Marine Pollution Bulletin 58 (2009) 418–423

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Note

A case study of *in situ* oil contamination in a mangrove swamp (Rio De Janeiro, Brazil) ☆

Elcia M.S. Brito [a,b,c,*], Robert Duran [b], Rémy Guyoneaud [b], Marisol Goñi-Urriza [b], T. García de Oteyza [d], Miriam A.C. Crapez [e], Irene Aleluia [f], Julio C.A. Wasserman [g]

[a] Departamento de Geoquímica Ambiental, Universidade Federal Fluminense, Niterói, RJ, Brazil
[b] Equipe Environnement et Microbiologie- UMR IPREM5254, Université de Pau et des Pays de l'Adour, Pau, France
[c] Grupo de Ingeniería Ambiental, Departamento de Ingeniería Civil, Universidad de Guanajuato. Unidad Belem, Centro, Guanajuato, Gto., México
[d] Department of Environmental Chemistry (ICER-CSIC), E-08034 Barcelona, Spain
[e] Departamento de Biologia Marinha, Universidade Federal Fluminense, Niterói, RJ, Brazil
[f] Departamento de Meio Ambiente, Instituto Nacional de Tecnologia, Rio de Janeiro, RJ, Brazil
[g] Programa de Pós-Graduação em Geologia e Geofísica Marinha, Universidade Federal Fluminense, Niterói, RJ, Brazil

ARTICLE INFO

Keywords:
Hydrocarbonoclastic bacterial consortium
Bioremediation
Petroleum
Mangrove sediments
In situ experiment

ABSTRACT

Mangroves are sensitive ecosystems of prominent ecological value that lamentably have lost much of their areas across the world. The vulnerability of mangroves grown in proximity to cities requires the development of new technologies for the remediation of acute oil spills and chronic contaminations. Studies on oil remediation are usually performed with *in vitro* microcosms whereas *in situ* experiments are rare. The aim of this work was to evaluate oil degradation on mangrove ecosystems using *in situ* microcosms seeded with an indigenous hydrocarbonoclastic bacterial consortium (HBC). Although the potential degradation of oil through HBC has been reported, their seeding directly on the sediment did not stimulate oil degradation during the experimental period. This is probably due to the availability of carbon sources that are easier to degrade than petroleum hydrocarbons. Our results emphasize the fragility of mangrove ecosystems during accidental oil spills and also the need for more efficient technologies for their remediation.

1. Introduction

Mangrove wetlands are intertidal ecosystems that grow in tropical and sub-tropical regions along the coastlines, constituting important nurseries for fishes, crustaceans, birds and small mammals. In these systems, sediments behave like a sink, retaining pollutants. Thus, the toxicity of pollutants will be magnified, intensively affecting the organism's health. Among these pollutants, the petroleum compounds are the most harmful, producing immediate damages to the organisms, particularly during acute spills (Nansingh and Jurawan, 1999). In order to mitigate the damage caused by accidental oil spills, the environmental impact, extent of damage caused by the spill and time for the ecosystem to naturally self-recover have been widely studied (Garrity et al, 1994; Duke and Pinzon, 1997; Abuodha and Kairo, 2001). The mortality and/or damage to plants and animals will depend not only on the type, quantity, quality and weathering state of the oil, but also on the prevailing climatic and tidal conditions. Furthermore, agrochemical environmental characteristics of the substrate and seasonal variations (such as hydrodynamics that splash oil on the root system and trunk) can contribute to the persistence of oil over or inside the sediments, increasing the environmental impact (Garrity et al, 1994; Burns and Codi, 1998). In such situations, recovery may be extremely long (up to 50 years), or the damage may be definitive.

Guanabara Bay (close to Rio de Janeiro City, Brazil) has few remaining mangrove areas, one of which is the Guapimirim Mangrove (GM). It is a protected and well-preserved mangrove area, part of the large mangrove stands that originally covered over 285 km^2 of Guanabara Bay littoral land. Presently, an area of only 135 km^2 remains. In January 2000, the rupture of a heavy oil pipeline within the northwestern portion of Guanabara Bay spilled a catastrophic 1.3 million tons of oil, contaminating large swaths of beaches and affecting the Environmental Protected Area of Guapimirim Mangrove. Furthermore, the periodic oil spills that originated in the large petrochemical complexes within the area constitute a significant threat. Guanabara Bay also receives

☆ "**Capsule**": *In situ* microcosm study for remediation with a bacterial consortium of mangrove sediments contaminated with petroleum.

* Corresponding author. Address: Departamento de Ing. Cívil, Cuerpo Académico de Ing. Ambiental, Universidad de Guanajuato. Av. Juarez 77, Centro, Guanajuato, Gto., México. Tel.: +52 473 102 0100.
E-mail address: emsbrito@gmail.com (E.M.S. Brito).

0025-326X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2008.12.008

discharges of untreated domestic effluent, wastewater input and other industrial discharge (Godoy et al, 1991).

Two years after the Guanabara Bay accident, we investigated the microbial diversity of culturable hydrocarbonoclastic bacteria isolated from this site (Brito et al, 2006). During the isolation process, we obtained a hydrocarbonoclastic bacterial consortium (HBC) with high degradation capacity. We report here a study to evaluate the *in situ* ability of the HBC to degrade oil under natural conditions. *In situ* microcosms were set up in the same contaminated site. They were seeded with the HBC, and oil degradation was followed over a three month period.

2. Materials and methods

2.1. The hydrocarbonoclastic bacterial consortium

The experimental area is located at the GM (Fig. 1), in Guanabara Bay. Initially, surface sediment samples (0–2 cm) were collected at low tide in order to isolate the bacterial consortium able to grow in petroleum-enriched media (Brito et al, 2006).

Growth media was prepared with 500 ml of sterile seawater (filtered through a 0.45 μm membrane and autoclaved at 1 bar for 30 min), supplemented with sucrose (15 g l^{-1}), urea (10 g l^{-1}) and Arabian Light® petroleum (3 ml) and then inoculated with 50 g of sediment. The culture was incubated under constant magnetic stirring, at room temperature. After 30 days of incubation, the consortium obtained was tested for its bioremediation capacity on the *in situ* microcosms.

2.2. In situ microcosm experiments

Nine 700 cm^2 surface area microcosms were installed in GM (Fig. 1), between 5 and 30 m from the edge of the mangrove fringe. The installation sites were randomly chosen, allowing variable exposure to tides, sunlight, run-off, and proximity to red and black mangrove trees. The microcosms were constructed with 40 cm long PVC cylindrical tubes (30 cm internal diameter). To better simulate the natural conditions inside the microcosms, the lower 15 cm of the PVC cylinders that were buried in the sediment were perforated.

Three different treatments (all in triplicate) were applied to the microcosms: (a) addition of 350 ml of petroleum and 500 ml of bacterial consortia (microcosms B1, B2 and B3), (b) single addition of 350 ml of Arabian Light® petroleum (P1, P2 and P3), and (c) control microcosms, without petroleum or bacterial consortia (C1, C2, and C3). The experiment was followed for 3 months; every 7 days, we obtained *in situ* measurements of pH, Eh and temperature, and we also collected surface sediments (using a solvent-cleaned stainless spoon) for microbiological and chemical analysis (Dehydrogenase Activity, Electron Transport System and Residual Hydrocarbons).

2.3. Characterization of bacterial diversity

The T-RFLP (Terminal Restriction Fragment Length Polymorphism) was used to verify the initial microbial structure of HBC. DNA was extracted with an Ultra Clean Soil DNA Isolation Kit® (Mo Bio Laboratories), following Precigou et al, 2001, verified by 1% agarose gel electrophoresis in Tris-Acetate-EDTA buffer (TAE). DNA solutions were preserved at −20 °C.

The 16S rRNA genes were amplified by PCR using primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 926R (5′-CCGTCAATTCCTTTRAGTTT-3′), labeled with the fluorochromes TET (5-tetrachloro-fluorescein) and HEX (5-hexa-chloro-fluorescein), respectively. The reactions were cycled in a PTC 200 Thermo-cycler (MJ Research) with an initial denaturation step at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1.5 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 15 min. PCR-products were purified with the GFX PCR DNA purification kit (Amersham) and digested with 10U

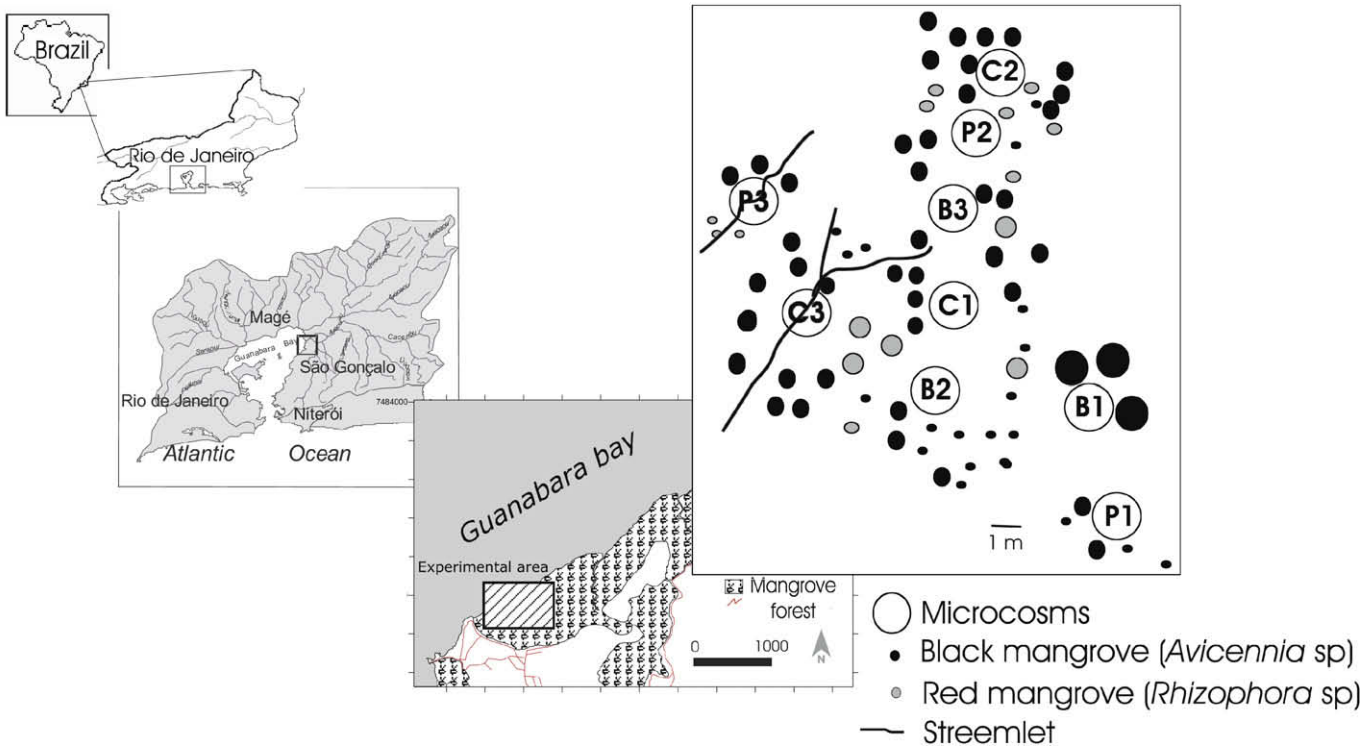

Fig. 1. Location of the experimental area in Guanabara Bay (Guapimirim Mangrove: 22°44'S, 43°02'W), where the position of the *in situ* microcosms are represented.

of restriction enzymes *HaeIII* or *HimpI1*, at 37 °C for 3 h. Approximately 50 ng of digested DNA was mixed with 15 μl of deionized formamide and denatured (94 °C for 5 min) and then chilled on ice. The lengths of the terminal restriction fragments were determined by capillary electrophoresis on an ABI prism 310 (Applied Biosystems). After an injection step of 10 s, the electrophoresis was carried out for 30 min at 15 kV. The T-RFLP profiles were analyzed using the Gene Scan Software (Applied Biosystems).

Data sets were constructed considering the peaks for which fluorescence was higher than 100 units for at least one sample, as previously described (Bordenave et al, 2004). Statistical analyses were carried out with a hierarchical cluster analysis, based on the Jaccard coefficient and UPGMA, using the MVSP software (MultiVariate Statistical Package 3.1, Kovach Computing Services).

2.4. Dehydrogenase activity

The measurement of intracellular dehydrogenase activity is a way to estimate the total microbial activity performed in soil by viable cells. The method used was described by Trevors (1982) and Houri-Davigton and Relaxans (1989) and is based on the production of formazan. The formazan concentration was determined spectrophotometrically at a wavelength of 475 nm. All assays were performed in triplicate; data points represent the average.

2.5. Fluorescein diacetate hydrolysis

Fluorescein diacetate (FDA) hydrolysis is a non-specific reaction that represents esterase activity, which involves proteases, esterases and lipases. Usually it is present during bacterial decomposition of organic material (Medzon and Brady, 1969). Microbial degrading activity was determined by measuring FDA hydrolysis, following the methodology described by Stubberfield and Shaw (1990).

2.6. Hydrocarbons

To quantify the residual hydrocarbons, the sediment samples collected in all microcosms at the beginning and end of the experiment were submitted to organic extraction in an open-vessel microwave apparatus (Pastor et al, 1997). Dichloromethane/isooctane extractions were performed at 665 W for 6 min in the microwave (*Microwave Digestion Microdigest 3.6*). The extracts were reduced to a small volume using a rotary evaporator and purified in an Al_2SO_3 silica gel clean-up column (Brito et al, 2005; Torres et al, 1999). The purified fraction was reduced to a few mL in isooctane and analyzed in a Fisons MD-800 CG/MS, with a temperature range from 60 °C to 310 °C at 4 °C min^{-1}. The capillary column used was an HP-5: 30 m × 0.25 mm ID.

3. Results and discussion

T-RFLP analysis of the HBC showed low bacterial diversity represented by four main populations with *Hinf*1I and three with *Hae*III (Table 1). The isolation of a few strains of HBC was consistent with this result: the isolated strains were related to two different genera, *Alcanivorax* sp. and *Micrococcus* sp., and to members of the Hydrothermal vent-like group. Despite the low diversity and the modest number of observed strains, these and other strains isolated from the same sampling site were able to degrade petroleum hydrocarbons efficiently (Brito et al, 2006). For example, the *Alcanivorax venusti* strain GPM2503 was able to degrade 45% of pyrene in only 21 days *in vitro*, suggesting that the strain and the HBC may have valuable potential for the remediation of oil-contaminated sediments.

Table 1
T-RFLP patterns of 16S rRNA genes amplified from total DNA extracts from the HBC digested with *Hea*III or *Hinf*1I. Fragment sizes of the T-RFs are given in base pairs.

*Hae*III digestion		*Hinf*1I digestion	
3′ fragment	5′ fragment	3′ fragment	5′ fragment
Length of T-RFs (relative abundance)			
59 (15%)	58 (31%)	59 (10%)	52 (61%)
190 (3%)	59 (69%)	61 (28%)	58 (3%)
478 (82%)	–	344 (5%)	59 (8%)
–	–	346 (57%)	204 (28%)

The results of the *in situ* remediation experiment are presented in Figs. 2 and 3. The 398/399, 835/836/837 and 1920/1921 peaks correspond to phthalate compounds that probably originated from the PVC materials used to set up the microcosm. These peaks are present in all chromatograms: from t_0 (before crude oil addition) to t_9 (the last measurement, 84 days after the oil addition). They are also the only peaks seen in the control chromatograms (see, for example, t_0). This last observation suggests that either no residual contamination remained from the abovementioned 2000 oil spill-accident or the sampling site was not affected by the oil spill. The t_1, t_6 and t_9 chromatograms presented in Fig. 2 shows that the light aliphatic fractions reduced slightly during this period. The residual peaks from the compounds that were attributed to the light aromatic hydrocarbon fraction (such as naphthalenes, phenanthrenes and dibenzothiophenes) were also observed (Fig. 3), suggesting that the degradation of the light aromatic hydrocarbons was in its initial phase after 84 days. This observation supports previous reports indicating that the order of petroleum compound biodegradation is: *n*-alkanes > iso-alkanes > medium molecular weight alkanes and aromatics > refractory compounds such as heavy polycyclic alkanes and aromatics, steranes, terpanes, resins and asphaltenes (Atlas, 1981; Oudot and Dutrieux, 1989).

Comparing the degradation patterns (Figs. 2 and 3) of the spiked crude oil in B and P microcosms (with and without the HBC) reveals similar behavior, indicating that the HBC could not stimulate oil degradation. This suggests that the minor degradation observed in all microcosms was a result of natural conditions. The microcosms were influenced by natural processes since they were semi-open systems that allowed water in and out through the perforations; they also received atmospheric inputs. Beyond microbial action, degradation under natural conditions can also be carried out by photodegradation and/or evaporation (Ramsay et al, 2000; Tam et al, 2002). Furthermore, our results demonstrate that natural oil degradation in mangrove ecosystems is very slow, which supports findings by Garrity et al. (1994) showing that the mangrove fields of Bahía las Minas (Panama) took more than 5 years to recover after they were contaminated in 1986 following a large oil spill.

The impact of oil treatment on the microcosms was also examined using dehydrogenase activity as an index, i.e., electron transport system activity (ETS) and FDA hydrolysis, also called esterase activity (Table 2). Viable microorganisms were detected in all microcosms. These data were analyzed with an ANOVA test that evaluated the variability of the stress caused by different treatments applied to the microcosms (only petroleum or petroleum + HCB). The noise was such that no significant difference in EST and FDA hydrolysis could be measured between the microcosms, suggesting natural variability greater than that imposed by the treatments themselves. Both parameters, dehydrogenase activity and FDA hydrolysis, were expected to decrease with increasing pollution, but this effect was not observed in our experiments. Our results suggest that either the amount of oil spilled was excessive enough to cause the microorganisms' death, or the seeding of HBC was enough to significantly augment microbial

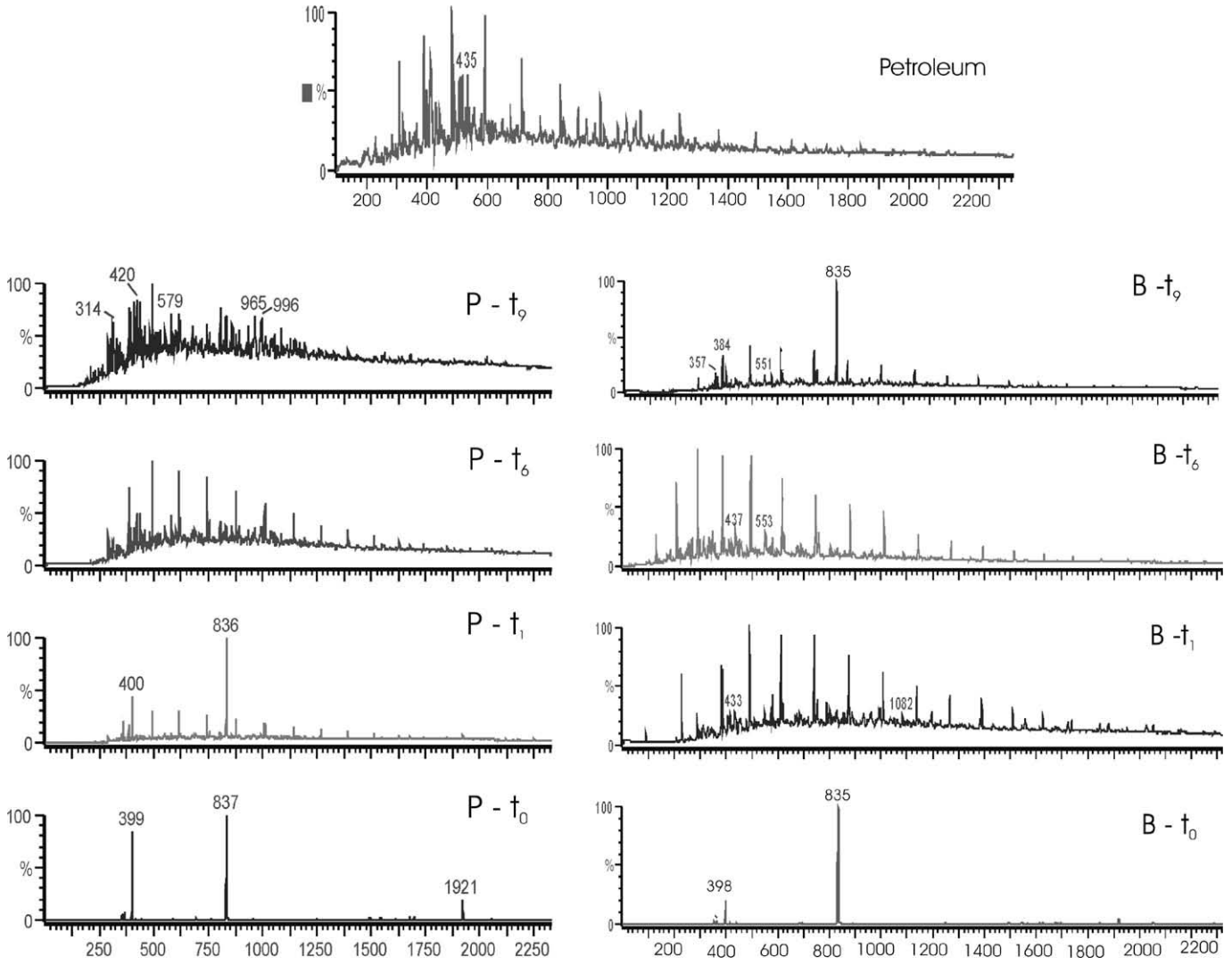

Fig. 2. B microcosms (contaminated with petroleum and seeded with HBC enrichment) and P microcosms (only petroleum applied) at t_0 (at the beginning before crude oil addition), t_1 (7 days after petroleum addition), t_6 (42 days after petroleum addition), and t_9 (84 days after petroleum addition); the natural Arabian Light petroleum chromatogram (upper).

activity. Although the inclusion of natural processes increases variability and renders interpretation more difficult, this type of experiment more accurately reproduces environmental reality.

It is also important to emphasize that just a week after seeding the microcosms with the HBC, oil emulsification was observed, implying microbial activity. These emulsions (little oil micelles) have also been suggested as indicative of oil degradation processes (Van Hamme et al., 2003; Van Hamme et al., 2006; Singh et al, 2007). In fact, the interactions between oil emulsification and biodegradation are not entirely known (e.g., Liu et al, 1995). Since the 1980s, this relationship has been observed and thought to be positive (i.e., emulsification was assumed to be part of the biodegradation process). The hypothesis is that the action of specific bacterial excreted compounds (exoenzymes or surfactants) can sufficiently reduce the interfacial tension of the oil, to allow for microemulsion formation, or pseudo-solubilization of the hydrocarbons, and subsequent hydrocarbon uptake (Reddy et al, 1982; Kearns and Losick, 2003). However, it is not easy to understand how the oil is incorporated or how different hydrocarbons travel across bacterial membranes to reach cytoplasmic metabolic enzymes (Singh and Desai, 1986; Pines and Gutnick, 1984; Sikkema et al., 1995). Various members of the microbial community probably react differentially to the effects of excreted surfactants (Bruheim and Eimhjellen, 1998; Van Hamme et al., 2001).

Usually microorganisms tracked *in vitro* do not have carbon sources other than the contaminant, so real oil degradation may be overestimated. In our experiments, there are natural carbon sources for the microorganisms (e.g., debris, leaves, etc), which are easier to use than hydrocarbons. In this case, the microorganisms have degraded hydrocarbons (spent energy) only to the extent necessary to make their surroundings habitable; they chose the less energy-intensive and more abundant natural carbon sources as their primary energy source. Furthermore, the presence of other contaminants in the natural environment (heavy metals, for instance) may also affect bacterial activity (Lin et al, 2007) and reduce the capacity for biodegradation.

It is not easy to estimate the ideal experimental period when previous comparable data are not available. Usually, the oil degradation experiments are carried out by *in vitro* microcosms (such as penicillin bottles, Erlenmeyer flasks, aquaria, bioreactors, etc.), and real-life *in situ* experiments are rare. Based on the published rate of *in vitro* hydrocarbon degradation, we designed our experiment for three months: this period was not long enough to demonstrate significant hydrocarbon degradation. Ashok et al. (1995) evaluated

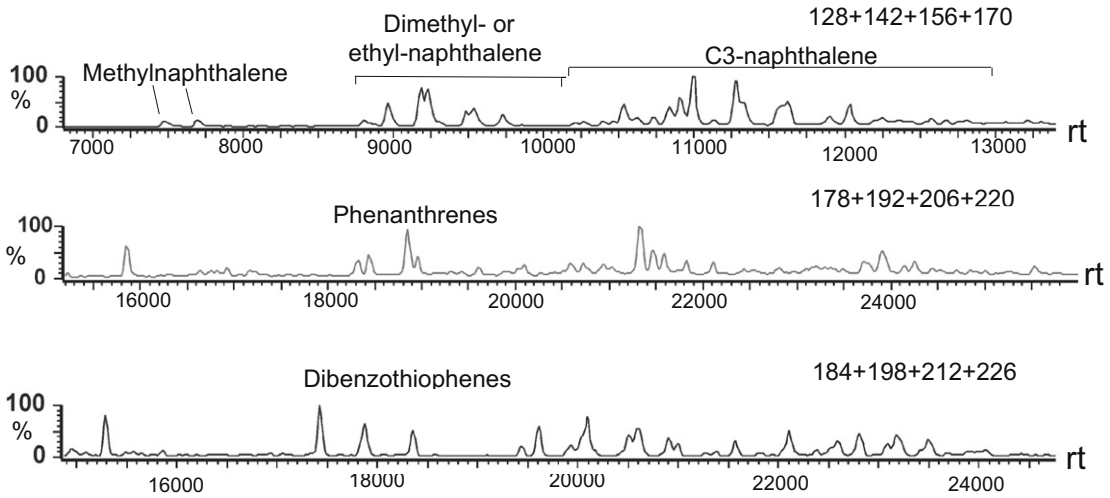

Fig. 3. P1 microcosm (only petroleum applied) 84 days after petroleum addition (t_9): The residual fraction of light aromatic hydrocarbons (naphthenes, phenanthrenes and dibenzophenes).

Table 2
Microbial activity of soil microcosms determined on intervals of 7 days (in triplicate): Mean and standard error of each microcosm ($n = 60$)

	EST controls	EST oil only	EST oil +HBC	FDA controls	FDA oil only	FDA oil + HBC
Microcosm 1	0.58 ± 0.3	0.79 ± 0.4	0.68 ± 0.3	1.2×10^{-2}	1.1×10^{-2}	1.2×10^{-2}
Microcosm 2	0.58 ± 0.4	0.69 ± 0.3	0.61 ± 0.3	8.9×10^{-3}	1.2×10^{-2}	1.1×10^{-2}
Microcosm 3	0.58 ± 0.3	0.69 ± 0.4	0.81 ± 0.3	1.0×10^{-2}	1.1×10^{-2}	1.0×10^{-2}

EST: Electron System Transport activity (μl $O_2.h^{-1}.g^{-1}$ of sediments); FDA (σ^2 0.003–0.004).

the degradation rates of several polycyclic aromatic hydrocarbons (PAHs) in soil samples. Using *in vitro* microcosms, they showed that 90% of naphtalene was degraded in only 10 days, while only 5% of chrysene was degraded after 120 days. Yuan et al. (2002) calculated that PAH half-lives ranged between 2 and 40 days in soil samples. Other researchers suggested a period of 12 months for *in situ* oil degradation and 2 months under optimal laboratory conditions (Oudot and Dutrieux, 1989). *In situ* experiments where bioremediation studies manipulate microorganisms to stimulate oil degradation are scarce among the published literature. This can be attributed to the fact that *in situ* microcosms yield complex results. Nonetheless, few biostimulation studies have examined physical and/or chemical parameters such as nitrogen, phosphorus, aeration, or the addition of surfactant substances to improve oil emulsification (Burns et al., 1999; Burns et al., 2000; Duke et al., 2000; Ramsay et al., 2000). Presumably, researchers believe that after oil spills, the microorganisms will adapt to the newer conditions and will improve oil degradation, so they try to simulate environmental conditions that will enhance microbial growth.

4. Conclusions

In spite of the low T-RFLP diversity observed in the HBC, the isolated strains were able to degrade petroleum hydrocarbons, suggesting that the HBC have valuable potential for the remediation of oil-contaminated sediments. HBC seeding on oil-contaminated mangrove sediments in *in situ* experiments did not significantly stimulate oil degradation; the microcosms exhibited extremely slow recovery. Our main conclusion is that the experiment should be done for a longer period (for one or two years) to validate these results. Although the experimental period was short (three months), some other conclusions can be formulated: the natural mangrove conditions significantly influenced degradation in the microcosms, due to the availability of carbon sources that probably competed with the hydrocarbon compounds. Since oil degradation was not stimulated by HBC, the observed slight reduction of the hydrocarbon fractions was a natural process and possibly resulted from photooxidation or other physicochemical reactions. An alternative to reduce the environmental influence on oil biodegradation stimulated by HBC would be the use of a microcosm as a sort of land farm to test the potential of bacterial consortia. The interference of phthalates in the hydrocarbon chromatograms should be avoided by using materials such as wood, steel or aluminum to set up the *in situ* microcosms.

Mangrove fields have immense ecological value, generating coastal stability and habitats for a wide range of marine beings. However, rather than preserving these rich natural resources, human activity has severely impacted them. In the proximity of large cities, hydrocarbons (from atmospheric deposition, shipping, oil spills or illegal oil-smuggling operations) assault mangrove vulnerability, highlighting the need for new remediation technologies. In this assay, the application of indigenous adapted microorganisms or consortia of microorganisms to *in situ* experiments represented a starting point and a promising approach that needs further efforts.

Acknowledgements

The authors are grateful to C. A. Caretta for helpful comments on the manuscript and to A. Demitchell for the English revision. The authors also thank Dr. R. E. Santelli for help in open-vessel microwave extractions, Dr. R. A. Epifânio for preliminary tests using Nuclear Magnetic Resonance Spectroscopy and Petróleo Brasileiro S.A., which provided the petroleum samples. This study was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Pesquisas), Conseil Régional d'Aquitaine and Conseil Général des Pyrénées

Atlantiques. E.M.S Brito received funding from Brazil and France with a Doctorate scholarship from CAPES.

References

Abuodha, P.A.W., Kairo, J.G., 2001. Human-induced stresse on mangrove swamps along the Kenyan coast. Hydrobiologia 458, 255–265.

Ashok, B.T., Saxena, S., Singh, K.P., Musarrat, J., 1995. Short communication: biodegradation of polycyclic aromatic hydrocarbons in soil around Mathura oil refinery, India. World J. Microbiol. Biotechnol. 11, 691–692.

Atlas, R.M., 1981. Microbial degradation of petroleum hydrocarbons and environmental perspectives. Microbiol. Rev. 45, 180–209.

Bordenave, S., Jézéquel, R., Fourçans, A., Budzinski, H., Merlin, F.X., Fourel, T., Goñi-Urritza, M., Guyoneaud, R., Grimaud, R., Caumette, P., Duran, R., 2004. Degradation of Erika fuel oil. Aquat. Living Resour. 17, 261–267.

Brito, E.M.S., Vieira, E.D.R., Torres, J.P.M., Malm, O., 2005. Persistent organic pollutants in two reservoirs along Paraíba do Sul-Guandu river system, Rio de Janeiro, Brazil. Quim. Nova 28, 941–946.

Brito, E.M.S., Guyoneaud, R., Goñi-Urriza, M., Ranchou-Peyruse, A., Verbaere, A., Crapez, M.A.C., Wasserman, J.C.A., Duran, R., 2006. Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. Res. Microbiol. 157, 752–762.

Bruheim, P., Eimhjellen, K., 1998. Chemically emulsified crude oil as substrate for bacterial oxidation: differences in species response. Can. J. Microbiol. 44, 195–199.

Burns, K.A., Codi, S., 1998. Contrasting impacts of localised versus catastrophic oil spills in mangrove sediments. Mangroves and Salt Marshes 2, 63–74.

Burns, K.A., Codi, S., Pratt, C., Duke, N.C., 1999. Weathering of hydrocarbons in mangrove sediments: testing the effects of using dispersants to treat oil spills. Org. Geochem. 30, 1273–1286.

Burns, K.A., Codi, S.C., Duke, N.C., 2000. Gladstone, Australia field studies: weathering and degradation of hydrocarbons in oiled Mangrove and Salt Marsh sediments with and without the application of an experimental bioremediation protocol. Mar. Pol. Bul. 41, 392–402.

Duke, N.C., Pinzon, M.Z.S., 1997. Large-scale damage to Mangrove forests following two large oil spills in Panama. Biotropica 29, 2–14.

Duke, N.C., Burns, K.A., Swannell, R.J.P., Dallahus, O., Rupp, R.J., 2000. Dispersant use and a bioremediation strategy as alternate means of reducing impacts of large oil spills on Mangroves: the Gladstone field trials. Mar. Poll. Bull. 41, 403–412.

Garrity, S.D., Levings, S.C., Burns, K.A., 1994. The Galleta oil spill. I. Long-term effects on the physical structure of the Mangrove fringe. Estuarine, Coastal and Shelf Science 38, 327–348.

Godoy, J.M., Moreira, I., Mendes, L.B., 1991. The Study of^{210} Pb method applicability to Guanabara sedimentation rates. Encontro Nacional de Aplicações Nucleares, 1. Recife. Anais, Recife UFPE 2, 285–304.

Houri-Davigton, C.H., Relexans, J.C., 1989. Measurement of the actual electrons transport system (ETS) activity in Marine sediments by incubation with INT. Env. Technol. Lett. 10, 91.

Kearns, D.B., Losick, R., 2003. Swarming motility in undomesticated *Bacillus subtilis*. Mol. Microbiol. 49, 581–590.

-Chi-Wen, Lin, -Hung-Chun, Lin, -Chi-Yung, Lin, 2007. MTBE biodegradation and degrader microbial community dynamics in MTBE, BTEX, and heavy metal-contaminated water. International-Biodeterioration-and-Biodegradation 59 (2), 97–102.

Liu, Z., Jacobson, A.M., Luthy, R.G., 1995. Biodegradation of naphthalene in aqueous nonionic surfactant systems. Appl. Environ. Microbiol. 61, 145–151.

Medzon, E.L., Brady, M.L., 1969. Direct measurement of acetylesterase in living protist cells. J. Bacteriol. 97, 402–415.

Nansingh, P., Jurawan, S., 1999. Envronmental sensivity of a tropical costlinine (Trindad, West Indies) to oil spills. Spill Sci. Tecnol. Bull. 5, 161–172c.

Oudot, J., Dutrieux, E., 1989. Hydrocarbon weathering and biodegradation in a tropical Estuarine ecosystem. Mar. Environ. Res. 27, 195–213.

Pastor, A., Vázquez, E., Ciscar, R., de la Guardia, M., 1997. Efficiency of microwave-assisted extraction of hydrocarbons and pesticides from sediments. Analusis 25, 286–292.

Pines, O., Gutnick, D., 1984. Alternate hydrophobic sites on the cell surface of *Acinetobacter* calcoaceticuls RAG-1. FEMS Microbiol. Lett. 22, 307–311.

Precigou, S., Goulas, P., Duran, R., 2001. Rapid and specific identification of nitrile hydratase (NHase)-encoding genes in soil samples by polymerase chain reaction. FEMS Microbial. Lett. 204, 155–161.

Ramsay, M.A., Swannell, R.P.J., Shipton, W.A., Duke, N.C., Hill, R.T., 2000. Effect of bioremediation on the microbial community in oiled Mangrove sediments. Mar. Poll. Bull. 41, 413–419.

Reddy, P.G., Singh, H.D., Roy, P.K., Baruah, J.N., 1982. Predominant role of hydrocarbon solubilization in the microbial uptake of hydrocarbons. Biotechnol. Bioeng., 24:1241–1269.

Sikkema, J., de Bont, J.A.M., Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59, 201–222.

Singh, M., M, Desai, J.D., 1986. Uptake of water insoluble substrates by microorganisms. J. Sci. Ind. Res. 45, 413–417.

Singh, A., Van Hamme, J.D., Ward, O.P., 2007. Surfactants in microbiology and biotechnology Part 2. Application aspects. Biotechnol. Advan. 25, 99–121.

Stubberfield, L.C.E., Shaw, P.J.A., 1990. A comparison of tetrazolium reduction and FDA hydrolysis with other measures of microbial activity. J. Microbiol. Methods 12, 151–162.

Tam, N.F.Y., Guo, C.L., Yau, W.Y., Wong, Y.S., 2002. Preliminary study on biodegradation of phenanthrene by bacteria isolated from mangrove sediments in Hong Kong. Mar. Poll. Bull. 45, 316–324.

Torres, J.P.M., Malm, O., Vieira, E.D.R., Japenga, J., Koopmans, G., 1999. Organochlorinated compounds and polycyclic aromatic hydrocarbon determination in sediments from tropical rivers in Brazil. Ciência e Cultura Journal of the Brazilian Association for the Advancement of Science 51, 54–59.

Trevors, J.T., 1982. Effect of pentachlorophenol on electron transport system activity in Soil. Bull. Environ. Contam. Toxicol. 29, 727–730.

Van Hamme, J.D., Singh, A., Ward, O.P., 2001. Physical and metabolic interactions of *Pseudomonas* sp. Strain JA5-B45 and *Rhodococcus* sp. Strain F9-D79 during growth on crude oil and effect of a chemical surfactant on them. Appl. Env. Microbiol. 67, 4874–4879.

Van Hamme, J.D., Singh, A., Ward, O.P., 2003. Recent advances in petroleum microbiology. Microbiol. Mol. Biol. Rev. 67, 503–549.

Van Hamme, J.D., Singh, A., Ward, O.P., 2006. Physiological aspects part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol. Advan. 24, 604–620.

Yuan, S.Y., Shiung, L.C., Chang, B.V., 2002. Biodegradation of polycyclic aromatic hydrocarbons by inoculated microorganisms in soil. Bull. Environ. Contam. Toxicol. 69, 66–73.