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Full Length Research Paper

Petroleum-hydrocarbon utilization by native bacterial population from a wastewater canal Southwest Nigeria

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The application of a consortium of native bacterial species in bioremediation processes has long been desired in Nigeria because they would be cost effective and efficient in terms of acclimation time. Two Nigerian crude oils (Bonny light and Escravos blend) were exposed to the wastewater canal via oil impregnated membrane filters (0.45 µm diameter) for 21 days in a microcosm experiment. Bacterial petroleum-hydrocarbon utilizers were later harvested from both the millipore membrane filters and laboratory biodegradative studies. Some of the striking discoveries made were that pH fluctuations in the reaction flasks were due to microbial activities, microbial enzymes, presence of crude oil degradation metabolites such as organic acids, surfactants and aldehydes. More petroleum-hydrocarbon utilizers were detected at 0 to 15 cm depth than at 15 to30 cm. The petroleum sample with higher fractions of saturated hydrocarbon was biodegraded faster (Bonny light) than the ones with higher fractions of asphaltenes and aromatics (Escravos blend). Escravos blend had C-14 component undegraded after the 3-week oil explosure to the waste water. The physico-chemical properties of the freshwater ecosystem were determined and it supported previous conclusion on the latent self purification capability of the aquatic ecosystem inspite of frequent oil pollution incidents. The mean temperatures of the freshwater ecosystem were within the mesophilic range 27-29°C and the pH of the environment supported acidophilic bacterial consortium. Gas chromatographic profiles of the mineralization process of Bonny light and Escravos blend gave the conclusive evidence for the capability of the native bacterial population to mineralize petroleum hydrocarbons in wastewater, at optimum physico-chemical conditions in the habitat. Petroleum-hydrocarbon utilizers detected included *Bacillus megaterium, Pseudomonas putida, Micrococcus luteus, Bacillus brevis, B. punilis* and *Enterobacter aerogenes.*

Key words: Biodegradation, bioremediation, petroleum-hydrocarbon.

INTRODUCTION

The demand for petroleum as a source of energy and as a primary raw material for chemical industries in recent years has resulted in an increase in world production (Gutnick and Rosenberg, 1977). This dramatic increase in production, refining and distribution of crude oil has brought with it an ever increasing problem of environmental pollution (Atlas and Bartha, 1992). Concern over the biological effects of increasing oil spillage on land, water and fish ponds has mounted from the beginning of oil prospecting in Nigeria (Odu, 1972). Although, scientific investigations into the effect of oil pollution in Nigeria only began after the Shell BP Bonu 11 blow-out of July 19, 1970.

The fate of petroleum hydrocarbons in the environment is largely controlled by abiotic factors which influence rates of microbial growth and enzymatic activities that determine the rates of petroleum hydrocarbon utilization (Atlas 1995; Leahy and Colwell, 1990). The persistence of petroleum pollution depends on the quantity and quality of hydrocarbon mixture and on the properties of the affected ecosystem. In one environment, petroleum hydrocarbon persists indefinitely whereas under another

set of conditions the same hydrocarbons may be completely biodegraded within a few hours or days (Atlas and Bartha, 1992). Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats (Atlas and Bartha, 1973). The ability to isolate high numbers of certain oil-degrading microorganisms from oil- polluted environment is commonly taken as evidence that these microorganisms are the active degraders of that environment (Okerentugba and Ezeronye, 2003). Although, hydrocarbon degraders may be expected to be readily isolated from an oil-associated environment, the same degree of expectation may be anticipated for microorganisms isolated from a total unrelated environment such as domestic wastewater.

The objectives of this study were to evaluate the potential of native bacterial population of wastewater with the latent ability to utilize petroleum hydrocarbons as well as characterize the petroleum-hydrocarbon utilizers.

MATERIALS AND METHODS

History of sampling site

The wastewater samples were drawn from the wastewater network situated west of Lagos State University (LASU), Ojo campus. The canal has the history of being fed with domestic wastewater from the staff quarters as well as the occasional discharge of diesel oil from the generator house near by.

Collection of water samples

Water samples for physico-chemical analysis were collected in sterile reagent bottles from the different spots at 0 to 15 cm and 0 to 30 cm depths along the course of the wastewater canal west of LASU Ojo Campus. Water samples for microbiological analysis were collected in sterile screw cap bottles and stored at 4°C in the refrigerator.

Sources of crude oil samples

Bonny Light and Escravos Blend Crude Samples were obtained from the Bonny Terminal of the Shell Petroleum Development Company of Nigeria and Escravos Terminal Chevron (Nigeria) Limited.

Measurement of physicochemical parameters

Mercury-bulb thermometer was used in measuring the temperature of the water while pH was measured in the laboratory with a pH Meter (pW 9504 philips pH meter). Salinity was measured by $AGNO_3$ titration, dissolved oxygen by the KI method, turbidity, conductivity, HPO_4^{3-}, NO_3^- and NH_4-N were determined using the portable spectrophotometric apparatus (Amund, 1984).

Exposure of oil to the environment

Typical microcosm experiments for the '*in situ*' determination of oil degradation rates were carried out as described (Gilbert and Higgins, 1978; Amund and Igiri, 1990). Oiled Millipore membrane filters (TYPE HA 0.45µm) were used as supports for the oil, allowing a thin film to be evenly absorbed over a large area. All oiled filters absorbed 120-130 mg of oil. The oiled filters were inserted through a slit into perforated plastic balls so that they formed an equatorial diaphragm. The balls were re-sealed with a hot knife and placed in a perforated plastic container, which was immersed in the wastewater canal in LASU, Ojo Campus. The plastic container was attached by a line to the stem of a near by tree. Controls were set up in the laboratory by immersing plastic balls housing the oiled filters into sterile wastewater in order to determine the effects of non-biological phenomena on oil leaching. Oiled-membrane samples were withdrawn from the field at two-week intervals and each membrane was then placed in 10 ml of sterile waste water in screw-cap bottles and shaken at 600 oscillations/min on a wrist action flask shaker to free the organisms from the filter. The total bacteria counts and the population of hydrocarbon-utilizing bacteria were determined in the resulting suspension (Amund and Akangbou, 1993; Amund and Igiri, 1990).

Enumeration of microorganisms

Total heterotrophic counts of bacteria were carried out on nutrient agar plates whilst the population of hydrocarbon-utilizing bacteria were determined by plating aliquots of water samples (o.1 ml) on Minimal salt agar and incubated at 28±2°C for 48 h. The resulting colonies were later counted (Amund et al., 1987; Amund and Igiri, 1990). Crude oils used as carbon sources were introduced by vapour-phase transfer by placing filter discs impregnated with the oils into the lids of petri dishes (Raymond et al., 1976). Hydrocarbon-utilizing bacterial strains were isolated and identified by the Bergey's Manual of Determinative bacteriology (Buchanan and Gibbons, 1974).

Laboratory biodegradation studies

The ability of the indigenous microbial flora of the wastewater to degrade crude oil was tested by introducing the oil to fresh (unsterilized) wastewater sample from the sampling points at 0.05% (v/v). The flasks (250 ml) were incubated, with shaking at 200 rev/min. at room temperature (28±2°C). Bacterial growth was monitored by viable counts on nutrient agar plates (Amund and Igiri, 1990). The residual oil was measured and the qualitative changes in the hydrocarbon profile of the oil were monitored by gas-liquid chromatography as previously described (Amund, 1984).

RESULTS AND DISCUSSION

The Physicochemical factors influenced the type and number of bacterial isolates from the wastewater (Table 1). The slow-moving, fresh water ecosystem had an acidic pH suggesting that the indigenous acidophilic bacterial population evolved because of the pH of the environment. Generally, the nutrient level in the water was not too low but for phosphorus which was found to be extremely low. The mean temperature of the wastewater during August rain-break monitored for 3 years were 27°C in the morning, 29°C in the afternoon and 28°C in the evening. This agrees with conditions earlier observed by previous researchers as regards mesophilic temperature range for petroleum hydrocarbon degradation in aquatic ecosystem (Zobell, 1964).

Table 1. Selected chemical and physical characteristic of two wastewater samples during august rain- break.

Sample	NH_3 (mg/l)	NO_3 (mg/l)	PO_4 (mg/l)	DO (mg/l)	pH	Salinity %	Turbidity (FTU)	Conductivity (ms/cm)
A	0.16	2.6	0.04	5.3	5.63	0.064	12	0.12
B	0.38	2.7	0.23	8.7	5.88	0.48	2.02	0.11

FTU: Formalin turbidity units.

Table 2. Mean heterotrophic bacterial population in wastewater (before the test crude oil was dispersed).

Depth (cm)	No of colonies per ml
0-15	1697
15-30	2257
Control	0

Table 3. Mean bacterial petroleum-degrader population in wastewater (0-30 cm depth).

Sample	No of colonies per ml	% Hydrocarbon utilizer	
		0 to 15 cm	15 to 30 cm
Bonny Light	517	30.5	22.9
Escravos blend	450	26.5	19.9
Control	0	0	0

The heterotrophic bacterial population in the wastewater were different (Table 2) at the two depths (0 to 15 cm and 15 to 30 cm) chosen. The two Nigerian crude oil samples (Bonny Light and Escravos Blend) accommodated varying population of petroleum-hydrocarbon utilizers (Table 3). Comparatively, more bacterial petroleum-hydrocarbon utilizers metapolized Bonny light than they did for Escravos blend (Table 3). This corroborated earlier discovery by researchers that the more saturated hydrocarbon petroleum contains the faster the rate of its hydrocarbon metabolism by microbes (Amund and Akangbou, 1993; Leahy and Colwell, 1990). The significance of dissolved oxygen as the rate limiting factor was prominently observed as the percent hydrocarbon utilizers were more at 0 to 15 cm depth than at 15 to 30 cm depth. The low rates of biodegradation of Escravos blend was predictable and can be attributed majorly to two factors; its higher fractions of asphaltenes and aromatics (Table 5) and lesser attraction of bacterial petroleum hydrocarbon utilizers. Bacterial crude oil hydrocarbon utilizers were detected more during laboratory biodegradative studies than on the field (Table 6). This presumably was due to the surface area available for colonization in the laboratory studies than in the membrane studies. The volume of petroleum available for bacterial metabolism was more compared to the meager 120 to 130 mg available on the millipore membrane for bacterial metabolism.

Table 4. pH of media during laboratory biodegradative studies.

Sample	pH		
	Day 5	Day 10	Day 21
Bonny Light	6.0	8.4	7.10
Bonny Light	6.0	7.86	7.25
Escravos Blend	6.9	7.30	7.10
Escravos Blend	7.0	7.65	7.10
Control (Bonny Light)	6.25	6.25	6.25
Control (Escravos Blend)	6.15	6.15	6.15

The monitoring of pH changes during the laboratory biodegradative studies (Table 4) confirmed that fluctuations in pH reading within the 21-day period was as a result of both microbial and chemical changes which must have been precipitated by microbial enzymes (Atlas and Bartha, 1972). Meanwhile, other crude oil metabolites such as surfactants, organic acids, and aldehydes were responsible for pH fluctuations in the reaction vessels (Atlas and Bartha, 1972). Morphological and biochemical characterization of petroleum-hydrocarbon utilizers supported by oiled membranes revealed the following organisms; *Bacillus megaterium, Bacillus brevis. Bacillus pumilis, Enterobacter aerogenes, Pseudomonas putida* and *Micrococcus luteus*. They were characterized following methods of Cowan and Steel (1985); Buchanan and Gibbons, (1974). The result of the measurement of the abiotic factors in this fresh water

Table 5. Fractional composition and specific gravity of Nigerian crude oils.

Crude oil Fractions	Saturation (%)	Aromatics (%)	Asphaltenes (%)	Residue (%)	Specific Gravity
Bonny Light	81.11	7.20	2.48	0.21	0.84
Medium	64.90	13.37	13.37	8.36	0.98
Escravos Light	69.74	22.05	2.56	5.65	0.78
Forcados Blend	58.89	11.10	340	26.61	0.88

Source: Amund and Akangbou (1993).

Table 6. Identification of hydrocarbon utilizing bacteria.

Isolates	Cellmorphology Gram reaction	Citrate utilliza tion	Gelatin liquefac tion	M-R test	V-P test	Indole	Starch	Oxidase	Catalase	Urease	Mannose	Suspected microbes
E1	+ rods Dull Surface	+	+	+	-	-	+	+	+	+	-	*Bacillus brevis*
E2	+ rods white raised	+	+	+	-	-	-	-	+	+	-	*Bacillus megaterium*
E3	+ rods cream mucoid	+	+	-	+	-	-	-	+	+	-	*Bacillus pumilis*
E4	- rods cream raise	+	+	+	-	-	+	+	+	-	-	*Pseudomonas putida*
M1	+ rods cream raised	+	+	+	-	-	-	-	+	+	-	*Bacillus brevis*
M2	+ rods cream raised	+	+	+	-	-	-	-	+	+	-	*Bacillus brevis*
M3	-ve rods white raises	+	+	+	+	-	+	-	+	+	-	*Enterobacter aerogenes*
M4	+ ve coci cream raised	-	-	-	-	-	-	+	+	+	-	*Micrococcus luteus*

+ = Positive
- = Negative
V-P = Voges Prauskauer
M-R = Methyl Red

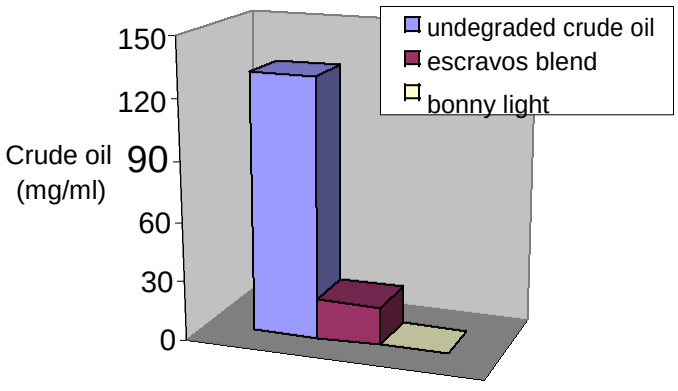

Figure 1. GC profile of crude oil biodegradation.

ecosystem suggested the presence of inherent capability for self-purification inspite of the frequent oil pollution incidents.

Comparatively, undegraded Bonny light hydrocarbon profile as shown by infrared (IR) spectrophotometric analysis revealed different bands characteristic of aromatics, triple bonds and phenols (Pavia et al., 1996). The gas chromatographic profiles of degraded Bonny light and Escravos blend (Figure 1) showed complete mineralization of Bonny light hydrocarbons and incomplete mineralization of Escravos blend, probably

due to the fact that Bonny light consists of more saturated fractions than Escravos blend. This corroborates the findings of previous researchers (Atlas and Bartha, 1972; Amund, 1984).

It is apparent from this investigation that the fractional composition of the Nigerian crude oil under study had a major effect on their biodegradation rates and bioremediation (Table 5). The use of native bacterial consortium with petroleum hydrocarbon utilizing capabilities as seed onto oil-impacted environment could prove a more environmentally-friendly approach to bioremediation which would on the long run enhance sustainable development rather than the use of exotic bacterial strains and chemicals.

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