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Full Length Research Paper

Rate of biodegradation of crude oil by microorganisms isolated from oil sludge environment

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The rate of biodegradation of crude oil by micro-organisms isolated from crude oil sludge environment in Eket, Akwa Ibom State of Nigeria was studied. Mineral salt medium supplemented with crude oil was used and three most abundant species isolated from a crude oil sludged soil - *Micrococcus varians*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were selected for the degradation test. The microbial counts used as index during the degradation process for each of the organisms ranged from 1.8 to 6.4 x10^5 cfu/g for *P. aeruginosa*, 1.5 to 5.2 x 10^5 cfu/g for *M. varians* and 1.3 to 4.7 x 10^5 cfu/g for *B. subtilis*. *P. aeruginosa* degraded 97.2% of the oil introduced into the medium followed by *M. varians* with 85.7% degradation. The least was *B. substilis* with 72.3% degradation of the oil. *P. aeruginosa* was found to have the highest rate of degradation.

Key words: Biodegradation, crude oil, hydrocarbonclastic-organisms, sludge-environment, bioremediation.

INTRODUCTION

The environmental impact of petroleum exploration in Nigeria and other oil producing countries has been on the increase. The increasing concern, however, of the environmental scientist is the destruction caused by oil spill both on cultivated and virgin lands. Oil spills on the land and sea has been on the increase with explorative activities. According to Awobayo (1981), between 1978 to 1980 there were 734 cases of oil spills in Nigeria. This has become alarming in the past decades. Oil spills are destructive to both vegetations and animals in the soil not only because of their contact toxicity but also because hydrocarbons in the soil reduces oxygen tension and increases anaerobiosis which is harmful to plant roots (Bossert and Bartha, 1984).

The biodegradation of pollutants in the environment is a complex process whose quantitative and qualitative aspects depend on the nature and amount of the pollutant present, the ambient and the seasonal environmental condition, and the constitution of the indigenous microbial community (Leahy and Colwell, 1990; Hinchee and Olfenbuttel, 1991a, 1991b). Microbial biodegradation of petroleum hydrocarbon in the environment is said to be comparatively slow because it is influenced by a number of factors which include the population of hydrocarbon biodegraders, temperature and nutrient availability (Atlas and Bartha, 1983, 1986). A number of reviews have been carried out on the scientific underpinning and engineering aspects of biodegradation (Atlas, 1981; Borden et al., 1994; Boulding; 1995; Gibson and Saylor; 1992).

Several methods have also been introduced to increase the rate of hydrocarbon biodegration in the soil and they include oxygenation by excavation of the soil, nutrient supplementation and microbial seeding (Atlas and Bartha, 1992). Microbial seeding involves the introduction of microorganisms into the natural environment for the purpose of increasing the rate or extent of biodegradation of pollutants. The rate of biodegradation by individual organisms therefore has been the limiting factor and hence the need for microbial seeding.

This study was therefore designed to monitor the rate of biodegradation of hydrocarbon in soil by microorganisms isolated from crude oil sludge environment of Qua Iboe Terminal (QIT), Eket, Akwa Ibom State, Nigeria.

MATERIALS AND METHODS

Isolation of test organisms

The test organisms were isolated from crude oil sludge environment of Qua Iboe Terminal (QIT), Eket. A mineral salt med- ium of Zajic and Supplison (1972) made up of the following (g/l): KH_2PO_4 (1.8), K_2HPO_4 (1.3), NH_4Cl (4.00) $MgSO_4.7H_20$ (0.2) and agar agar (15.0)

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was supplemented with 1% Bunny light crude oil (from Exxonmobil Producing Nigeria Unlimited, Eket). The pH of the medium was adjusted to 6.5 using HNO_3.

The medium was sterilized and then supplemented with crude oil. A pour plate method was used with 1ml aliquot from the diluted soil sample obtained from the crude oil sludge environment. After 48 h of growth, three most abundant species from the three plates incubated were subcultured and identified using the method of Cowan (1985) and Collins and Lyne (1976). These isolates were used as test organisms.

Microbial biodegradation test

Soil samples belonging to the Ultisol soil, used in this experiment were collected from the University of Uyo research farm, air dried, sieved and sterilized in hot air oven at 160°C for 48 h, cool for 24 h and then destabilized for another 48 h. This was to ensure elimination of indigenous soil microorganisms. About 20 g each of the soil sample was weighed and introduced into twenty-four sterilized bottles. Then 4 ml of sterilized Bonny Light crude oil was poured into all the bottles and 2 ml of 48 h old nutrient broth culture of each of the test organisms were introduced into eighteen bottles.

A set of six bottles with soil samples and 4 ml of crude oil but without micro organisms served as control. The four sets of bottles were thoroughly mixed, moistened with distilled water to field capacity and monitored for 24 days at ambient temperature (28 ± 2°C) and aerated at intervals of four days for the period of biodegradation.

Determination of microbial load

The pour plate method of Zajic and Supplision (1972) was used in determining the microbial load of the treated soil in the bottles with an interval of 1, 4, 8, 12, 16, 20 and 24 days. From each bottle 1 g of soil was removed and serial dilution ranging from 10^{-1} to 10^{-3} were prepared. Three plates from each dilution were inoculated and incubated at ambient temperature for 24 h. Counts for the different test organisms were recorded.

Extraction of residual crude oil

A preliminary extraction was carried out with 4 ml of the crude oil mixed with 20 g of the soil. This was used to determine the percentage recovery rate and subsequently the fractional lost with respect to the recovery method. The modified method of Toogood and MeGill (1977) was used for the extraction. 20 ml of methylene chloride was poured into each bottle containing the crude oil polluted soil with the seeded organisms. It was shaken vigorously and decanted into sterile test tube. The extraction was repeated twice with 10 ml each of the methylene chloride. The total extract which contains the residual crude oil was then heated over a hot water bath for the evaporation of the methylene chloride. The residual crude oil left in the test tube was then weighed and the volume calculated based on the fractional recovery rate (FRR). Two extractions were done for each set and the mean value recorded.

Fractional recovery rate (FRR)

It is generally known that most extraction procedures, fall short of 100% efficiency. In many cases this percentage lost are not considered and this, to a great extent affects the final result. In biodegradation work, except the fractional lost in extraction is known, a false assessment of the biodegradation can be recorded. It was on this premise, therefore, that the crude oil extraction method of McGill (1977) was subjected to series of trials. This resulted in the establishment of a recovery rate and the establishment of the fractional lost. 4 ml of crude oil was introduced into 20 g of soil. Using the method of Mc Gill (1977), the crude oil was extracted from the soil. It was discovered that the percentage recovery rate was consistently 62.5 ± 2% (that is, from the 4 ml introduced into the soil, 2.5 ± 0.16 ml was consistently recovered with the remaining adsorbed to the soil particles). There was a consistent percentage lost of about 37.5% which amounts to 1.5 ± 0.16 ml of the 4 ml used.

From the above, it was possible to calculate the fractional lost per ml of the crude oil recovered as:

Amount of oil introduced - 4 ml
Amount of oil recovered - 2.5 ml
Recovery per ml of the oil - 2.5/4 = 0.625/ml.
Percentage recovery = 0.625 x 100 = 62.5%
The fractional recovery rate (FRR) = 0.625
The fractional lost = 0.375

The implication of the fractional recovery rate (FRR) is that, after a biodegradation study, the residual crude that is extracted in order to determine the quantity degraded by the organisms is always less than the actual by the fraction lost. The fractional lost needs to be computed and added to the recovery residue before the exact quantity biodegraded is known. The fractional recovery rate of 0.6 is therefore a constant with respect to the method used here for crude oil residue recovery.

RESULTS

Microbial isolates

The bacterial isolates from the crude oil sludge soil environment were characterized and identified based on their morphological and biochemical properties. The isolates which were selected on the basis of their counts and growth on the mineral salt medium supplemented with Bonny light crude oil and used for the biodegradation work were *B. subtilis*, *M. varians*, and *P. aeruginosa*.

Growth of seeded microorganisms in crude oil polluted soil

The growth of hydrocarbonoclastic organisms inoculated into the crude oil polluted soil is shown in Figure 1. *P. aeruginosa* had the highest growth with the count of 6.4 x 10^5 cfu/g of soil. This was followed by *M. varians* with 5.2 x 10^5 cfu/g. *B. subtilis* had the least growth in the polluted soil with the count of 4.7 x 10^5 cfu/g.

Weight loss of crude oil in soil seeded with the biodegraders

Table 1 shows the weight loss of crude oil in soil seeded with hydrocarbonoclastic microorganisms. *P. aeruginosa*-treated soil had the highest weight loss of crude oil on the first four days of incubation and this decreased with the incubation period. This was followed by *M. varians* and *B.*

Table 1. Weight loss (g) of crude oil in soil seeded with hydrocarbonclastic organisms.

Isolate	Incubation period (days)					
	4	8	12	16	20	24
M. varians	0.4	0.5	0.3	0.4	0.5	0.5
P. aeruginosa	0.6	0.5	0.5	0.4	0.4	0.4
B. subtilis	0.2	0.3	0.5	0.3	0.4	0.4
Control	0.1	0.1	0.1	0.1	-	-

Standard error of mean (S_x) = 0.033.
LSD = 0.0142.

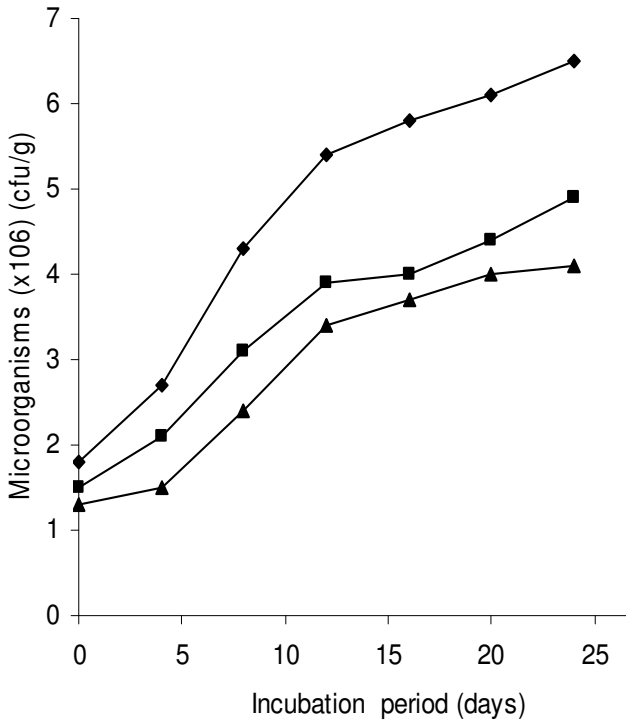

Figure 1. Growth patterns of isolates, *P. aeruginosa* (♦), *M. varians* (■) and *B. subtilis* (▲), in bonny light crude oil-polluted soil (cfu/g).

subtilis treated soils. Unlike *P. aeruginosa,* the crude oil weight loss increased with incubation period in *M. varians* and *B. subtilis.*

Crude oil biodegradation in polluted soil seeded with hydrocarbonoclastic organisms

The crude oil degradation per day is shown in Table 2. *P. aeruginosa* had the highest biodegradation rate per day and this decreased with incubation to a constant from the 16th day of incubation. On the other hand, the rate of biodegradation of the crude oil increased from the 4th day of incubation in both *M. varians* and *B. subtilis.* The amount of oil degraded after 24 days for *P. aeruginosa* was 3.83 ml while that of *M. varians* and *B. subtilis* were 3.43 and 2.89 ml, respectively.

The percentage biodegradation is shown in Figure 2. There was a sharp decline in the percentage biodegradation rate of the crude oil per 100 cell per day. *P. aeruginosa* had a high rate of degradation per 100 cell the first 4 days and declined very sharply till the 12th day of incubation. The same trend was exhibited by *M. varians* but with a gradual decline to the 12th day. On the other hand, *B. subtilis* increased in their biodegradation rate to the 12th day of incubation after which there was a decline in the biodegradation rate.

DISCUSSION

The rate of biodegradation of crude oil by hydrocarbonoclastic organisms isolated from crude oil sludge environment was assessed. The biodegraders which were *P. aeruginosa*, *M. varians* and *B. subtilis*, showed different abilities in the breakdown and utilization of the crude oil. *P. aeruginosa* had the highest growth in the sterilized soil supplemented with crude oil. This was followed by *M. varians* and then *B. subtlis.* The highest growth exhibited by *P. aeruginosa* was not surprising not only because it was isolated from oil sludge environment but also because it is known to possessed a more competent and active hydrocarbon degrading enzymes than other biodegraders (Walker et al., 1976). It is known to be fast growing and are capable of degrading a wide variety of organic compound (Ijah and Okonga, 1993).

The biodegradation process which was monitored by the weight loss of the crude oil introduced into the soil revealed that *P. aeruginosa* within the first 4 days of incubation caused an average weight loss of 0.6 g crude oil per day and this decreased to 0.5 g per day by the 8th through the 12th day. This further decreased from the 16th day through the 24th day to an average of 0.4 g/day. Unlike *P. aeruginosa*, the average weight loss of crude oil per day for *M. varians* increased from 0.4 to 0.5 g/day to the 8th day of incubation and thereafter fluctuated between 0.3 to 0.5 g. This same trend was also found in *B. subtilis* which however had the lowest rate of biodegradation.

In considering the crude oil biodegradation per day, it was observed that *P. aeruginosa* had the highest bio-

Table 2. Crude oil biodegradation (ml/day) in polluted soil seeded with hydrocarbonoclastic organisms.

Isolate	Oil left in soil (ml)	Incubation period (days)						Mean biodegradation per day
		4	8	12	16	20	24	
M. varians	0.57	0.53	0.66	0.39	0.53	0.66	0.66	0.143
P. aeruginosa	0.17	0.79	0.66	0.66	0.53	0.53	0.53	0.154
B. subtilis	1.11	0.26	0.39	0.66	0.39	0.53	0.53	0.115

Standard error of mean (S_x) = 0.033.
LSD = 0.15.

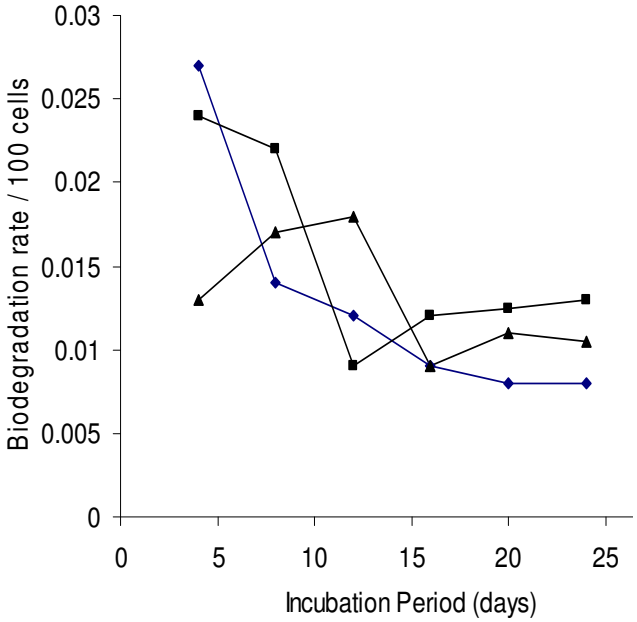

Figure 2. Rate (%) of crude oil degradation per 100 cells per day in polluted soil seeded with hydrocarbonclastic organisms; *P. aeruginosa* (♦), *M. varians* (■) and *B. subtilis* (▲).

degradation. This was followed by *M. varians* and *B. subtilis.* This is in agreement with report of Ekpo and Umoh (2001), using the carbon dioxide evolution technique to monitor hydrocarbon biodegradation; they noted that *P. aeruginosa* had the highest cumulative CO_2 production in a bioremediation experiment. It has also been reported that *Pseudomonas* species, because of their ability to degrade wide range of pollutants, exhibited an increase rate of removal of the pollutant trichloroethylene (TCE) from groundwater (Munakata-Marr et al., 1996).

Further observation revealed that the rate of degradation by *P.aeruginosa* decreased sharply with increase in the incubation period from the 4th day to the 12th day and then stabilized to the 24th day. The same trend was observed with *M. varians* while *B. subtilis* showed an increase in degradation up to the twelveth day of incubation and thereafter decreased. When relating this trend to the increase in microbial cell count, it was observed that there was a rapid increase in the cell biomas of *P. aeruginosa* from 1.8 to 5.26 x 10^5 cfu/g within the first 12th days of incubation. The increase in cell biomas between the 12th to the 24th day of incubation was only from 5.26 to 6.4 x 10^5 cfu/g. The initial high rate of biodegradation observed is attributed to the increase in microbial biomass, and nutrient availability. This is in agreement with earlier report that added nutrient increases the rate of biodegradation (Dibble and Bartha, 1976). In a similar experiment, Jones and Greenfield (1991) reported quite promising result with an average rate of degradation of 30 ppm per day in a fertilizer supplemented soil incubated with specific microorganism.

Contrary to this observation, *B. subtilis* showed an initial increase in biodegradation rate upto the 12th day of incubation and then a decline. This trend was also reflected in the increase in the cell biomass which was only 1.3 to 3.4 x 10^5 cfu/g on the 12th day; and to 4.7 x 10 cfu/g on the 24th day of incubation.

From the forgoing it is important to point out that in bioremediation programme, the efficiency of the seeded organism and its stage of growth should be taken into consideration in clean up or removal of pollutant from the environment. The two methods of bioaugmentation, the seeding of an active biodegrader like *P. aeruginosa* and the enrichment of the medium is of great advantage. This study has also revealed that the removal of pollutant from an environment is effective within the first two weeks of microbial seeding and therefore suggests that a continuous seeding of biodegraders with addition of appropriate nutrient will prove very effective in any bioremediation activity.

REFERENCES

Atlas RM (1981). Microbial degradation of petroleum hydrocarbons. An Environment perspective. Microbiol. Rev. 45: 180-120.
Atlas RM, Bartha R (1983). Fate and effect of polluting petroleum in marine environment. Microbiol. Rev. 49: 49-85.
Atlas RM, Bartha R (1983) Biotechnology of petroleum pollutant. Biodegradation Microb. Ecol. 12: 155-157.
Atlas RM, Bartha R (1992). Hydrocarbon biodegradation and oil spill bioremediation. Adv. Microb. Ecol. 12: 278-338.
Awobayo SA (1981). An analysis of oil spill incidence in Nigeria (1976-1980). Proceeding of An international seminar on the petroleum industry in Nigeria, 111: 55-61.
Borden RC, Vogel TM, Thomas JM, Ward CH (1994). Handbook on bioremediation, Lewis publishers, Boca Roton, FL.
Bossert I, Bartha R (1984). The fate of petroleum in the soil ecosystem.

In: Atlas RM (ed) petroleum microbiology Macmillan, new York pp. 435-475.
Boulding JR (1995). Practical handbook of soil vacuole zone and Ground-water contamination. Assessment, prevention and remediation. Lewis publisher, Boca, Raton FL.
Dibble JI, Bartha R (1979). Rehabilitation of oil polluted agricultural land. A case study. Soil Sci. 123: 56-60.
Ekpo MA, Umoh IF (2001). Bioremediation activity of seeded microorganism on crude oil polluted soil supplemented with organic fertilizer (goat dung) J. Appl. Sci. 4: 2277-2284.
Gibson DT, Saylor GS (1992). Scientific foundations of bioremediation: current status and future needs: American academy of microbiology, Washington D.C.
Hinchee RE, Olfenbuttel RF (1991a). (eds). Instu bioremediation: Applications and investigations for hydrocarbon and contaminated site remediation. Butterworth – Heinemann, Boston.
Hinchee RE, Olfenbuttel RF (1991b). (eds). Onsite bioremediation: process for xenobiotic and hydrocarbon Treatment. Butterworth – Heineman, Boston.
Ijah UJ, Okang LI (1993). Petroleum hydrocarbon degrading bacteria isolated from soil. W. Afr. J. Biol. Appl. Chem. 38: 1-4.
Jones M, Greenfield JH (1991). Greenfield, in-situ comparison of bioremediation methods for a number 6 residual fuel oil spill in Lee country, florida. In: proceedings of the 1991 international oil spill conference, American petroleum institute, Washington D.C. pp. 533-540.
Leahy JG, Colwell RP (1990). Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54: 305-540.
Munakata-Mar J, MaCarty PL, Shields MS, Region M, Francesco SC (1996). Enhancement of trichloroethylene degradation in aquifer microsomers bioaugmented with wild type and genetically altered Burkholderia (*Pseudomonas cepacia*). Environ. Sci. Technol. 30: 2045-2053.
Toogood JA, McGill WB (1977). The reclamation of Agricultural Soils after Oil Spills: II Extension. The University of Alberta, A. I. P. Publishers, Edmonton.
Walker JA, Colwell RP, Petrakis L (1976). Biodegradation rate of component of petroleum. Can. J. Microbiol. 22: 1209.
Zajic E, Suplison B (1972). Emulsification and degradation of Bunker C fuel oil by Micro Organism. Biotechnol. Bioeng. Rev. 10: 1-49.