Microbial degradation of petroleum hydrocarbons in a polluted tropical stream

ARTICLE *in* WORLD JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY · JULY 2007

Impact Factor: 1.78 · DOI: 10.1007/s11274-007-9345-3

Microbial Degradation of Petroleum Hydrocarbons in a Polluted Tropical Stream

Sunday A. Adebusoye [1*], Matthew O. Ilori [1], Olukayode O. Amund [1], Olakunle D. Teniola [2], S. O. Olatope [2]

[1] Department of Botany and Microbiology, University of Lagos, Lagos, Nigeria

[2] Biotechnology Division, Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria,

Email: olakunleteniola@yahoo.com, Telephone: (234)-8023533428

Abstract: Enrichments of water samples from a polluted stream with crude oil resulted in the isolation of nine bacteria belonging to the following genera: *Acinetobacter, Alcaligenes, Bacillus, Corynebacterium, Flavobacterium, Micrococcus* and *Pseudomonas*. A mixed culture was developed from the assemblage of the nine bacterial species. The defined microbial consortium utilized a wide range of pure HCs including cycloalkane and aromatic HCs. Utilization of crude oil and petroleum cuts i.e., kerosene and diesel resulted in increase in total viable count (till day 10) contaminant with drops in pH and residual oil concentration. Crude oil, diesel and kerosene were degraded by 88%, 85% and 78% respectively in 14 days. Substrate uptake studies with axenic cultures showed that growth was not sustainable on either cyclohexane or aromatics while degradation of the petroleum fraction fell below 67% in spite of extended incubation period (20 day). From the GC analysis of recovered oil, while reductions in peaks of n-alkane fractions and in biomarkers namely n-C17/pristane and n-C18/phytane ratios were observed in culture fluids of pure strains, complete removal of all the HC components of kerosene, diesel and crude oil including the isoprenoids was obtained with the consortium under 14 days. Study shows that assemblage of microbial cultures offer a more extensive degradation than pure cultures. [The Journal of American Science. 2006;2(3):48-57].

Key Words: Mixed culture; Hydrocarbons; Degradation; Bacteria; Phytane; Pristane; Microbial strains; Residual oil

Introduction

Continual crude oil spills in the Niger Delta area of Nigeria due to pipeline bursting and due to oil tanker accident and similar occurrences elsewhere have drawn attention to the problem of petroleum hydrocarbons (PHCs) contamination in the environment. Whatever the origin of contamination, some petroleum or decomposition products may reach groundwater reserves, lakes or water courses providing water for domestic and industrial use. Apart from possible hazards to health such as liver damage and skin problems (Okonkwo, 1984), such contamination is objectionable because of the very low concentration at which PHCs and associated materials can be detected by their smell and taste (Anyaegbu, 1987; Nwankwo and Irrechukwu, 1987). This problem is most serious in areas which rely on groundwater and rivers as major sources of drinking water; as constantly experienced in Lagos and Niger Delta areas of Nigeria. The pollutant may also inhibit some microbial communities that are important in some biogeochemical cycles of that ecosystem and this affects the productivity of such ecosystems (Rhodes and Hendricks, 1990).

The present-day methods of ridding the environment of spilled oil most especially in Nigeria include mechanical collection, use of sorbent materials, sinking, burning, dispersion, etc., all of which have undesirable ecological consequences (Ekundayo and Obire, 1987). Microbial degradation is the major and ultimate natural mechanism by which one can clean-up the PHC pollutants from the environment (Atlas, 1992; Amund and Nwokoye, 1993; Lal and Khanna, 1996). However each individual strain is usually characterized by an ability to utilize only a few kinds of hydrocarbons (HCs) Yeasts, for example, can oxidize only the aliphatic HCs (West *et al*., 1984; Okpokwasili and Ibe, 1987). Such bacterial genera as *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Vibrio* and *Pseudomonas* contain species that together can degrade most constituents of crude oil, including the aliphatic, alicyclic, aromatic, and polycyclic HCs (Atlas, 1992; Ko and Lebeault, 1999). It has been observed that pure cultures of the individuals species have only limited substrate ranges and are of little help in consuming the complex HC mixtures found in crude oil (Colwell and Walker, 1977;

Okpokwasili and Ibe, 1987; Adams and Jackson, 1996). Since the HC mixtures differ markedly in volatility, solubility and susceptibility to degradation, it is therefore evident that the necessary enzymes needed cannot be found in a single organism. Therefore, a mixed culture of microbial community is required for complete biodegradation of oil pollutants.

A great deal has been learned about the microbiology of PHCs by pure cultures under laboratory conditions (Amanchukwu *et al*., 1989; Amund and Adebiyi, 1991; Dixit and Pant, 2000). But to understand the fate of petroleum in soil and aquatic environments, natural assemblages of organisms must be examined. Use of natural populations as in ocular will enable individual species in the consortium to consume different HC components of the oil and also permit some of the interactions that occur in nature to occur in the laboratory: competition among organisms, commensalism, and possible sequential co-metabolic events (Lal and Khanna, 1996). The use of consortia of known microbial composition has gained recent attention owing to its effectiveness over natural mixed populations of unknown species. Lal and Khanna (1996) showed that a combination of *Alcaligenes calcoaceticus* and *Alcaligenes odorans* effected higher degradation rates than that shown by consortia of unknown microbial populations. In earlier study, Okpokwasili and James (1995) observed better utilization of kerosene by a pure culture than a mixed culture. One of the possible reasons given by the authors was antagonistic properties of individual organisms in the consortium.

Oil pollution is a continuous phenomenon most especially in oil producing countries. Thus oil pollution despite the progress of recent years will remain a considerable problem. The microbes' scavenging versatility need to be harnessed to a greater extent than at present. In this communication, the petroleum degrading potentials of axenic cultures as well as assemblage of pure bacterial strains from a polluted stream was examined with the hope of isolating and stocking useful organisms with high crude oil degrading potentials as candidate organisms for clean-up of petroleum contaminated systems.

Materials and Methods

Water samples

Water Samples were collected during dry season from three locations along the course of a polluted stream in Lagos, Nigeria. These were Shomolu, Abule-Oja and Iwaya tagged I, II, and III respectively. These stations were about few kilometres from one another. Two replicate samples were collected from each site and were transported immediately to the laboratory for further work which commenced upon arrival. The stream had the following characteristics: pH, 6.2 – 6.7; conductivity, 117.0 – 624.0 μs/cm; total dissolved solids, 363 – 719 mg/L; total suspended solids, 442 – 719 mg/L; total acidity, 45 – 79 g/L; nitrate, 44 – 79 mg/L; sulphate, 8 – 13 mg/L; salinity, 50 – 715 mg/L. All heavy metals were below the detection limit. The water was pale-brown in colour and had an offensive odour particularly at station III.

Chemicals and crude oil

Higher purity n-alkane, cycloalkane, and aromatic HCs were obtained from Farmex Nigeria Limited, Sango-Otta. Escravos high crude oil and petroleum cuts were obtained from Chevron Nigeria Limited.

Utilization of crude oil by microorganisms in the polluted stream

The water samples were aseptically dispensed into two sets of conical flasks in two replicates. Sterile Escravos light crude oil at 1.0% (v/v) was added to one set while the other set served as control. All the flasks were incubated at ambient temperature (29.0 ± 1.0 ^{o}C) in a gyratory shaker incubator operated at 120 rpm for 14 days. The total viable counts (TVCs) in each flask were monitored at intervals. TVCs were obtained after serial dilutions in sterile distilled water, spread-plating of appropriate aliquots in tryptone soy agar (TSA) and incubation of plates for 24 – 36 h at room temperature.

Assessment of bacterial populations

Total viable heterotrophic bacterial count (TVC) was determined by spread-plating each water sample (after appropriate dilution in sterile distilled water) onto TSA. The spread plates were incubated at 30 ^{o}C and examined for bacterial growth at 24 h. Enumeration of HC-degrading bacteria was performed by spread inoculation on mineral salts medium (MSM) formulated according to Mills *et al*. (1978). The medium contained the following in g/L of distilled water; NaCl, 10.0; KCl, 0.29; $MgSO_4.7H_2O$, 0.42; KH_2PO_4, 0.83; $NaNO_3$, 0.42; Na_2HPO_4, 1.25. The medium was solidified with purified agar (20.0 g). The pH was adjusted to 7.2 prior autoclaving. Crude oil (as carbon source) was supplied by vapour phase transfer as described by Raymond *et al*. (1976). Sample dilution and plate inoculation were handled as described for TVC analysis while inoculation was done for one week.

Isolation of HC-degrading bacteria and development of defined mixed cultures

The isolation of HC-degrading bacterial species was performed by enrichment on crude oil in an MSM described above. To isolate the organisms 2 mL of water sample was placed in 250 mL conical flask containing 99 mL of MSM. The medium was supplemented with 1% (v/v) crude oil as the sole source of carbon and energy and incubated with shaking at room temperature for 12 days after three repeated transfers, aliquots of appropriate dilution of the enriched culture was plated on MSM agar wherein crude oil was supplied by vapour phase transfer and incubated for one week. The resulting colonies were purified on TSA and screened for HC utilization. The mixed culture was developed by assemblage of the isolated pure strains.

HC degradation studies

Growth of the defined microbial consortium and three pure isolates was monitored in 250-mL flasks containing 99 ml MSM with 1mL of sterile crude oil as substrate. Both the consortium and pure strains were pre-grown in peptone water for 18 h before seeded into the flasks. The experiments were carried out in two replicates. Flasks containing crude oils but without inoculation served as control. Utilization of petroleum cuts, kerosine and diesel was also setup in similar manner. The pH, TVC and residual oil analysis served as biodegradation indices and were all monitored at determined intervals of time.

Extraction of residual oil

Undegraded oil (residual oil) was extracted from the culture fluids with ethylene chloride. Extraction was done by adding 10 mL of thoroughly shaken culture to a separating funnel. To this, was added 10 mL of ethylene chloride. The funnel was vigorously shaken, after which contents were allowed to settle in order for the phases to separate. The organic phase was drawn off, and thereafter quantified gravimetrically and chromatographically. The control flasks were also extracted similarly.

Gas chromatographic (GC) analysis of oil

Fresh and residual oils (1.0 μL) were analyzed by GC (Hewlett Packard 5890 Series II) fitted with flame ionization detector, and AJ & W Scientific DB-1 fused silica 15 m long column (internal diameter, 0.32 mm; film thickness, 1.0 μm). The injector and detector temperatures were maintained at 300^{o}C and 325^{o}C respectively. The column temperature was programmed to rise from 50 – 500^{o}C for 27 min.

Results

Enumeration of microbial populations

The frequency of occurrence of HC utilizes relative to the total heterotrophs is presented in Table 1. It was observed that the proportions of the HC-utilizing bacteria within the heterotrophic communities were generally less than 1.0%. However, the highest population density of heterotrophs and HC-utilizers were obtained from sample originating from station I, but sample III gave the highest percent of degraders.

Utilization of crude oil by indigenous microflora

Figure 1 illustrates the growth dynamics and population increase of microbial communities indigenous to the various samples polluted with crude petroleum as well as the undisturbed stream water. The layout of the growth patterns indicates that the population of the microbial communities increased consistently for the next 12 days before declining. Samples obtained from sites I and II attained relatively similar population densities before falling to 1.91×10^{7} and 1×10^{10} cfu/mL (cfu = colony forming units) respectively. However, the highest cell increase was obtained from the sample originating from site III. The microbial counts of this sample peaked at day 12 at 1.12×10^{11} cfu/mL and thereafter decreased to 9.12×10^{9} on the 15th day. In the case of the undisturbed control samples, a consistent decrease in population size was observed for sample III from the onset of experiment to the end (Figure 1). In the case of the other samples, a slight growth was observed between day 0 and day 6 after which it dropped sharply. This is likely due to continued cell division by the robust inoculum or utilization of endogenous substrates or exogenous nutrients in the water. In the experimental samples, the increase in population of microbial communities was accompanied by visual gradual decrease in crude oil and total disappearance on day 15.

Identification of bacterial strains

The enrichment of the water samples with crude oil resulted in the isolation of nine bacterial strains. The organisms were identified by morphological and biochemical techniques using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), as *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffi*, *Flavobacterium* sp.,

Micrococcus roseus, and *Corynebacterium* sp. However, only three of these isolates namely *Corynebacterium*, *Acinetobacter lwoffi* and *Pseudomonas aeruginosa* were selected for further studies as representative pure cultures. The mixed culture used for the biodegradation studies consist of an assemblage of the nine HC-degrading bacterial isolates.

Growth characteristics on petroleum hydrocarbons

The ability of the selected strains and the defined consortium to grow on spectrum of hydrocarbon substrates was tested in MSM amended with selected carbon substrate as the sole source of carbon and energy. Incubation was carried out at room temperature on a gyrating shaker incubator for 7 – 14 days. In our systems, growth was defined as increase in turbidity and TVC, reduction in residual oil concentration determined gravimetrically as well as disappearance of individual HC peaks by GC analysis. The mixed bacterial culture grew on all the HCs tested though to varying degrees. In the case of pure strains, growth was sustainable on long chain n-alkane, dodecane; petroleum fractions including kerosene, diesel, AP SAE 40 lubricating oil and crude oil. All the strains failed to utilize, benzene, naphthalene and toluene with exception of *A. lwoffi* which showed a slight growth on the former aromatic. Similarly, growth of the isolates was not sustainable on hexane and cyclohexane.

Changes in pH and population counts of bacterial stains as well as percent degradation in respect of residual oil concentration during utilization of crude oil and petroleum cuts are illustrated in Figures 2 – 4. All the organisms including the mixed cultures grew on these substrates without lag phase. In the mixed culture system, relatively high turbidity was observed in less than three days of incubation while it was longer in the pure culture systems. The oil layers in all cases were slowly emulsified and eventually disappeared with incubation. The growth of the organisms on the PHC substrates generally resulted in a gradual decrease in pH of the culture medium contaminant with a systematic increase in TVC with *P. aeruginosa* exhibiting it characteristic pigment on diesel and kerosene.

During degradation of crude oil, more than 60% of the oil was depleted by the pure strains during the 20-day incubation period. Mean generation times ranged between 1.97 and 2.92 days (Table 2). Expectedly, a more extensive utilization was accomplished by the mixed bacterial culture. The defined consortium achieved the highest growth yield (0.49 $day^{-1)}$ while nearly 90% of the crude petroleum was utilized (Figure 2, Table 2) within the 14-day cultivation period.. The results obtained from degradation of diesel followed almost similar sequence with those of crude oil. The mean generation time ranged insignificantly ($P < 0.05$) from 2.03 to 2.27 days for the pure strains (Table 2). The growth dynamics show a consistent increase in TVC from the day 0 until day 14 (Figure 4) after which it declined. For instance, cell of *A. lwoffi* increased from 1.2×10^5 to 7.59×10^8 cfu/mL at day 14 after which it decreased gradually apparently due to depletion in substrate level or accumulation of toxic metabolites. In the case of kerosene, generation times were slightly higher than crude and diesel oils. Similarly, cell increase was relatively less (i.e., 3 – 4 order of magnitude) while percent degradation obtained was below 80%. Growth rates obtained for the consortium and pure cultures ranged from 0.32 – 0.35 day^{-1} with the highest obtained for the mixed culture system. Generally, it would appear that diesel was utilized best on the basis of growth data obtained even though the highest degradation rate was obtained when isolates were grown with crude oil. One phenomenon worthy of note is that in spite of the apparent decrease in cell densities observed in all flasks, percent degradation increased consistently (Figures 2 and 3).

Also biomarkers namely, nC17/pristane and nC18/phytane ratios extrapolated from GC profiles decreased at a faster rate in the flasks containing mixed cultures than pure cultures (Table 3). The former ratio decreased by 89% between 0 and 20 days during growth of *P. aeruginosa* with crude oil. The equivalent decrease in *A. lwoffi* for the same period was 30%. For the latter ratio, it was initially 6.14 but decreased to 0.38 and 2.41 respectively in flasks inoculated with *P. aeruginosa* and *A. lwoffi*. The corresponding values for diesel were 1.07 and 0.24 respectively for both isolates (Table 3). In contrast to these observations, growth of the consortium on both crude and diesel oils resulted in complete lost of all alkane peaks including the isoprenoids in 14 days. Although complete lost of n-alkane and isoprenoid peaks was also observed during growth of *Corynebacterium* sp. with crude oil, however, this was not achieved until after 20 days of incubation (Table 3). The qualitative changes in the HC profiles inherent in kerosene were also revealed by GC analysis. When the mixed culture was grown with this substrate, similar to observations on crude and diesel oils, there was total disappearance of all detectable alkane peaks in less than 14 days (Chromatogram not shown) while representative peaks were still detectable after 20 days of growth with pure strains though significant reduction in concentration was nonetheless obtained.

Table 1. Population counts of heterotrophic and HC-utilizers in water samples

Sample	Total heterotrophs (cfu × 10^7)	Total HC-utilizers (cfu × 10^4)	% HC counts
I	4.5	5.7	0.124
II	2.53	1.3	0.051
III	1.53	5	0.327

Table 2. Growth potentials of hydrocarbon-utilizing bacterial strains

	Crude oil			Diesel			Kerosine		
Isolate	Tg[a]	μ[b]	% oil degradation	Tg[a]	μ[b]	% oil degradation	Tg[a]	μ[b]	% oil degradation
Corynebacterium sp.	1.97	0.35	62.8	2.03	0.34	61.43	2.09	0.33	61
Acinetobacter lwoffi	2.07	0.34	64.7	2.11	0.33	64.03	2.39	0.32	63.3
Pseudomonas aeruginosa	2.92	0.32	65.8	2.27	0.31	60	2.07	0.34	65.4
Mixed culture	1.4	0.49	88.1	1.21	0.51	85.3	1.99	0.35	77.8

[a]Mean generation time; [b]specific growth rate.

Table 3. nC17/Pristane and nC18/Phytane Ratios of Recovered Hydrocarbons During Growth of Bacterial Strains

	Crude oil				Diesel			
	Day 14		Day 21		Day 14		Day 21	
Isolate	nC17/pris[a] ratio	nC18/phy[b] ratio	nC17/pris ratio	nC18/phy ratio	nC17/pris ratio	nC18/phy ratio	nC17/pris ratio	nC18/phy ratio
Corynebacterium sp.	1.76 (18.56)	4.46 (27.36)	0 (100)	0 (100)	0.67 (50.74)	1.11 (67.73)	0.34 (75)	1 (70.93)
Acinetobacter lwoffi	1.77 (18.09)	2.91 (52.61)	1.51 (30.13)	2.41 (60.75)	1.07 (21.32)	2.37 (31.11)	0.06 (95.59)	1.07 (68.9)
Pseudomonas aeruginosa	0.87 (59.74)	0.76 (87.62)	0.24 (88.89)	0.38 (93.81)	0.4 (70.59)	0.67 (80.52)	0.1 (92.65)	0.24 (93.02)
Mixed culture	0 (100)	0 (100)			0.08 (94.12)	0.11 (96.8)		

[a]nC17/pristane ratio; [b]nC18/phytane ratio. Percent reduction of ratios (written in parentheses) have been calculated with reference to the amount recovered from uninoculated control tubes.

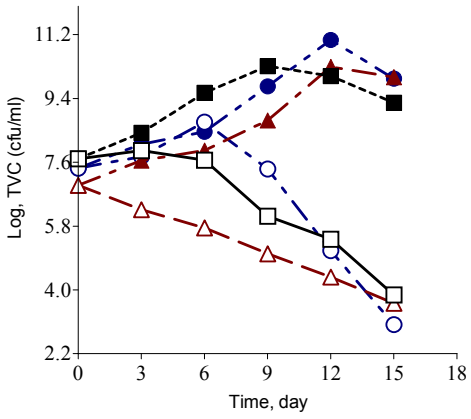

Figure 1. Crude Oil Degradation by Microbial Communities in the Polluted Streams. ■, I; ▲, II; ● III. Respective control samples are represented with open symbols.

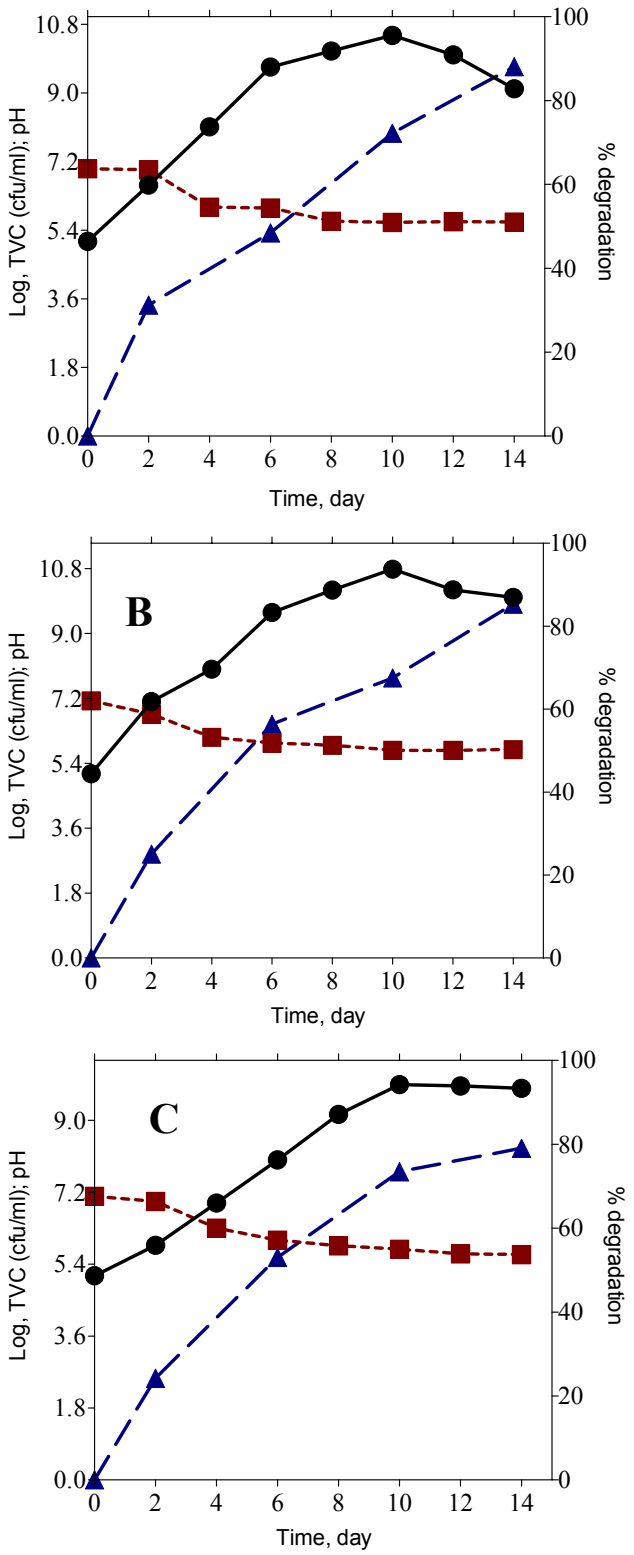

Figure 2. Degradation of Crude Oil (A), Diesel (B) and Kerosene (C) by Defined Microbial Consortium. ●, log TVC (cfu/ml); ▲, percent degradation; ■, changes in pH.

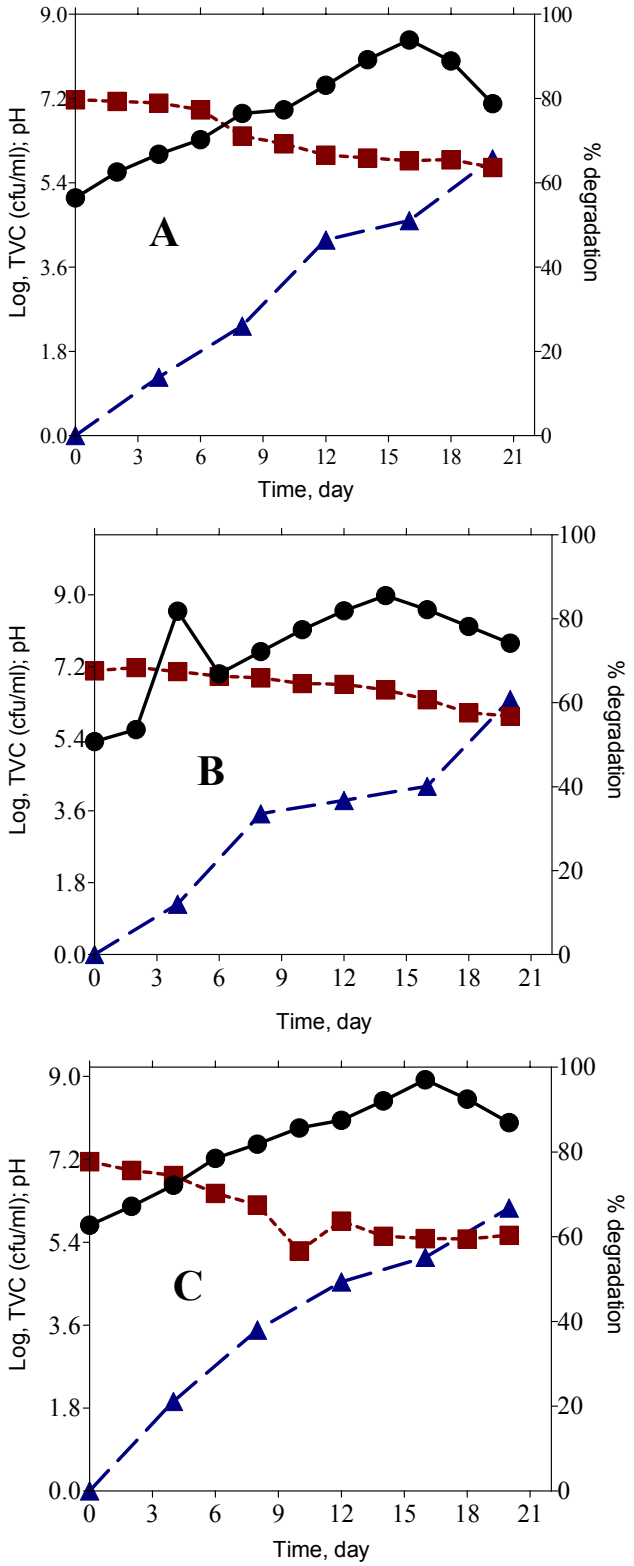

Figure 3. Degradation of Crude Oil by Pure Cultures of *Acinetobacter lwoffi* (A), *Corynebacterium* sp. (B) and *Pseudomonas aeruginosa* (C). ●, log TVC (cfu/ml); ▲, percent degradation; ■, changes in pH.

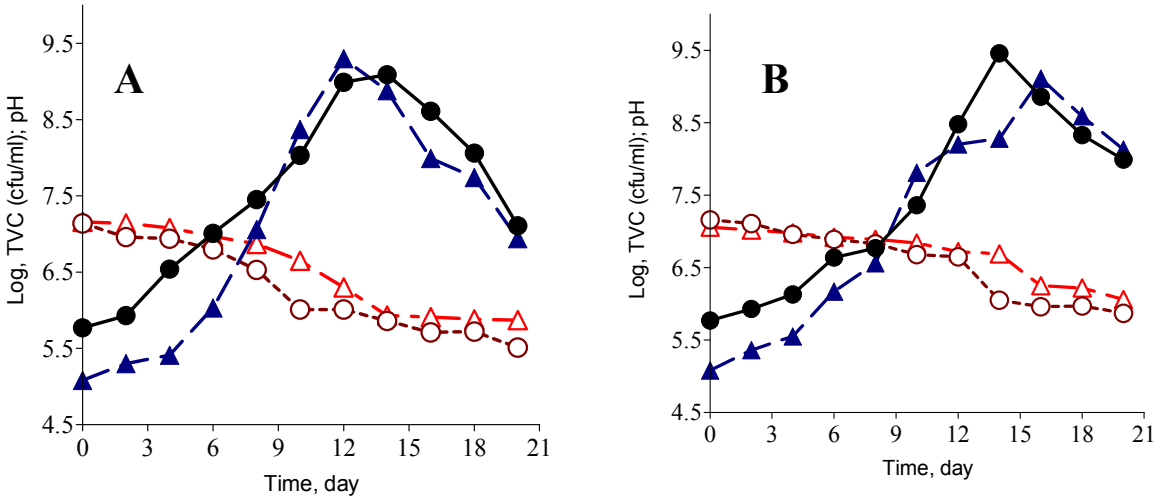

Figure 4. Growth Dynamics of Pure Cultures on Diesel (A) and Kerosene (B). Log TVC (cfu/ml) of *Pseudomonas aeruginosa*, ●; log TVC of *Acinetobacter lwoffi*, ▲; open symbols represent respective changes in pH values of the culture media.

Discussion

Land polluted with petroleum hydrocarbons (PHCs) is a major problem throughout the world. Apart from forming breeding centres for mosquitoes, the vector of the causative agent of many tropical diseases such as malaria, many economic plants and marine lives are destroyed by oil pollution (Nwankwo and Irrechukwu, 1987). The need to clean-up these sites has led to the development of effective bioremediation techniques (Hopper, 1989). One of such involves the use of competent microbial flora as inoculum to degrade the contaminant by a process referred to as bioaugmentation. Crude oil in the environment is primarily biodegraded by bacteria and fungi, which all appear to be ubiquitously distributed in aquatic and terrestrial ecosystems. Microbial degradation of crude oil is often shown to occur by attack on alkanes or light aromatic fractions, while the higher molecular weight aromatics, resins and asphaltenes are considered recalcitrant (Lal and Khanna, 1996). The results obtained during substrate spectrum analysis and growth studies are comparable to these findings. The three isolates were all long-chain n-alkane utilizers. Usually, the use of crude oil or refined petroleum as substrates for enrichment has often led to the isolation of microorganisms that metabolize n-alkanes (Atlas, 1992). Although other substrates such as naphthalene, cyclohexane, toluene that readily supported the growth of the mixed culture were recalcitrant to the pure strains, however, studies on individual HCs has been shown not to be necessarily a good guide to the microbial response to mixtures as obtained in crude oil and other petroleum cuts, since there are several reports of co-metabolism (Mechalas *et al*., 1973; Atlas, 1995; Ko and Lebeault, 1999).

As depicted in Figures 2 – 4, all the isolates including the consortium grew on all the HC substrates logarithmically concomitant with reduction in residual oil concentration and pH of the culture fluids. The growth dynamics may either be due to the constitutive nature of HC assimilating capabilities in the organisms or reflects the adaptation of the strains as a result of previous exposure to exogenous HCs. This may be followed by a concomitant development of the ability to use the oil and/or its catabolic products as carbon and energy sources. Usually microbial utilization of HCs often leads to production of organic acids (Amund and Adebiyi, 1991; Okpokwasili and James, 1995). Thus, the acids probably produced account for the reduction in pH levels. The mixed culture exhibited a superior degradative competence on all the HC substrates tested than the pure bacteria strains (Figs. 2 – 4, Tables 1 and 2). Generally mixed cultures have been most commonly found to degrade oil (Leahy and Colwell, 1990). The degradation of crude oil by these mixed populations was reported to be in the range of 21 – 68% using 1g/L of the crude oil. In the present study, nearly 90% degradation has been reported at 1 g/L concentration under 14 days. Hydrocarbon degradation particularly of crude oil, by heterotrophic consortia has been

extensively reported (Leahy and Colwell, 1990; Okpokwasili and James, 1995). It has been observed that individual organisms could metabolize only a limited range of HC substrates (Britton, 1984). This has also been shown in *Brevibacterium* and *Flavobacterium*, which degrade 40 and 75% of the crude oil in 12 days respectively, analysis by GC revealed both isolates were capable of degrading the aliphatic fractions only (Atlas and Bartha, 1972). Although our isolates degraded between 60 and 66% of the crude oil and petroleum cuts, GC analysis showed that significant peaks of alkane were left undegraded even after 20 days incubation whereas, all the detectable HC peaks inherent in crude oil, diesel and kerosene were completely utilized within 14 days by the defined bacterial consortium.

It can be inferred from the growth data that growth of the isolates and the mixed culture was most abundantly supported by crude and diesel oils than kerosene. Preference for higher molecular weight HCs has been reported previously (Amanchukwu *et al*., 1989; Okpokwasili and James, 1995). The reason for better growth on crude oil than kerosene in part may be attributed to the more complex chemical composition of the oil than diesel and kerosine. A refined petroleum product such as kerosine contain short chain HCs of 5 – 14 carbon atoms in length, and is less likely to support growth than crude and diesel oils.

From the GC profiles of residual oils, reductions in peaks and in values for biomarkers namely nC17/pristane and nC18/phytane ratios were much more pronounced in *Pseudomonas aeruginosa* than *Acinetobacter lwoffi* and *Corynebacterium* sp. with the exception of the latter on crude oil where total disappearance of alkane peaks was obtained after 20-days cultivation (Table 2). Most importantly, these chromatographic characteristics correspond to criteria used to quantify petroleum degradation due to microbial activities (Wang et al., 1994; Yveline *et al*., 1997). The growth of mixed bacteria culture with crude and diesel oils resulted in the total disappearance of all the representative HC peaks including pristane and phytane peaks in 14 days. This revelation however, shows that these compounds are not as resistant to biodegradation as once believed. Previously, Ward *et al*. (1980) also observed rapid degradation of pristane and phytane by microorganisms during growth on Amoco Cadiz oil and therefore cautioned against using them as internal standards for biodegradation. Similarly in a recent publication, Ko and Lebeault (1999) observed rapid degradation of these isoprenoids in an HC mixture by co-culture of *Pseudomonas aeruginosa* K1 and *Rhodococcus equi* P1, than by pure cultures of these organisms.

In conclusion, this work has shown the occurrence of various strains of bacteria with capability to utilize crude oil and petroleum cuts in a polluted tropical stream. The data reported here supported the premise that faster rate of degradation of HCs is achieved by the action of assemblages of pure strains of microorganisms with overall broad enzymatic capabilities rather than by a single versatile organisms as seen in *Pseudomonas aeruginosa, Acinetobacter Iwoffi* and *Corynebacterium* sp. and mixed cultures of unknown composition. Consortia of unknown composition might result in poor growth and hence less degradation as documented by Okpokwasili and James (1994), Lal and Khanna (1996) and recently by Okerentugba and Ezeronye (2003). More importantly, the release of enrichment cultures of unknown composition for bioremediation would have licensing difficulties due to their effect on the receiving ecosystem. Therefore it necessary to construct effective mixed cultures of known microorganisms as demonstrated in this study for effective bioremediation strategy.

Acknowledgements

We are grateful to Chevron Nigeria Limited for the opportunity to use their facilities particularly, Mr. Brook Patterson for his technical assistance. Adebusoye S.A. gratefully acknowledges Federal Institute of Industrial Research, Oshodi, Nigeria for a laboratory space.

Corresponding Author:
Sunday A. Adebusoye
Faculty of Science
Department of Botany and Microbiology
University of Lagos
Akoka, Yaba, Lagos, Nigeria
Phone: (234) 80-3438-8871
Email: sadebusoye@yahoo.com

Information of Authors
Prof. N. A. Olasupo
Dept of Microbiology,
Lagos State University,
Ojo, Lagos. Nigeria
Tel: +234 802 319 8533
E mail: naolasupo@yahoo.com

Dr. A. O. Olaniran
Dept of Microbiology,
University of Durban – Westville,
P. Bag X 54001, Durban 4000.
South Africa.
Tel: +27 31 204 4401
E mail: adeolaniran@yahoo.com

Dr G. Soberon – Chavex

Dept de Microbiologia Molecular,
Instituto de Biotecnologia,
Universidad Nacional Autonoma de Mexico
Apdo. Postal 510-3, Cuernavaca, Mor 62250, Mexico
Tel: +52 7 3291634
E mail: gloria@ibt.unam.mx

Dr R. Swannell,
National Environmental Technology centre
AEA Technology, 353 Harwell,
Didcot, Oxon OX11 ORA, UK.
Tel: +44 1235 463974
E mail: richard.swannell@aeat.co.uk

Sunday A. Adebusoye
Faculty of Science
Department of Botany and Microbiology
University of Lagos
Akoka, Yaba, Lagos, Nigeria
Phone: (234) 80-3438-8871

Received: June 30, 2006

References

1. Adams, P., and Jackson, P. P. 1996. Bioremediation of oil spills, theory and practice. In the Proceedings of International Seminar on Petroleum Industry and the Nigerian Environment, pp 30-42.
2. Amanchukwu, S. C., Obafemi, A., and Okpokwasili, G. C. 1989. Hydrocarbon degradation and utilization by a palm-wine yeast isolate. FEMS Microbiol. Lett. (57): 151-154.
3. Amund, O. O., and Adebiyi, A. G. 1991. Effect of viscosity on the biodegradability of automotive lubricating oils. Tribol. Intern. (24): 235-237.
4. Amund, O. O., and Nwokoye, N. 1993. Hydrocarbon potentials of yeast isolates from a polluted Lagoon. J. Sci. Res. Dev. (1): 65-68.
5. Anyaegbu, B. A. 1987. Medical perspectives of the impact of environmental pollution. In the Proceedings of International Seminar on Petroleum Industry and the Nigerian Environment, pp 352-357.
6. Atlas, R. M., and Bartha, R. 1972. Degradation and mineralization of petroleum by two bacteria isolated from coastal waters. Biotech. Bioeng. (14): 297-308.
7. Atlas, R. M. 1992. Petroleum microbiology. In Encyclopedia of Microbiology. pp. 363-369. Baltimore, MD: Academic Press.
8. Britton, L. N. 1984. Microbial degradation of aliphatic hydrocarbons. In Microbial Degradation of Organic Compounds, ed. D. T. Gibson, pp. 89-129. New York: Marcel Dekker.
9. Colwell, R. R., and Walker, J. D. 1977. Ecological aspects of microbial degradation of petroleum in the marine environment. Crit. Rev. Microbiol. (5): 423-445.
10. Dixit, V. S., and Pant, A. 2000. Hydrocarbon degradation and protease production by *Nocardiopsis* sp. NCIM 5124. Lett. Appl. Microbiol. (30): 67-69.
11. Ekundayo, J. A., and Obire, O. 1987. Use of indigenous microorganisms in ridding the environment of spilled oil. In the Proceedings of International Seminar on Petroleum Industry and the Nigerian Environment, pp 139-148.
12. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T., and William, S. T. 1994. Bergey's Manual of Determinative Bacteriology. Baltimore: William and Wilkins.
13. Hopper, D. R. 1989. Cleaning up contaminated wastesites. Chem. Engine. (96): 94-110.
14. Ko, S. H., and Lebeault, J. M. 1999. Effect of a mixed culture on co-oxidation during the degradation of saturated hydrocarbon mixture. J. Appl. Microbiol. (87): 72-79.
15. Lal, B., and Khanna, S. 1996. Degradation of crude oil by Acinetobacter calcoaceticus and Alcaligenes odorans. J. Appl. Bacteriol. (81): 355-362.
16. Leahy, J. G., and Colwell, R. R. 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. (54): 305-315.
17. Mechalas, B. J., Meyers, T. J., and Kolpack, R. L. 1973. Microbial decomposition patterns using crude oil. In The Microbial Degradation of Oil Pollutants, eds. D. G. Ahearn, and S. P. Meyers, Baton Rouge, LA: Center for Wetland Resources Publication No. LSU-SG-73-01.
18. Mills, A. L., Breuil, C., and Colwell, R. R. 1978. Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. Canad. J. Microbiol. (24): 552-557.
19. Nwankwo, J. N., and Irrechukwu, D. O. 1987. Problems of environmental pollution and control in the Nigerian petroleum industry. In the Proceedings of International Seminar on Petroleum Industry and the Nigerian Environment, pp 102-107.
20. Okerentugba, P. O., and Ezeronye, O. U. 2003. Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. Afr. J. Biotechnol. (2): 288-292.
21. Okonkwo, P. O. 1984. Toxicological effects of petroleum products pollution: a summary of some observations in Nigeria. In the Proceedings of International Seminar on Petroleum Industry and the Nigerian Environment, pp 258-259.
22. Okpokwasili, G. C., and Ibe, S. N. 1987. Genetically engineered microbes and oil degradation. In the Proceedings of International Seminar on Petroleum Industry and the Nigerian Environment, pp 97-103.
23. Okpokwasili, G. C., and James, W. A. 1995. Microbial contamination of kerosene, gasoline, and crude oil and their spoilage potentials. Material. u. Organismen (29): 147-156.
24. Raymond, R. L., Audson, J. O., and Jamison, V. W. 1976. Oil degradation in soil. Appl. Environ. Microbiol. (31): 522-535.
25. Rhodes, A. N., and Hendricks, C. W. 1990. A continuous - flow method for measuring effects of chemicals on soil nitrification. Toxicity Assessment (5): 77-78.
26. Wang, Z., Fingas, M., and Sergy, G. 1994. Study of 22 year-old Arrow spill oil samples using biomarker compounds by GC/MS. Environ. Sci. Technol. (28): 1733-1746.
27. Ward, D. M., Atlas, R. M., Boehm, P. D., and Calder, J. A. 1980. Microbial biodegradation and chemical evolution of oil from the Amoco Cadiz Spill. Ambio. (9): 277-283.
28. West, P.A., Okpokwasili, G. C., Brayton, P. R., Grimes, D. J., and Colwell, R. R. 1984. Numerical taxonomy of phenanthrene-degrading bacteria isolated from the Chesapeake Bay. Appl. Environ. Microbiol. (48): 988-993.
29. Yveline, L. D., Frederick, J., Pierre, D., Michael, G., Jean, C. B., and Gilbert, M. 1997. Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum wastes of refinery from 1956 to 1997. Mar. Pollut. Bull. (22): 103-109.