CFU/g of soil, whereas that of oil-polluted soil amended with white melon shell ranged from 1.3 x 10^7 CFU /g to 3.4 x 10^7 CFU /g of soil (Table 2). The results indicates an increase in the counts of total aerobic heterotrophic bacteria in soil amended with melon shells, the results agrees with the findings of Westlake *et al.* (1978) and in sharp contrasts to the work of Ijah *et al.* (2003b).

Table 1. Biological and physiochemical properties of melon shells used for bioremediation studies.

Parameter	Yellow melon shell	White melon shell
pH	7.06	7.02
Moisture (%)	1.95	1.93
Nitrogen (%)	10.25	9.92
Phosphorus (ppm)	17.33	4.21
Organic carbon (%)	1.06	1.08
Total aerobic bacteria	6.0x 10^6 CFU/g	7.0x106 CFU/g
Crude oil utilizing bacteria	3.0x 10^2 CFU/g	4.0x10^2 CFU/g
Total fungi	6.0x 10^1 CFU/g	8.0x10^1 CFU/g

Table 2. Counts of total aerobic heterotrophic bacteria in oil polluted soil amended with shell.

Incubation period (days)	Bacteria counts (10^7 CFU/g)			
	A	B	C	D
0	2.0	1.5	1.2	1.3
7	2.4	1.0	1.4	1.6
14	3.5	1.1	2.0	2.8
21	2.6	1.4	4.2	3.4
28	2.9	1.5	3.0	3.1

Legend:
A = unpolluted control soil;
B = unamended polluted soil;
C = polluted soil amended with brown melon shell;
D = polluted soil amended with white melon shell.

Table 3 show the counts of crude oil utilizing bacteria CUB with soil amended with melon shells having higher counts of CUB than those of unamended and unpolluted control soil, this may be as a result of high nitrogen and phosphorus contents present in the amended soil. The crude oil utilizing bacteria isolates were identified as species of *Bacillus*, *Micrococcus*, *Pseudomonas* and *Acinetobacter* with *Bacillus* sp, been frequently isolated than other species. This is in agreement with the findings of different authors (Ijah 1998: Leahy and Colwell 1990; Bossert and Bartha 1984).

Table 3. Counts of crude oil utilizing bacteria in oil polluted soil amended with melon shells.

Incubation period (days)	Crude oil utilizing bacterial counts (10^4 CFU/g)			
	A	B	C	D
0	2.0	2.5	3.0	3.5
7	1.0	1.0	4.0	4.0
14	2.5	2.0	3.0	5.0
21	2.0	3.5	6.0	7.5
28	2.0	2.5	8.5	10.0

Table 4 also revealed the counts of fungi in both polluted and unpolluted soil. The counts of fungi in amended polluted soil and those of unamended polluted and oil free soil does not show any significant difference which indicates that the amendment does not have much effect on fungal growth. The fungi identified are species of *Aspergillus*, *Mucor* and *Rhizopus* with *Aspergillus* having frequent occurrence. This is in agreement with the findings of Ijah *et al.* (2003b).

Table 4. Counts of total fungi in polluted soil amended with melon shells.

Incubation period (days)	Fungal vounts (10^1 CFU/g)			
	A	B	C	D
0	16.0	13.0	7.0	10.0
7	14.0	9.0	6.0	7.0
14	17.0	8.0	10.0	6.0
21	19.0	10.0	13.0	12.0
28	10.0	8.0	16.0	14.0

Table 5. Utilization of crude oil by bacterial isolates.

Bacterial isolates	Growth in crude oil medium
Pseudomonas sp.	++
Micrococcus sp.	+++
Bacillus sp.	+++
Acinetobacter sp.	+

Legend:
+++ = Heavy growth;
++ = Moderate growth;
+ = Minimal growth.

The result on the ability of CUB isolates to utilize crude oil revealed that *Micrococcus* sp. and *Bacillus* sp. utilize crude oil at higher rate than other isolates (Table 5). This may be due to efficient hydrocarbon-degrading enzyme systems that these organisms posses.

The results of bioremediation study also show that the rates of oil breakdown in oil-polluted soil with time. In unamended polluted soil 45% of the oil was biodegraded after 28 days whereas in polluted soil amended with melon shells 75% of oil was degraded within the same period of time (Table 6). This may be due to high nitrogen and phosphorous contents in the melon shells which favor the growth of crude oil utilizing bacteria in the amended soil.

The pH of the polluted soil amended with melon shell was raised from 7.67 to 8.03 (Table 7). This may be one of the conditions that increase the rate biodegradation of crude oil in amended soil since crude oil degrading bacteria grow and utilize hydrocarbons better at slightly alkaline pH (Dibble and Bartha 1979).

Table 6. Rate of biodegradation of crude oil in soil amended with melon shells.

Sampling period (days)	Weight loss of crude oil (%)		
	A	B	C
7	16.0	20.0	20.0
14	20.0	25.0	37.5
21	40.0	50.0	50.0
28	45.0	75.0	75.0

Legend:
A = Unamended polluted soil;
B = Polluted soil amended with brown melon shell;
C = Polluted soil amended with white melon shell.

Table 7. pH of oil polluted soil amended with melon shells.

Sampling period (days)	pH Values			
	A	B	C	D
0	7.67	7.68	7.73	7.75
7	7.67	7.75	7.80	7.77
14	7.69	7.77	7.81	7.87
21	7.69	7.79	7.88	7.96
28	7.72	7.80	7.89	8.03

Conclusion

From the results of this study, it can be concluded that brown and white melon shell possess the ability to enhance the biodegradation of crude oil in soil by 30%, hence brown and white melon shell can serve as good materials for reclaiming crude oil polluted soil. Their use in reclaiming oil polluted soil will also solve the problem of solid waste disposal in the Nigerian environment.

References

Alexopoulus, C.J.; and Mims, C.W. 1979. Introductory mycology, 3rd ed., Wiley, New York, NY, USA.

Bossert, I.; and Bartha, R. 1984. The fate of petroleum in soil ecosystem. *In:* R.M. Atlas (Ed.) Petroleum microbiology, Macmillan, New York, NY, USA, pp. 435-73.

Chang, Z.; Weaver, R.W.; and Rhykerd, R.L. 199). Oil bioremediation in high and low phosphorus soil. J. Soil Contam. 5: 215-24.

Cowan, S.T. 1974. Manual for identification of medical bacteria. 2nd ed., Cambridge University Press, Cambridge, England, pp. 56-82.

Dibble, J.T.; and Bartha, R. 1979. Effects of environmental parameters on biodegradation of oil sludge. Appl. Environ. Microbiol. 37: 729-739.

Ijah, U.J.J.; and Ukpe, L.I. 1992. Biodegradation of crude Oil by *Bacillus* strains 28A and 61B isolated from oil spilled soil. Waste Management 123: 55-60.

Ijah, U.J.J. 1998. Studies on relatives capabilities of bacterial and yeast isolates

from tropical soil in degrading crude oil. Waste Management 18: 293-9.

Ijah,U.J.J.; and Antai, S.P. 2003. The potential use of chicken-drop micro-organisms for oil spill remediation. The Environmentalist 23: 89-95.

Ijah, U.J.J.; and Ndana, M. 2003. Stimulated biodegradation of crude oil in soil amended with periwinkle shells. The Environmentalist 23: 249-54.

Jobson, A.; McLaughlin, M.; Cool, F.D.; and Westlake, D.W.S. 1974. Effects of amendments on the microbial utilization of oil applied to soil. Appl. Environ. Microbiol. 27: 166-71.

Kokub, O.; Shafeeq, M.; Kahlid, Z.M.; and Malik, K.A. 1989. Isolation, screening and characterization of biosurfactant producing bacteria. *In:* K.A. Malik; S.H.M. Maqui; and M.I.H. Aleem (Eds). Proc. Int. Symp. Biotech. for Energy, 12-16 Dec. 1989, NIAB/NIBGE, Faisalabad, Pakistan, pp. 221-32.

Leahy, J.C.; and Colwell, R.R. 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54: 305-15.

Okoh, I.A.; Ajisebutu, S.; Babalola, G.O.; and Trejo-Hernandez. M.R. 2001. A Study of the potentials of *Burkholderia cepacill* strain (RQ1) in the biodegradations of heavy crude oil (Maya). Int. Microbiol. 4: 83-7.

Song, H.; Wang, X.; and Bartha, R. 1990. Bioremediation potential of terrestrial fuel spills. Appl. Enviro. Microbiol. 56: 652-6.

USEPA. 1990. Alaskan Bioremediation Project. Publication EPA/600/889/073, 1-21.

Zajic, E.; and Supplisson, B. 1972. Emulsification and degradation of “Banker C” fuel oil by microorganisms. Biotech. and Bioengin.14: 331-43.