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Biodegradation of petroleum hydrocarbons under tropical estuarine conditions

O.O. Amund and C.O. Igiri

The physico-chemical parameters of water samples collected from three points in the Lagos lagoon were studied for 12 months. Salinity varied seasonally but the temperature, pH, dissolved O_2, conductivity, NO_3^- and HPO_4^{2-} concentrations were relatively constant. There was a direct proportionality between the population density of hydrocarbon-utilizing bacteria and the oil content of water samples. Twelve hydrocarbon-utilizing bacteria were isolated by selective enrichment and characterized as species of *Pseudomonas, Alcaligenes, Acinetobacter* and *Bacillus*. The organisms grew mainly on long-chain aliphatic hydrocarbons. Laboratory and field biodegradation studies showed both quantitative and qualitative changes in the hydrocarbon content of crude oil due to microbial degradative activities and a faster rate of oil depletion from the Lagos lagoon during the rainy season. The results obtained could offer a predictive model for estimating the rate of disappearance of petroleum hydrocarbons from the tropical estuarine environment.

For French summary, see next page.

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Introduction

The problem of petroleum pollution of coastal waters has resulted in intense investigations into the sources, quantity, fate and biological impact of this major form of pollution. The widespread ability of microorganisms to assimilate hydrocarbons is of great significance in the natural environment. Estimates of both the distribution of hydrocarbon-utilizing microorganisms and their biodegradative potential are needed in order to assess the environmental impact of polluting hydrocarbons.

The Lagos lagoon (Fig. 1) is a wide expanse of estuarine water extending from Lagos harbour to the Niger Delta in South-west Nigeria. The lagoon is extensively polluted at the harbour by seepages from the oil discharge terminal and it also receives an unquantifiable amount of spent lubricating oils from the adjoining drainage systems. In most of the studies that have been published on petroleum biodegradation, major consideration has been given to the utilization of oil by pure and mixed cultures under laboratory and simulated field conditions (Kator *et al.* 1971; Jobson *et al.* 1972; Horowitz & Atlas 1977). There have been few reports on the *in situ* degradation of spilled oil in the natural environment (Gilbert & Higgins 1978; Westlake *et al.* 1978). The purpose of the present study was to assess the petroleum-degrading potential of indigenous microorganisms of the Lagos lagoon so that the results obtained could provide a baseline estimate for predictive modelling of the fate of polluting oils in the tropical estuarine environment.

Materials and Methods

Collection of Samples

Three sampling points were used in the Lagos lagoon (Fig. 1): Lagos harbour, the University of Lagos jetty and the Ikorodu end of the lagoon. These points represent marine, brackish and freshwater conditions respectively (Hill & Webb 1958). Water samples for physico-chemical analyses were collected in reagent bottles whilst those for microbiological analyses were collected in sterile screw-cap bottles and stored at 4°C.

On a étudié les paramètres physico-chimiques d'échantillons d'eau récoltés en trois endroits du lagon de Lagos pendant 12 mois. La salinite a varié avec les saisons mais tant la température que le pH, l'oxygène dissous, la conductivité et les teneurs en nitrates et en phosphates sont restés relativement constants. Il y avait une proportionnalité directe entre la densité de population des bactéries utilisant les hydrocarbures et le contenu en pétrole des échantillons d'eau. Douze souches de bactéries utilisant les hydrocarbures ont été isolées par enrichissement sélectif et caractérisées au niveau de l'espèce comme des *Pseudomonas, Alcaligenes, Acinetobacter* et *Bacillus.* Ces organismes croissent principalement sur des hydrocarbures aliphatiques à longue chaîne. Des études de biodégradation au laboratoire et sur le terrain ont montré des changements tant quantitatifs que qualitatifs dans le contenu en hydrocarbures du pétrole brut, dus aux activités de dégradation microbienne ainsi qu'un appauvrissement plus rapide en pétrole du lagon de Lagos pendant la saison des pluies. Les résultats obtenus pourraient offrir un modèle prédictif pour l'estimation de la vitesse de disparition des hydrocarbures du pétrole dans l'environnement d'estuaires tropicaux.

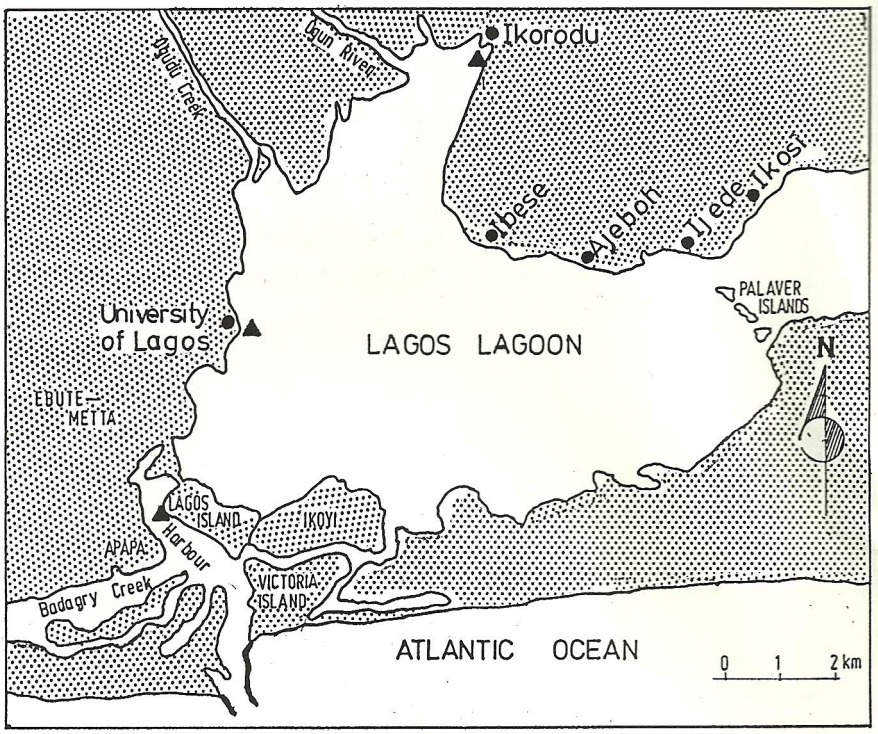

Figure 1. Map of the Lagos lagoon showing the sampling stations (▲).

Measurement of Physico-chemical Parameters

Water temperature was measured on site using a mercury bulb thermometer whilst the pH was measured in the laboratory with a pH meter. Salinity was measured by $AgNO_3$ titration, dissolved oxygen by the KI method, whilst turbidity, colour, conductivity, HPO_4^{2-}, NO_3^- and ammonia-nitrogen were determined using the portable spectrophotometric apparatus. Oil content was measured using the method of Simrad *et al.* (1951).

Enumeration of Microorganisms

Total heterotrophic counts of bacteria were carried out on nutrient agar plates whilst the population of hydrocarbon-utilizing bacteria was determined by plating aliquots of water samples on the minimal medium of Mulkins-Phillips & Stewart (1974) using *n*-hexadecane as the carbon source. Inoculated agar medium was inverted over sterile filter paper that was moistened with *n*-hexadecane and held in the lid of a Petri dish. Incubation was at 30°C for 72 h.

Isolation of Hydrocarbon-utilizing Bacteria

Liquid enrichments of water samples were carried out on the mineral salts medium of Mulkins-Phillips & Stewart (1974) using Nigerian crude oil (Forcados blend) as the carbon source. Isolates were tested in pure culture for ability to assimilate crude oil as well as pure hydrocarbon substrates such as *n*-decane, *n*-dodecane, *n*-hexadecane, naphthalene, phenanthrene and anthracene. Other substrates tested were hexadecan-1-ol, palmitic acid and salts of succinic and acetic acids.

Exposure of Oil to the Environment

Nigerian crude oil (Forcados blend) was exposed to the lagoon using the method of Gilbert & Higgins (1978). Millipore membrane filters (pore size 0.22 μm, 47 mm dia.) were used as supports for the oil, allowing a thin film to be evenly absorbed over a large area. All oiled filters absorbed 120 to 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 aluminium bucket which was suspended in the lagoon water in a creek adjacent to the University of Lagos jetty. The aluminium bucket was attached by a line to probe roots of the mangrove vegetation. Controls were set up in the laboratory by emersing plastic balls housing the oiled filters into sterile lagoon water in order to determine the effects of non-biological phenomena on oil leaching. Membrane samples were withdrawn from the field at two-week intervals and each membrane was then placed in 10 ml of sterile lagoon 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 bacterial counts and the population of hydrocarbon-utilizing bacteria were determined in the resulting suspension. Residual oil on the membrane filters was measured by infrared spectrophotometry (Simrad *et al.* 1951). Each filter was extracted with carbon tetrachloride (5.1 ml) and the absorbance of the oil extract was read in a Pye Unicam SP100 infrared spectrophotometer using a KBr liquid cell of path length 0.5 mm. The sum of peak heights at 3.38, 3.42 and 3.50 μm (representing CH, CH_2 and CH_3 stretching frequencies, respectively) was taken as a quantitative measurement of the amount of oil.

Laboratory Biodegradation Studies

The ability of the indigenous microbial flora of the lagoon water to degrade crude oil was tested by introducing the oil to fresh (unsterilized) water samples from the sampling points at 0.1% (v/v). The flasks (250ml) were incubated, with shaking at 200 rev/min, at room temperature (28 $\pm$ 2°C). Bacterial growth was monitored by viable counts on nutrient agar and hexadecane/agar plates. The residual oil was measured by infrared spectrophotometry (Simrad *et al.* 1951) while the qualitative changes in the hydrocarbon profile of the oil was monitored by gas–liquid chromatography as previously described (Amund 1984).

Results

Physico-chemical Factors and the Distribution of Hydrocarbon-utilizing Bacteria

The data for physico-chemical analyses of the Lagos lagoon waters (Table 1) showed that salinity was the most variable environmental factor showing both

Table 1. Physico-chemical parameters and the oil content of water samples from three locations* in the Lagos lagoon (January–December 1986).

	Temperature (°C)			Dissolved oxygen (mg/1)			Salinity (%)			pH			Ammonia-nitrogen (mg/1)			Phosphate ion concentration (µg/1)		
	HB*	IK	UL	HB	IK	UL	HB	IK	UL	HB	IK	UL	HB	IK	UL	HB	IK	UL
January	28.0	29.0	30.0	2.4	3.7	3.9	3.2	1.1	2.0	8.4	7.6	8.2	6.00	4.03	5.24	1.33	0.89	0.65
February	30.7	32.9	34.0	8.2	6.4	8.0	3.2	1.5	2.7	8.8	8.2	8.4	6.91	6.53	6.92	1.65	1.50	2.50
March	31.0	31.0	32.0	6.3	7.4	5.3	3.7	2.1	3.2	9.6	7.6	8.0	2.03	3.00	2.53	1.52	1.80	2.53
April	31.0	35.5	34.0	4.8	3.5	2.8	3.3	1.4	2.3	8.8	8.0	8.2	4.41	5.04	4.00	0.52	0.71	0.55
May	31.0	31.0	31.5	7.0	5.1	6.9	3.2	0.9	1.9	9.6	8.0	9.2	4.23	1.08	2.15	0.71	0.43	0.45
June	26.5	27.5	25.5	5.8	4.5	5.2	1.5	0.5	0.96	8.4	7.2	7.9	1.15	2.23	1.03	0.50	0.40	0.48
July	27.6	28.2	28.4	6.2	3.9	4.0	1.0	0.3	0.8	8.2	7.2	8.6	2.31	1.57	1.05	0.45	0.40	0.35
August	26.2	27.7	28.0	8.6	3.2	3.0	1.7	0.12	1.22	8.0	7.2	7.8	2.52	1.50	0.82	0.51	0.34	0.35
September	25.5	27.0	26.0	5.2	5.8	3.9	0.97	0.2	0.24	8.6	7.4	8.2	2.01	0.73	1.23	0.22	0.51	0.45
October	26.2	27.0	28.0	2.4	2.0	2.0	0.98	0.3	0.8	9.0	7.8	8.9	1.83	1.64	1.45	1.24	0.53	0.95
November	29.5	27.0	31.0	2.0	2.8	2.6	1.42	0.05	0.65	8.9	7.6	8.4	1.41	2.53	1.53	1.13	0.34	0.53
December	30.8	33.0	34.2	2.9	3.5	3.5	1.82	0.5	1.05	8.6	7.8	8.4	2.11	2.94	2.54	0.84	0.41	0.45

* HB: Lagos harbour; IK: Ikorodu; UL: University of Lagos jetty.
† FTU: Formazin turbidity units.

Table 1 continued.

	Nitrate ion concentration (mg/l)			Colour (platinum-cobalt units)			Conductivity (Us/cm × 10^3)			Turbidity† (FTU)			Oil content (mg/l)		
	HB	IK	UL	HB	IK	UL	HB	IK	UL	HB	IK	UL	HB	IK	UL
January	3.34	2.91	3.27	50	175	250	52.50	20.00	34.00	17.5	48	75	130	70	147
February	3.93	3.20	3.75	40	450	1000	50.50	280.00	43.00	18	60	1050	110	85	95
March	7.22	7.03	6.90	25	150	249	49.00	246.00	450.00	10	32	250	90	77	92
April	6.81	5.91	5.43	75	150	380	54.40	18.00	288.00	18	25	120	70	65	70
May	7.25	6.95	6.21	10	60	50	78.90	22.00	36.00	8	121	20	185	145	140
June	7.81	7.23	6.80	50	260	2500	64.00	27.00	60.00	15	62	15	190	136	135
July	7.63	7.25	6.51	45	62	162	43.00	16.00	38.00	6	9	50	150	150	125
August	7.24	6.78	5.31	16	180	28	38.00	0.304	28.00	12	50	80	155	120	165
September	7.90	2.91	2.16	25	75	60	10.01	0.463	10.00	5	18	22	160	173	175
October	6.24	2.73	2.62	32	96	67	13.38	0.850	12.26	8	32	43	175	180	180
November	5.41	2.45	2.23	43	275	75	18.50	36.39	18.50	10	50	37	180	185	198
December	4.33	3.01	2.81	125	15	125	11.50	28.00	11.75	20	15	40	210	190	200

seasonal and site variations. Salinity values were generally higher at the harbour, which is the point where the lagoon opens into the sea, relative to the inner segment of the lagoon and the freshwater areas of Ikorodu, where the Ogun river enters the lagoon. During the dry season (January to April), the salinity at Lagos harbour ranged between 3.2% and 3.5% while the values were correspondingly lower at the University of Lagos jetty (2.0% to 3.2%) and the Ikorodu end of the lagoon (1.13% to 2.1%). During the rainy season (May to October) the lagoon water was more dilute, resulting in lower salinity readings at the three sampling stations. However, a similar trend was observed whereby the highest salinity readings were recorded at the harbour. There was no significant difference between the values obtained for temperature, dissolved oxygen, HPO_4^{2-}, NO_3^- and ammonia-nitrogen. However, the water at the three sites was always alkaline (pH 7.2 to 9.6) throughout the sampling period.

The population counts of hydrocarbon-utilizing bacteria and their percentages within the heterotrophic populations are shown in Table 2. The hydrocarbon-utilizing species constituted less than 1.0% of the total heterotrophic populations of the lagoon water. However, there was a positive correlation between the oil content of the water and the population of hydrocarbon-utilizing bacteria at the

Table 2. Bacterial population of hydrocarbon-utilizing bacteria at three locations* in the Lagos lagoon (January–December 1986).

	Hydrocarbon degrading bacterial population (colony forming units × 10^{-2})			Percentage of hydrocarbon utilizers		
	HB	IK	UL	HB	IK	UL
January	19.0 ± 2.6	2.0 ± 0.6	22.0 ± 0.7	0.01	0.02	0.001
February	22.0 ± 2.3	7.0 ± 1.2	9.0 ± 1.2	0.08	0.01	0.01
March	9.0 ± 1.4	4.0 ± 1.7	5.0 ± 1.3	0.03	0.01	0.01
April	8.0 ± 1.3	2.0 ± 0.8	3.0 ± 0.9	0.08	0.02	0.003
May	33.0 ± 2.2	18.0 ± 2.4	29.0 ± 2.4	0.21	0.10	0.03
June	47.0 ± 4.3	16.0 ± 2.2	12.0 ± 2.3	0.01	0.11	0.02
July	21.0 ± 2.4	18.0 ± 2.6	12.0 ± 2.4	0.5	0.10	0.02
August	23.0 ± 2.1	33.0 ± 3.6	40.0 ± 3.0	0.08	0.03	0.03
September	28.0 ± 2.2	40.0 ± 3.4	42.0 ± 3.3	0.2	0.01	0.02
October	35.0 ± 2.9	42.0 ± 3.3	44.0 ± 3.3	0.03	0.03	0.2
November	45.0 ± 2.2	44.0 ± 2.4	46.0 ± 3.8	0.03	0.02	0.02
December	59.0 ± 2.3	43.0 ± 2.3	46.0 ± 3.2	0.01	0.02	0.02

* HB: Lagos harbour; IK: Ikorodu; UL: University of Lagos jetty.

three sampling sites (correlation coefficient, $r = +0.91$, $+0.95$ and $+0.92$ for the harbour, University of Lagos jetty and Ikorodu, respectively).

Twelve hydrocarbon-utilizing bacterial species were isolated from enrichment cultures developed from water samples collected from the sampling stations. The organisms were identified in accordance with the taxonomic schemes of Cowan (1974) as *Pseudomonas* (five strains), *Bacillus subtilis* (three strains), *Acinetobacter* (two strains) and *Alcaligenes* (two strains). The *Pseudomonas* and *Bacillus* species were found at the three locations in the lagoon. All the isolates grew extensively on long-chain *n*-alkanes such as *n*-dodecane, *n*-tetradecane, *n*-hexadecane and their corresponding alcohol and fatty acid derivatives. None of the organisms grew on aromatic hydrocarbons.

Degradation of Crude Oil under Laboratory and Field Conditions

The results of batch culture studies on the degradation of crude oil under laboratory conditions using unsupplemented lagoon water are shown in Figs 2 and 3. The introduction of crude oil into the lagoon water resulted in a progressive increase in the total microbial load and in the population of hydrocarbon-utilizing bacteria with time and was accompanied by a gradual depletion of the oil. About 80 to 90% of the applied oil was removed within 21 days (Fig. 2); the paraffinic components appeared to be completely removed (Fig. 3).

Exposure of oiled filters to the lagoon water resulted in the colonization of the membrane surfaces by heterotrophic bacteria including hydrocarbon utilizers. The population of these organisms on the membrane surfaces increased with time (Fig. 4).

The results indicated that crude oil was degraded faster in the lagoon during the rainy season (May to July 1986) than during the dry season (January to March 1986), as evidenced by the relative rates of oil depletion from the membranes which were computed at 170 $\mu g/cm^2$ per day and 130 $\mu g/cm^2$ per day, respectively.

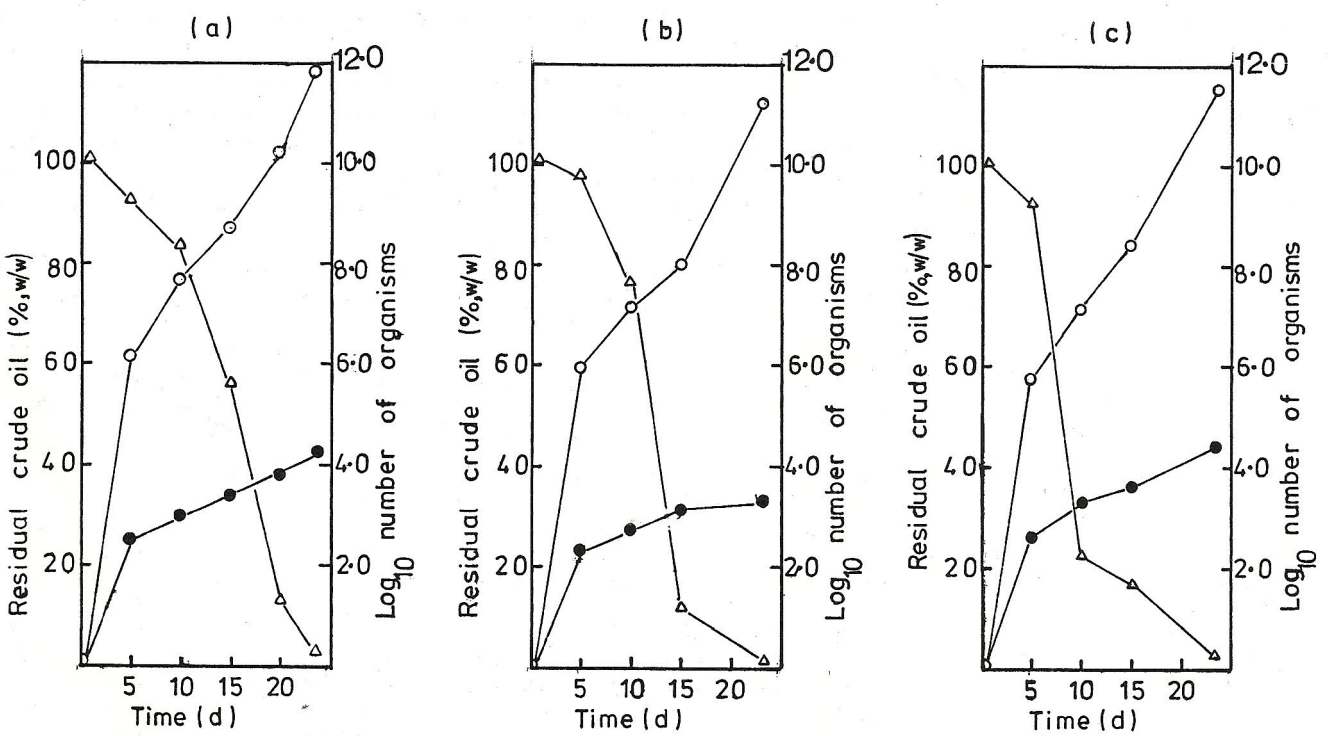

Figure 2. Progressive changes in the microbial load and oil content of lagoon water samples deliberately contaminated with Nigerian crude oil. (a) University of lagos jetty; (b) Ikorodu sampling station; (c) Lagos harbour. ○—Total heterotrophs; ●—hydrocarbon utilizers; △—crude oil concentration.

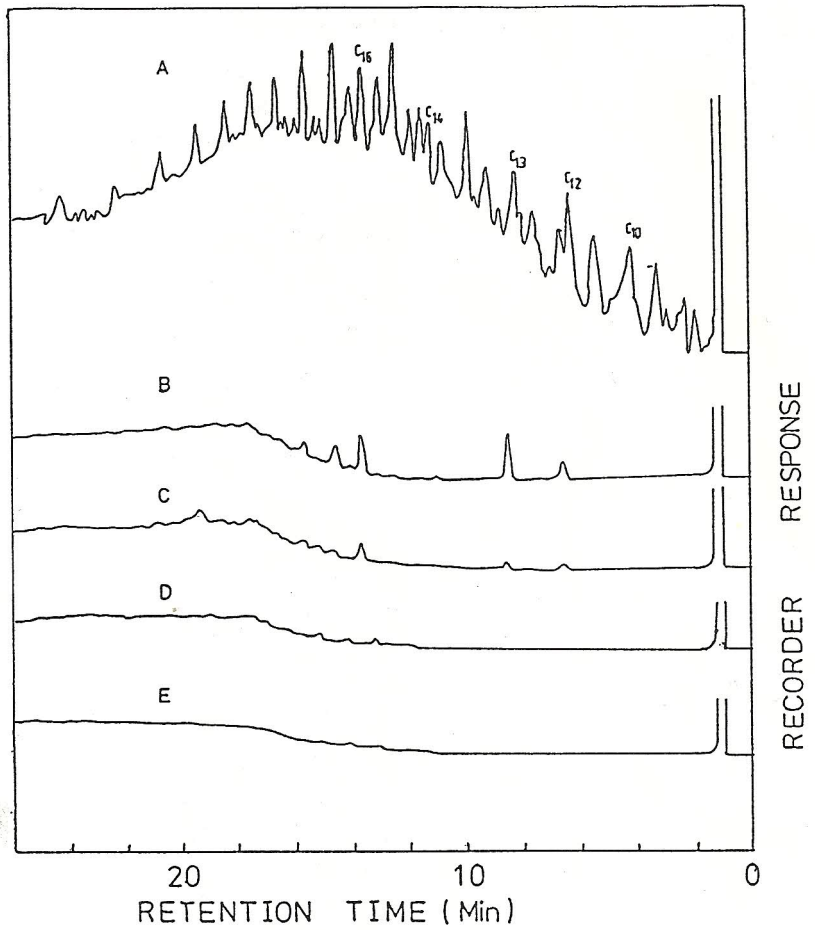

Figure 3. Gas chromatographic profiles of Nigerian crude oil during the laboratory biodegradation study with a water sample from the Ikorodu sampling station of the Lagos lagoon. A, fresh crude; B, C, D and E, profiles for the residual crude after degradation for 7, 11, 15 and 21 days, respectively.

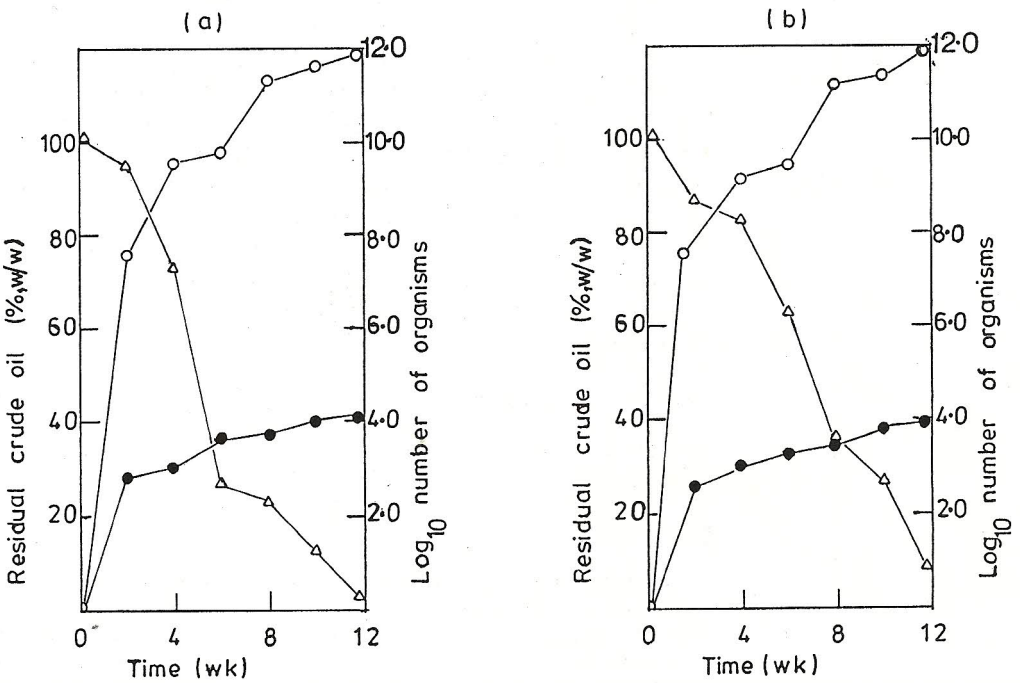

Figure 4. Changes in the bacterial population density and crude oil concentration on oil-impregnated membranes exposed to the lagoon water during the *in situ* biodegradation studies. (a) Rainy sason; (b) dry season. ○—Total heterotrophs; ●—hydrocarbon utilizers; △—crude oil concentration.

Discussion

The seasonal variation in salinity of the Lagos lagoon waters observed in this study corroborated previous observations made by other investigators that the lagoon is a typical estuarine ecosystem (Hill and Webb 1958; Olaniyan 1969). The seasonal changes in salinity have been attributed to seasonal changes in the direction of water movement. During the rainy season there is an influx of freshwater from the Ogun river and other contiguous smaller rivers, resulting in the overall dilution of the lagoon water. During the dry season there is a low level of water in the rivers, resulting in a net movement of water into the lagoon from the sea, leading to an increase in salinity along the entire length of the lagoon. However, there was an inverse relationship between the salinity profile and the distribution pattern or population density of hydrocarbon utilizers in the lagoon (correlation coefficient, $r = -0.13$, -0.40 and -0.62, respectively, for the three sampling stations). The faster degradation rate of crude oil in the lagoon during the rainy season is attributable to the low salinity values. Salinity has been shown to have an adverse effect on bacterial survival through a general osmotic effect or by specific ion toxicity (Carlucci & Pramer 1959; Jones 1963). The seasonal variation in oil degradation rate in the lagoon would also indicate that the fate of polluting oil will be highly dependent on the time of contamination. Similar observations were made by Ward & Brock (1976), who reported a general reduction of metabolic rate at extreme salinities, which therefore raises doubts about biodegradation of hydrocarbons in hypersaline environments.

The numbers of hydrocarbon utilizers at different points in the lagoon correlated with the degree of hydrocarbon contamination. Similar increases in populations of hydrocarbon utilizers have been reported when environmental samples are exposed to petroleum hydrocarbons (Atlas & Bartha 1972; Calomiris *et al.* 1976). The fate of petroleum hydrocarbons in the aquatic environment is largely influenced by abiotic factors which include temperature, dissolved oxygen content and mineral nutrients such as nitrates and phosphates. The temperature range in the Lagos lagoon during the period of study was between 25°C and 33°C. Hydrocarbon degradation has been found to occur at an order of magnitude faster at mesophilic temperatures than at low water temperatures (Gunkel 1967; Zobell 1969). The temperature regimes of the Lagos lagoon are characteristic of the tropical climatic condition. It is also noteworthy that the essential nutrients, nitrates and phosphates, were present in non-limiting concentrations during the period of study. The relatively high nutrient levels in the lagoon water may be due to the dumping of other organic pollutants, especially faecal matter, which is ultimately mineralized. However, the dissolved oxygen content of water samples from the sampling stations was not lower than normal and so does not indicate a gross pollution of the ecosystem. All these factors, therefore tend to indicate that the Lagos lagoon has an inherent capability for self-purification in spite of the frequent pollution incidents.

Acknowledgements

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