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OIL POLLUTION ON SOIL ECOSYSTEM AND THE USE OF INDIGENOUS SOIL BACTERIA FOR BIO-REMEDIATION SYNTHESIS

[1]Okpara O.N and [2]Akpoturi Peters
[1]Department of Biochemistry, Federal University Otuoke, Bayelsa State
[2]Department of Petroleum Engineering, Federal University of Petroleum Resources, Effurun

ABSTRACT
A bioremediation strategy based on Azotobacter- seeding of oil polluted medium containing indigenous bacterial degraders was used to enhance the removal of the contaminating crude oil. In this study, biological nitrogen fixation was co- optimized with crude oil degradation. Increase in cell number for both Azotobacter (from 0.5 – 4.3 x 10^6 cells/ml) and the oil degrading bacteria from 0.3- 0.6 x 10^6 cells/ml) in 10 days indicated significant nitrogen fixation and oil degradation in the two bacteria respectively. The low percentage, remaining crude oil (20 - 30%) in the polluted mixed cultures within 14 days confirmed the bioremediation efficiency of the bacterium-Azotobacter consortium. In the field demonstration of this bioremediation and Biological nitrogen fixation processes in an oil polluted soil microcosm, increase in growth parameters of the planted sorghum cultivar was significant in the Azotobacter - seeded plots, relative to non-seeded portion. Hence this bioremediation strategy hold strong potential for a large-scale clean-up of oil-polluted degraded soils and other ecosystems in the natural environment.
Keywords: Soil, Crude oil, Pollution, fixation, bacteria, degraders, clean-up, ecosystem, bioremediation contamination and environment.

INTRODUCTION
For some time now pollution of air, land and water have been posing staggering and challenging problems to mankind. In fact, industrialization is the bane of all sorts of pollution and the adverse effects associated with it. It is a known fact, that about 55% of the world's present energy supply comes from crude oil, the liquid form of petroleum (Brown and Skipsey 2001), which is a mixture of many thousands of organic compounds, more than three quarters usually being hydrocarbons (Wittle et al, 1996). Refining of crude oil requires considerable transportation through pipelines from the oil-fields, with the consequent possibilities of environmental contamination and pollution through accidental spill. The details of the potential biological damage depends on where the spill occurs. The primary concern in petroleum contamination and pollution of soil has been its effects on ground water and the subsequent mopping up (Calabrese and Kostecki, 1988). However, the effects of crude oil on the germination and growth of some selected plants have

been reported (Klokk, 2001). Contamination of land is of paramount importance to man in that it is on this portion of the earth that the anvil for man's existence and activities lie. Land is the home of multitude of micro-organisms that are involved in the maintenance of adequate ecological cycle needed for survival of nature. It is well known, in oil mineral producing area of Nigeria, that crude oil damage the soil and prevent crop growth for varying periods of time. This damaging effects are due to suffocation and toxicity of the crude oil (Plice 1984). For example, mangroves are both susceptible and vulnerable to oil because they grow mainly in anaerobic mud environment (Odum and Johannes, 1995). Their respiratory surfaces arc readily clogged by oil which presumably kills the plant by blocking supplies to the roots (Baker, 1992). Accordingly, a mangrove community can take many years to recover from exposure to oil especially if death of the mangrove is preceded by erosion (Moriarty, 1983). Several observations made on actual oil spill on land due to pipeline blow-out revealed that the nature and extent of the damage done by the oil were influenced by the moisture content of the soil. During the rains, the oil mainly flowed on the soil surface and collect in pools where there were depressions, however, in the dry season, the oil soaked downward and saturate the soil depending on the amount spilled, soil porosity or permeability and topography Paraffinic oils penetrate soils more readily than asphaltic (heavy) oils. Apart from the suffocating and toxicity tendencies of crude oil to plants, it has been reported that nitrate formation was reduced. Murphy (1999) noted that even 1.0% (v/w) of oil when mixed with soil practically checked nitrate formation and this is inimical to soil fertility and crop production.

Restoration of the loss soil fertility of agricultural land previously polluted by oil (spill) is of paramount Importance. For example, Adeniyi et al (1993), observed that non-agricultural occupation among household heads (7%) in gbokprem area of Cross River State, Nigeria, was due to the poor physical condition of the soil after oil spillage. Odu (1998) reported an increase in bioremediation of oil-polluted soil after applying paraffin supplemented (nitrogenous) fertilizer (PSF) to the soil. Nitrogen and phosphorus are the most limiting nutrients to oil degrading bacteria (Odu l 998 Atlas and Bartha 1993). The relative importance of azotobacters as nitrogen-fixers in some ecosystems have been reported (Dicker and Smith, 1998; Pshenin, 1983). Several reviews (Gadgil and Bhide 1980 Jensen, 1999; of the literature on biological nitrogen fixation, have shown than Azotobacter when grown in association with some bacteria fixed more nitrogen than it did in pure culture.

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Also it has been shown that unavailable carbon source can be made available to Azotobacter by some bacterial activity (Jensen, 1999. This experimentwork therefore emphasizes co-optimization of BNF and bio-remediation of crude oil polluted soil through 'Mutualistic' association between oil-degrading bacteria and Azotobacter which is a free-living aerobic nitrogen-fixing bacterium in soils.

METHODOLOGY

(a) Azotobacter Isolation: The Azotobacter Sp used in this study was selectively isolated from the top 1cm sandy-loamy soil of study area (XYZ), using the modified Burk's nitrogen-free medium described by Newton et al (1953). The basal medium consists of the following salts (in g/l): K_2HPO_4, 064; KH_2PO_4, 0.16 $MgSo_4.7H_2o$, 020; Nacl, 020; $CaSo.2H_2o$, 0.05 and trace elements, molybdenum and iron added a $NaMoO_4$, l. omg; and $PeSO_4$, 3.0mg. The carbon source, mannitol (10.0g/1) was added to the mixture and then autoclaved at 1.5lbpsi for 15 minutes. The pH was adjusted to 7.4.

(b) Isolation procedure was essentially that described by Dicker and Smith (1995) except that the initial carbon source of the medium were 0.9g!l of mannitol and 0.lg/l of glucose, and a little amount of nitrogen source. About 1.0g of the soil sample was introduced into sterile test tube and the total volume made up to 10ml with this medium and incubated for 24 hours. On the second day, 1.0ml aliquots of the supernatants were dilutes to 10ml with fresh sterile Azotobacter medium containing only mannitol as the carbon source. At the fourth serial dilution (4th day), 0.1 ml of atmosphericin (10mg/l) was added before plating onto solid plates as described by Ibrahim (1986) Amphotericin forestalled the growth of fungi (Wilding, et al 1998).

(c) Characteristics of the Isolate- The properties of the isolate include the following, rods and irregular in shape non—motile Gram-negative, slightly yellow house-hold pigmented and strickly aerobic. It did not ferment carbohydrates nor reduced nitrate; no hydrolysis of gelatin, starch or lipids, nor coagulation of blood. The crude protein extract fixed atmospheric nitrogen as ammonia, the pH and temperature optima were respectively 65 - 8.0 and 27 - 306. By reference to Key to the species of Azotobacter' (Jensen 1999) the isolate was suspected to be mor' of Azotobacter vinekandii than . beiierinckii.

(d) Crude oil degrading bacteria: The four bacterial strains which were proven to be good degraders of Bonny Light crude oil were isolated from the Universitystudy area soil previously polluted by Simulation (insitu).

These strains were labeled (a) KKK2O, (b) PPCS21 (c) LLPS28 and (d) NS76D16. They were each grown to optical density of about 0,65 at 546nm, using Azotobacter medium amended with Kcl, (O.29glo) and $NaNO_3$ (o 42s/l). Mannitol served as the carbon source.

(e) Biodegradation and Nitrogen - fixation assays To determine the relative "In vitro' biodegradation and N_2- fixation potential of the bacterium/Azotobacter consortium in the mixed ulturs culture son training Bonny light crude oil, the following experimental steps were taken. Twenty ml of each oil degrading bacterium (1.6 x 10 cells/ml) were taken from various pure cultures at the exponential growth phase, and aseptically transferred into 16 flasks (4 x 4) To each was added 2.0ml of crude oil and 8.Oml each, of fresh degraders medium devoid of $NaNO_3$ and mannitol. The flasks were incubated at room temperature (28 ± 2°) with shaking in an orbital in an orbital shaker with knob at position 3. The growth was monitored in all the flasks by optical density measurement at 24 hourly intervals, at about the maximum OD. 20m1 of Azotobacter inoculrnn (4.0 10 cells/ml) at the exponential growth phase was introduced into 2 each of the 4 different degraders cultures (2 x 4). The remaining 8 cultures were made up to 5.Oml with sterile mixed culture medium, Prior to the seeding of the cultures with Azotobacter, the cell numbers of degraders in each were enumerated by the plate dilution frequency technique of Harris and Sommers (1998) using the appropriate agar plates. At this point also the crude oil concentration in each medium was determined by optical density measurement at 520nm. This was done by taking O.5m1 of the oiled culture and extracting the crude oil with CCL4. The OD was taken against a blank (CCL4 without crude oil). The percent concentration of the oil in the extract was then extrapolated from a standard curve constructed by plotting different percent concentration of the oil in CCL4 against their corresponding ODs at 520nm. Nitrogen content in each culture was also assayed from cell-free samples taken from each medium, by Kjeldah method. These determinations were continued at 24 hourly time intervals, except for the remaining crude oil determination which was repeated at the end of 14th day.

(f) Azotobacter growth in the mixed cultures: The growth of Azotobacter in each mixed culture was measured at 48 hourly intervals after its inoculation by the plate-dilution frequency technique of Harris is and Sommers (1998) in which enumeration of colony forming units (CFU) in solid agar plates were prepared by dissolving l .50g of agar powder in 100ml of Azotobacter liquid medium containing mannitol and sodium azide and then autoclaved at 151b psi

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for minutes before pouring. The inoculum was prepared by taking 0.1ml samples from the mixed cultures and then serially diluted (10-fold) with the basal Azotobacter medium contain sodium azide which is toxic to other bacterial growth. The plates were then inoculated with 0.01ml of the appropriate dilutions, using a 0.1-mi pipette graduated in hundredths. Plates were incubated for 9 days before colonies were counted and the number of cells in the mixed cultures determined by reference to appropriate tables (Harris and Sommers, l 998).

Microcosm Experiment; In a delineated area of the study area (20cm x 10cm in 5 places), oil spills were simulated as follows: In two 'plots", about 5% (v/w) of oil was evenly layered on each of the soil surfaces and one was evenly mixed to a depth of about 5.0cm with a trowel. Another 10% (v/w) of the oil was applied to each of another two plots as above. The control had no oil applied on it. The plots were left undisturbed for 3 weeks except for the sprinkling of distilled water on them at three intervals of 4 days. Orr tb 14th day, 100 ml of Azotobacter (approximately 108cells/ml) was seeded on one half of each plot and then left to stand for another 27 days. Thereafter, 50 viable sorghum grains, previously soaked overnight in distilled water, were planted in each plot (25 grains per half plot). Ten days later, the soil was carefully tilled and the grains gradually uprooted for the measurement of the growth variables which included the number of visible germination, number of leaves, the length of plumule and radical.

RESULTS AND DISCUSSION

The ecology of the azotobacters have been studied extensivery (Molder, 1995; jensen, 1999). This group of microorganisms are of special interest based on their wide spread and relative importance in contributing to the nitrogen economy of both soil and marine ecosystems (Dicker and Smith, 1998):. This present work equally showed that Azotobacter could contribute substantially to the nitrogen requirement of oil-degrading bacteria in the soil. As is shown in Figs. 1 and 2, the growth of crude oil degrading bacteria in the various oiled media that were deficient in nitrogen source, was enhanced by seeing the polluted medium with Azotobacter. Nitrogen is one of the limiting inorganic nutrients required by oil degrading bacteria (Odu, 1998; Atlas and Bartha, 1993) and this effect was manifested in Fig. l when the $NaNO_3$ became

exhausted. This deficiency in nitrogen source was ameliorated through BNF exemplified by Azotobacter (Fig.2).

The development of free-living nitrogen-fixing bacteria in- soils in favoured by the presence of high amount of "available' carbon compounds and very low level of combined nitrogen such that the C/N ratios are high. Crude oil contains high proportion of C/N ratio and when these were made available by the oil degrading bacteria, the growth of Azotobacter, and hence nitrogen fixation was enhanced (Fig. 3 and Table 1 respectively).. Biological nitrogen fixation (BNF) takes place under low redox potential in the soil. In a redox study of oiled soils (Plice, 1984), it was revealed that petroleum also lowered the cedox potential of the soil and this played a part in nitrogen fixation. In all the mixed cultures, there was significant growth of the microorganisms due to availability of carbon and nitrogen sources (Fig. 2 and 3). However the growths of Azotobacter in the mixed cultures were not consistent (Fig. 3), and this was due to changes in the growth environment (e.g. production of metabolic intermediates and $NaNO_3$ remaining before Azotobacter seeding), which may be stimulator)' or antagonistic to growth and BNF. The numbers of Azotobacter cells in each mixed culture ranged from 1.0- 3.2 x, 106 between the 8th and 14th days of incubation (Fig. 3). The occurrence of azotobacters in numbers to cells/g soil indicate that considerable amounts of nitrogen are fixed (Mulder, 1995), so that in this case, the Azotobacter seed contributed substantially to the nitrogen economy of the polluted medium (Table 1). The data in Table 1 equally showed that nitrogen fixation in the mixed cultures, as determined by Kjeldah analyses, was not consistent for the same reason explained for growth. There is a linear relationship between nitrogen fixation and cell proliferation (Jensen, 1999).

CONCLUSIONS

Attempts are often made to reduce or prevent wide scale damage of the environment by oil spill, and the strategy adopted depends on the type of oil spilt and the habitat endangered. Apart from doing nothing, which sometimes the best choice, we can physically remove, disperse with chemicals, sink or burn the oil. Any action or inaction usually has disadvantages. Ideally, contingency plans for dealing with oil spills should be discussed, agreed upon and rehearsed before the need arises. From the standpoint of this investigation, Azotobacter-seeding of oil polluted soils, after few weeks or months of the spill (depending on the extent) offers an interesting possibility

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of enhancing the contamination of the soil by the indigenous soil microorganisms. The low oxygen tension created by crude oil is somewhat advantageous to Azotobacters which exhibit maximal activity at low Po_2 value (4%) (Molder, 1995). The possibility of up scaling this bioremediation strategy to bulk dimension when a large expanse of land is polluted is therefore very promising. Apart from the envisaged cheapness, the process co optimizes bioremediation and biological nitrogen fixation which enhances soil recovery. At the moment, the biochemical oxygen demand (BOD) exertion curves of Azotobacter/oil degrading bacteria consortia in crude oil polluted microcosms are being modeled in our pollution control and biotechnology laboratory. This will enable a fairly accurate prediction of the time frame for a particular type of oil to biodegrde completely when seeded with a known Azotobacter/oil degrading-bacteria consortia.

REFERENCES

Adeniyi, E.G., Olosule, R. and Angaye. (1993), Environmental and socio-economic impacts of spillage in the Petroleum producing riverine areas of Nigeria. In "The Petroleum industry and Nigerian Environment", Proceeding of the 2003 NNPC Seminar, pp. 233-248.

Atlas, R.M. and Bartha, R. (1993). Biodegradation of in sea water; limiting factors and artificial stimulation. In "The Microbial Degradation of Oil Pollutants, Ed. by DO. Ahearn & S.P. Meyers LSU, Baton Rouge, LA., pp. 147-152.

Brown, G.C. and Skipsy, E. (2001). Energy Resources, Geology, Supply and Demand. Open University Press, Milton Keynes.

Calabrese, E.J. and Kostecki, PT. (1988). Soils contaminated by petroleum; environmental and public health effects, New York. pp. 1-10.

Dicker, H.J, and Smith, D.W. (1995). Enumeration and relative importance of nitrogen-fixing bacteria in Delaware salt marsh. Appl. Environ. Microbiol. , 1019-1025.

Gadgil, PD. and Bhide, V.P. (1980). Nitrogen fixation by Azotobacter in association with some associated soil microorganisms. Proc. Natn. InSt. Sd. India. 26(B), 60-63. Harris, R.F. and Sommers, L.E. (1968). Plate-dilution frequency technique for assay of microbial ecology. App!. Microbiol. ,j(2), 330-334.

Ibrahim, R.S. (1990). Non-symbiotic nitrogen fixation Azotobacter as influenced by soil salinity. Acta Agron. Acad. Sci. Hung. 113-119.

Jensem, H.L. (1999). Nitrogen fixation and cellulose decomposition by soil microorganisms. (1) Aerobic cellulose-decomposers in association with Azotobacter. Proc. Linnean Soc. New South Wales, 65, 543-556.

Klokk, J. (2001). Effect of oil pollution on the germination and vegetative growth of five species of vascular plants. Oil and Petrochem. Poll, 2(1), 25-30.

Mahmoud, S.A.S., El-Sawy, M., Ishac, Y.Z. and El oil Safty, MN. (1978). The effects of salinity and alkalinity in the distribution and capacity of N2-fixation by Azobacter in Egyptian soils. E. Col. Boll. NF (Natuvetensk Forskingsradet) 99-109.

Moriarty, F. (1999) Ecotoxicology. In 'The study of Pollutants in Ecosystems". Second Edition, Academic Press, Harcourt Brach Jovanovich.

Molder, E.G. (1995). Physiology and ecology of free-living nitrogen-fixing bacterial. International Biological Programme, Vol. 6 WDP Steward ed. Cambridge University Press. pp. 3-28.

Murphy, H.P. (1996). Some effects of crude petroleum on nitrate production, seed germination and growth. Soil Sci. 22. 117-120.

Odu, C.T.I (1998). The effect of nutrient application and aeration on oil degradation on soil Environ. Poll. 15, 235-240.

Odum, WE, and Johannes, RE. (1995). The response of mangroves to man-induced environmental stress. In 'Tropical Marine Pollution", (EIF. Wood and RE. Johannes, Eds). Elsevier,Amsterdam, pp. 52-62.

Onwurah, I.N.E. (1996). Effect of crude oil on a soil Azotobacter isolate from an oil-mineral producing area of Nigeria; simulation and mathematical modelling. Ph.D. Thesis, University of Nigeria, Nukka.

Plice. M.J (1984). Some effects of crude petroleum on soil fertility. Soil Sci. Soc. Amer. Proc. 13. 413-416.

Pshenin, L.N. (1963). Distribution and ecology of Azotobacter in Black Sea. In 'Symposium On Marine. Microbology' CH. Opp heimer d. Chark C Thomas Publisher, Spring field, pp. 383-391.

Whittle, KJ., Hardy, R, Makier, PR. and McGl, A S. (1996). A quantitative assessment of the sources and fate of petroleum compounds in the marine environment. Phil. Trans. Royal Soc. London (B) 93-2 8.

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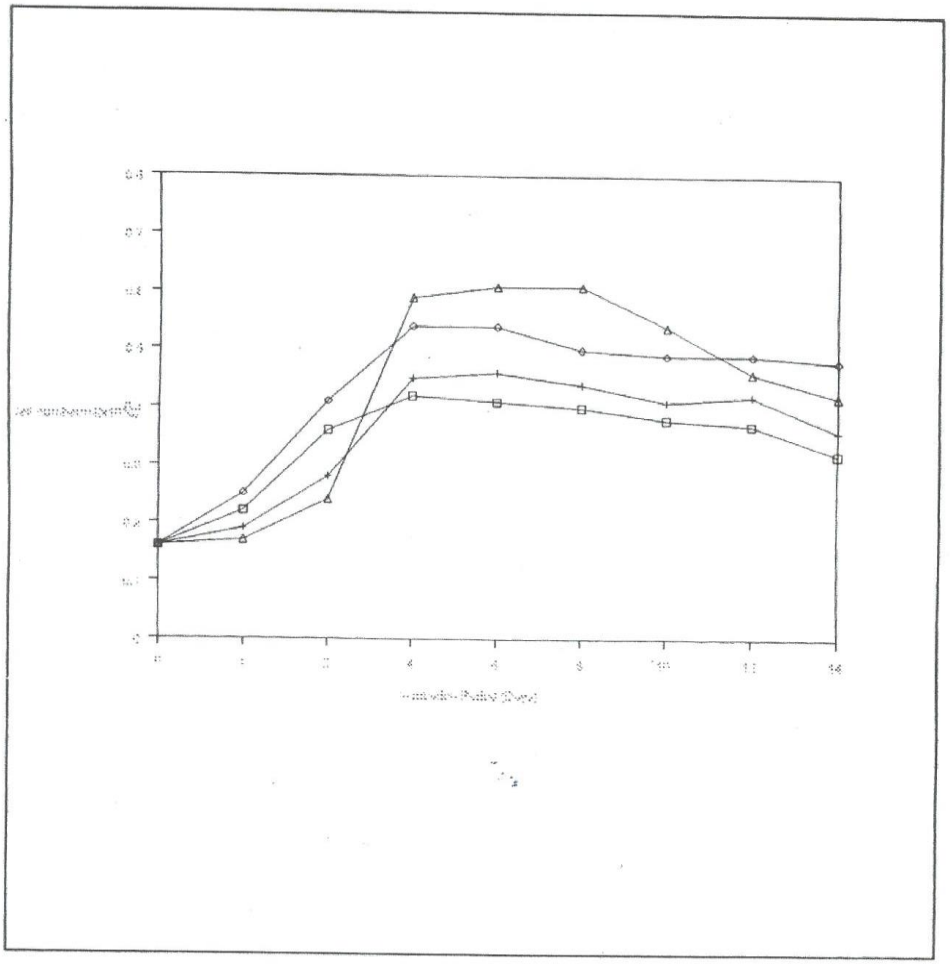

Fig 1. Growth of oil degrading bacteria isolates in crude oil culture medium devoid of adequate nitrogen source. Growth was measured by increase in viable cell number on appropriate solid agar plates.
Symbols :-o-$NS_{50}C_{10}$; -•-$NS_{50}C_{11}$; -▲-$NS_{75}D_7$; -_-$NS_{75}D_{10}$;

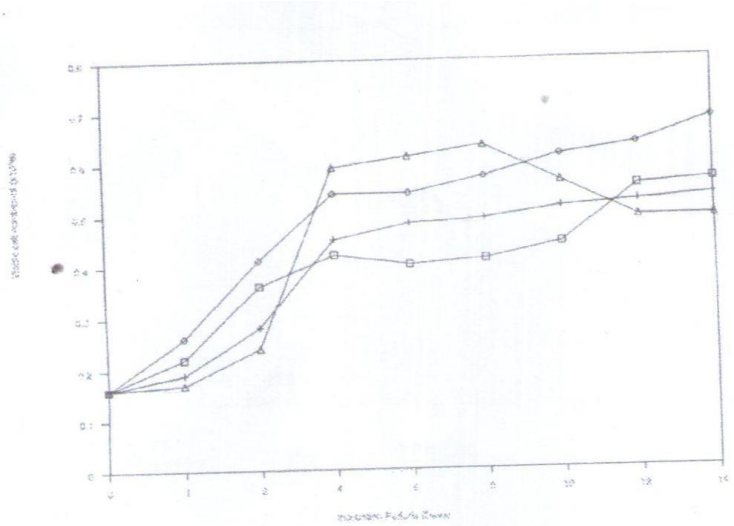

Fig 2. Effect of Azotobacter seed on the growth of oil degrading bacteria isolates in crude oil culture medium devoid of adequate nitrogen source. Growth was measured by increase in viable cell number of the degraders alone on appropriate solid agar plates.

Symbols : -o-$NS_{50}C_{10}$/Azotobacter; -•-$NS_{50}C_{11}$/Azotobacter;
-▲-$NS_{75}D_{7}$/Azotobacter; -_-$NS_{75}D_{10}$/Azotobacter;

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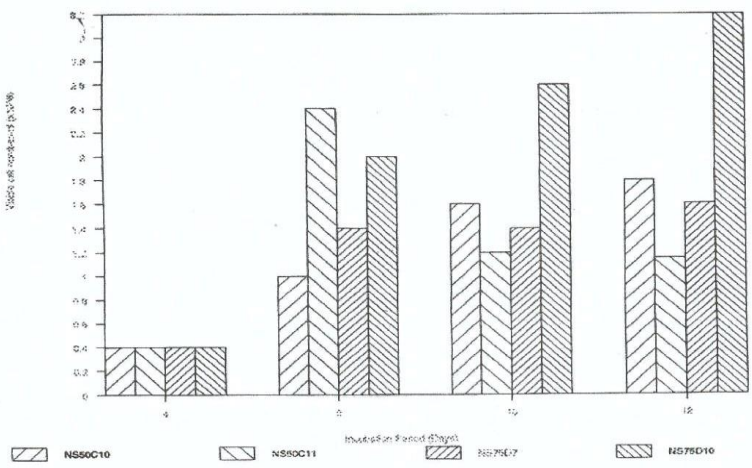

Fig 3. Growth of Azotobacter seed in association with crude oil degrading bacteria isolates in oiled culture medium devoid of any other carbon source. The four columns from left to right represent growth in association with $NS_{50}C_{10}$; $NS_{50}C_{11}$; $NS_{75}D_{7}$ and $NS_{75}D_{10}$ respectively.

Table 1: Estimate of Nitrogen Supplying potential (N_2-fixation) of *Azotobacter* grown together with oil degrading bacteria, and percent of crude oil remaining in various cultures. Values are mean ± SEM of 2 replicate experiments

Time (days)	% Oil Remaining				Pure Cultures				Mixed Cultures of Azotobacter and oil degrading bacteria							
					Nitrogen Content(mg/100ml)				NS$_4$C$_{IV}$/Azotobacter		NS$_4$C$_{IV}$/Azotobacter		NS$_4$D$_V$/Azotobacter		NS$_4$D$_V$/Azotobacter	
	NS$_4$C$_{IV}$	NS$_4$C$_{III}$	NS$_4$D$_V$	NS$_4$D$_{III}$	NS$_4$C$_{IV}$	NS$_4$C$_{III}$	NS$_4$D$_V$	NS$_4$D$_{III}$	% Oil Remaining	N_2 fixed (mg/100ml)	% Oil Remaining	N_2 fixed (mg/100ml)	% Oil Remaining	N_2 fixed (mg/100ml)	% Oil Remaining	N_2 fixed (mg/100ml)
0	100	100	100	100	6.9	6.9	6.9	6.9	NA	NA	NA	NA	NA	NA	NA	NA
4	71.6 ± 6.4	75.8 ± 4.0	72.3 ± 2.8	71.1 ± 5.2	2.21** ±0.14	0.91 ±0.16	1.24 ±0.04	1.55 ±0.02	77.0 ± 6.4	(2.21)** ± 0.11	75.8 ± 4.6	(0.91) ± 0.16	72.4 ± 2.8	(1.24) ± 0.12	71.1 ± 5.2	(1.55) ± 0.14
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.47 ± 1.14	ND	5.90 ± 1.43	ND	4.68 ± 1.24	ND	5.92 ± 0.46
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.18 ± 2.08	ND	3.85 ± 0.96	ND	4.83 ± 1.96	ND	7.40 ± 1.39
14	56.5 ± 4.1	52.4 ± 5.0	51.0 ± 1.2	52.8 ± 4.0	0	0	0	0	23.0 ± 2.8	6.94 ± 2.02	27.3 ± 4.1	3.71 ± 0.31	24.9 ± 3.4	5.14 ± 1.54	20.4 ± 4.72	8.97 ± 1.42

*Figures in parentheses show the remaining N_2 content due to initial $NaNO_3$ added into the degraders medium

NA Not applicable since there were no mixed cultures at this time

ND Not determined at this interval

Table 2: Effect of *Azotobacter* seed on the germination and growth of sorghum grains planted in a soil microcosm previously contaminated by crude oil. Details are described under microcosm experiment. Results are mean ± SEM of 5 replicate experiments

Soil Amendment	Soil without crude oil (control)				Soil with 5% of crude oil evenly layered on it				Soil with 5% crude oil homogenously mixed			
	% of viable germination	No of viable leaves per seedling	Length of plumule per seedling (cm)	Length of radical per seedling (cm)	% of viable germination	No of viable leaves per seedling	Length of plumule per seedling (cm)	Length of radical per seedling (cm)	% of viable germination	No of viable leaves per seedling	Length of plumule per seedling	Length of Radical Seedling (cm)
With *Azotobacter* seed	100	3 ± 1	15.4 ± 4.1	10.8 ± 2.2	36.9 ± 2.5	2 ± 1	5.9 ± 2.7	6.8 ± 3.4	0	None	1.2 ± 0.2	3.0 ± 1.4
Without *Azotobacter* seed	100	3 ± 1	9.2 ± 3.0	9.4 ± 2.8	12.3 ± 1.8	None	1.4 ± 0.6	2.6 ± 0.2	0	None	-	-

*Oil application on the soil stimulated bacterial counts. Also the crude oil degrading bacteria studied were isolated from this soil microcosm.

NB There was no growth of any type on all the sorghum grains planted in 10% crude oil-contaminated soil.