Anais da Academia Brasileira de Ciências (2014) 86(2): 745-754
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-37652014108012
www.scielo.br/aabc

Protists and bacteria interactions in the presence of oil

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Manuscript received on June 20, 2012; accepted for publication on September 2, 2013

ABSTRACT

Little is known about the role of protists and bacteria interactions during hydrocarbon biodegradation. This work focused on the effect of oil on protists from three different locations in Guanabara Bay and bacteria from *Caulerpa racemosa* (B_{Cr}), *Dictyota menstrualis* (B_{Dm}) and *Laurencia obtusa* (B_{Lo}) during a 96 h bioassay. *Cryptomonadida* (site 1, 2 and 3), *Scuticociliatida* (site 2) and *Euplotes* sp.1 and *Euplotes* sp.2 (site 3) appeared after incubation. The highest biomass observed in the controls was as follows: protist site 3 (6.0 µgC.cm^{-3}, 96 h) compared to site 3 with oil (0.7 µgC.cm^{-3}, 96 h); for bacteria, 8.6 µgC.cm^{-3} (B_{Dm}, 72 h) and 17.0 µgC.cm^{-3} (B_{Cr} with oil, 24 h). After treatment, the highest biomasses were as follows: protists at site 1 and B_{Lo}, 6.0 µgC.cm^{-3} (96 h), compared to site 1 and B_{Lo} with oil, 3.31 µgC.cm^{-3} (96 h); the bacterial biomass was 43.1 µgC.cm^{-3} at site 2 and B_{Dm} (96 h). At site 3 and B_{Lo} with oil, the biomass was 18.21 µgC.cm^{-3} (48 h). The highest biofilm proportions were observed from B_{Cr} 1.7 µm (96 h) and B_{Lo} with oil 1.8 µm (24 h). B_{Cr}, B_{Lo} and B_{Dm} enhanced biofilm size and reduced the capacity of protists to prey.

Key words: bacterial consortia, biomass, free living protist, Guanabara Bay, microbial loop, petroleum.

INTRODUCTION

Protists are unicellular eukaryotes that are widespread in all types of habitats (Buck et al. 2000, Laybourn-Parry et al. 2000, Pedros-Alio et al. 2000, Scott et al. 2001). They are an essential component of microbial food webs, and their phagotrophic activities release waste products into the environment, both as dissolved and particulate organic matter from the undigested components of prey bacteria (Nagata and Kirchman 1992a, b) and as dissolved inorganic nutrients, particularly ammonium and phosphate (Caron and Goldman 1990, Dolan 1997). Thus, protist grazing provides substrates for the further growth of prey, which include both heterotrophic bacteria (Jumars et al. 1989, Christaki et al. 1999) and autotrophic cells (Dolan 1997).

Protists are similar in size to their microbial prey, including bacteria, algae, and other heterotrophic protists. Their growth potential is the same as their microbial prey, and the high rate of protist metabolism facilitates carbon and energy flux through ecosystems (Fenchel 1987, Sherr and Sherr 1994).

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Bacteria are present in sediment in large numbers (approximately 10^{10} cells.g^{-1}). Their biomass is greater than the biomass of all other benthic organisms due to the structure and function of microbial biofilms. They possess a high surface to volume ratio, which is indicative of high metabolic rates. Dissolved inorganic and organic substrates can be metabolized with high substrate affinity and specificity. Particulate organic matter can be decomposed in close contact with the substrate by using hydrolytic enzymes (Silva et al. 2010, Guerra et al. 2011). Other than oxygen, microorganisms use alternative electron acceptors (nitrate, manganese, iron, sulfate, and carbon dioxide) for the assimilation of organic material (Edwards et al. 2005).

Oil is a complex mixture of recalcitrant and toxic substances (Wilkinson et al. 2002) and is able to disrupt cellular homeostasis (Sikkema et al. 1995, Crapez 2001). Some microorganisms utilize oil as a carbon and energy source (Crapez et al. 2001, Ron and Rosenberg 2002), and oil degradation is a multi-step process in which each step is performed via distinct processes performed by different functional groups from various microbial organisms (Dalby et al. 2008).

Despite the ecological importance of protists in aquatic and terrestrial ecosystems, relatively little is known about their role in hydrocarbon degradation compared to the reported role of bacteria. A more detailed knowledge of the role of protists in microbial interactions with hydrocarbons is essential to trace and model the fate of hydrocarbon contaminants in terrestrial and aquatic ecosystems.

This work aims to study the influence of oil (Light Arabian oil) on bacteria isolated from three seaweed samples and protists isolated from the sediment of Guanabara Bay during a 96 hour bioassay.

MATERIALS AND METHODS

Study Area

Guanabara Bay is located in the state of Rio de Janeiro in Southeast Brazil, between 22°40`-23°00`S latitude and 043°00`–043°18`W longitude. It is one of the largest bays along the Brazilian coastline and has an area of approximately 384 km^2 including its islands. The bay has a complex bathymetry with a relatively flat central channel that is 400 m wide that stretches more than 5 km into the bay and is defined by the 30 misobath. The deepest point of the bay (58 m) is located within this channel (Kjerfve et al. 1997). According to the same authors, north of the Rio de Janeiro -Niterói Bridge, the channel loses its characteristic features as the bay rapidly becomes shallower due to high rates of sedimentation, with an average depth of 5.7 m, which has accelerated in the past century due to anthropogenic activities in the catchment area.

The drainage basin of Guanabara Bay has an area of 4,080 km^2 and consists of 32 separate subwatersheds (Kjerfve et al. 1997). However, only six of the rivers are responsible for 85% (JICA 1994) of the 100 $m^3.s^{-1}$ of the total mean annual freshwater input. Currently, 11 million inhabitants live in the greater Rio de Janeiro metropolitan area, which discharges tons of untreated sewage directly into the bay (Carreira et al. 2002). There are more than 12,000 industries located in the drainage basin accounting for 25% of the organic pollution released into the bay. The bay also hosts two oil refineries along its shore that process 7% of the national oil. At least 2,000 commercial ships dock in the port of Rio de Janeiro every year, making it the second largest harbor in Brazil. The bay is also the homeport of two naval bases, a shipyard, and a large number of ferries, fishing boats, and yachts (FEEMA 1990). Recently, the chronically stressed environment has selected microorganisms able to cleave toxic compounds and form inter- or intra-specific microbial consortia to improve their survival (Crapez 2001, Crapez 2002, Fontana et al. 2006, Krepsky et al. 2007).

In this study, sediments at three different locations were sampled (Fig. 1). Site 1 is located in the northwest of the bay at Ilha do Governador (22°46.589' S, 43°11.424' W). This site has fine sand, coarse silt, a total organic carbon value near 5.2% of

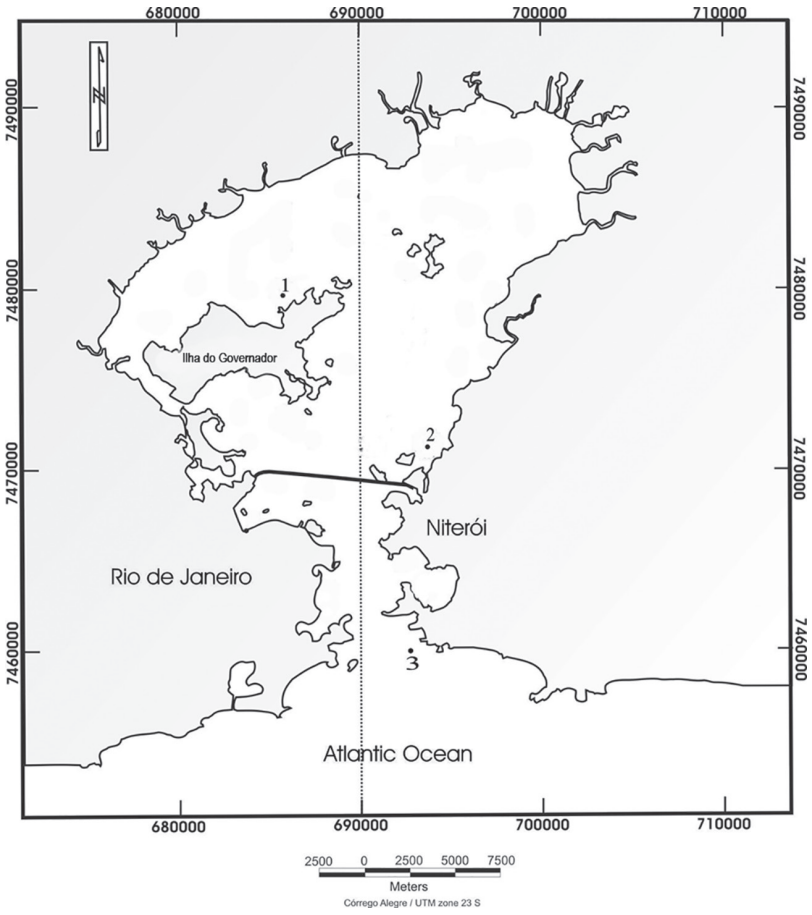

Figure 1 - Guanabara Bay and sample area.

the sediment dry weight, and an input of 458 g.C.m^{-2}. year^{-1} of organic matter (Carreira et al*.*, 2002).

Site 2 is located in the east of the bay at São Gonçalo's eutrophic river (22°51.064' S, 43°06.697' W). This site has medium silt and large space heterogeneity in sediment properties. The organic carbon measured was approximately 1.6% in this sediment (Carreira et al. 2002).

Site 3 is located at the entrance of the bay (22°59'01.1"S, 43° 04'59.8" W). This site has moderately well-sorted medium sand and a very low percentage of total organic carbon, ranging from 0.82-3.1% (Carreira et al. 2002).

Medium and Selection of Protists

The sediment samples were prepared according to Dragesco and Dragesco-Kernéis (1986) 3-4 hours after sampling. When free-living protists excysted, they were stored at 30°C for the maintenance of living bacteria and the short microbial loop (Fenchel 1987, Krepsky et al. 2007). All protist specimens were selected using glass micropipettes after six months of incubation. The protists were maintained in the laboratory in liquid medium containing coarse powdered rice at room temperature (Dragesco and Dragesco-Kernéis 1986). The identification of the protists was performed with a light microscope (Dragesco and Dragesco-Kernéis 1986), a BX 41 from Olympus, using the Protargol technique (Silva-Neto 2000).

Medium and Selection of Bacterial Consortia

The bacterial consortia were sampled from the surface of the seaweeds *Caulerpa racemosa*

(Forsskål) (Agardh 1873), *Laurencia obtusa* (Huds) (Lamouroux 1813), and *Dictyota menstrualis* (Hoyt) (Shnetter et al. 1987), according to Silva et al. (2005). They were maintained according to Krepsky et al. (2007), and oil was the source of both carbon and energy (Silva et al. 2005). Throughout the manuscript, the bacterial consortia are termed B_{Cr} (*C. racemosa*), B_{Lo} (*L. obtusa*) and B_{Dm} (*D. menstrualis*).

Bioassay

The organic carbon or biomass ($\mu gC.cm^{-3}$) of the protists and the bacteria B_{Cr}, B_{Lo} and B_{Dm} were quantified according to Gomes et al. (2007), Mauclaire et al. (2003), Carlucci et al. (1986) and Kepner and Pratt (1994), respectively. The bioassay was performed at 0, 24, 48, 72 and 96 h at room temperature (Krepsky et al. 2007) in absence of oil. In the bioassay including oil (treatment group), 1 mL of oil was added (Light Arabian oil, from PETROBRAS S.A) and the sample was incubated with shaking for 1 minute (Krepsky et al. 2007). The control groups were analyzed protists with or without consortia. The bacterial proportion, including the capsule that forms the biofilm, and the bacterial cell size were measured with an Olympus microscope, model BX 41, using the phase contrast, PH3 filter (1000 X) (Madigan et al. 2004).

Statistical Analyses

The data were analyzed with Statisoft Statistica 7, using ANOVA and MANOVA tests to analyze the carbon and biofilm results, respectively. Organic carbon data were transformed with arc-sen. Data were considered significant at $p \leq 0.05$.

RESULTS

The sediment samples from Guanabara Bay did not contain vegetative protists. They appeared after 3 weeks of laboratory incubation. In the northwest of the bay, at Ilha do Governador (site 1), *Cryptomonadida* (Senn 1900) was isolated (Kugrens et al. 2000). In the east of the bay, at São Gonçalo`s eutrophic river, (site 2), *Cryptomonadida* and *Scuticociliatida* (Small 1967) were isolated (Lynn and Small 2000). At the entrance of the bay, *Cryptomonadida* and two different ciliate species, *Euplotes sp.1* and *Euplotes sp.2*, were isolated (Lynn and Small 2000).

At site 1, *Cryptomonadida* produced 1.5 $\mu gC.cm^{-3}$ (48 h) of biomass in the bioassay control, but in the presence of oil, it only produced 0.4 $\mu gC.cm^{-3}$ (24 h). At site 2, *Cryptomonadida* and *Scuticociliatida* produced 0.7 $\mu gC.cm^{-3}$ (72 h) of biomass in the bioassay control, but in the presence of oil, they produced 0.4 $\mu gC.cm^{-3}$ (96 h). At site 3, *Cryptomonadida*, *Euplotes sp.*1 and *Euplotes sp.*2 produced 6.0 $\mu gC.cm^{-3}$ of biomass in the bioassay control, but this value was reduced to 0.7 $\mu gC.cm^{-3}$ in the presence of oil. The highest biomass was generally observed at the end of the bioassay (72-96 h) (Fig. 2a).

On the surface of *C. racemosa* (B_{Cr}), *L. obtusa* (B_{Lo}) and *D. menstrualis* (B_{Dm}) bacterial consortia, hydrocarbon-degrading bacteria was observed. The bacterial organic carbon values for the bioassay control were 8.4, 8.4 and 8.6 $\mu gC.cm^{-3}$ produced by B_{Cr} in 96 h, and by B_{Lo} and B_{Dm} in 72 h, respectively. In the presence of oil, the organic carbon produced by B_{Cr}, B_{Lo} and B_{Dm} was 17.0 $\mu gC.cm^{-3}$ in 24 h, 7.1 $\mu gC.cm^{-3}$ in 72 h and 10.9 $\mu gC.cm^{-3}$ in 24 h, respectively (Fig. 3a).

At site 1, *Cryptomonadida* in contact with the bacterial consortia showed enhanced biomass at the end of the bioassay. In the presence of B_{Cr}, B_{Lo} and B_{Dm}, *Cryptomonadida* produced 5.0, 6.0 and 0.2 $\mu gC.cm^{-3}$ of biomass, respectively, but in the presence of oil, *Cryptomonadida* produced 2.0, 3.3 and 0.4 $\mu gC.cm^{-3}$, respectively, at 96 h (Fig. 2b, c, d).

At site 1, the organic carbon produced by B_{Cr} and B_{Lo} was 5.5 and 5.9 $\mu gC.cm^{-3}$ of biomass at 96 h. B_{Dm} produced 1.1 $\mu gC.cm^{-3}$ of biomass in 72 h, but in the presence of oil, B_{Cr}, B_{Lo} and B_{Dm} produced 1.8 and 3.3 $\mu gC.cm^{-3}$ at 96 h and 0.7 $\mu gC.cm^{-3}$ at 72 h, respectively (Fig. 3b, c, d).

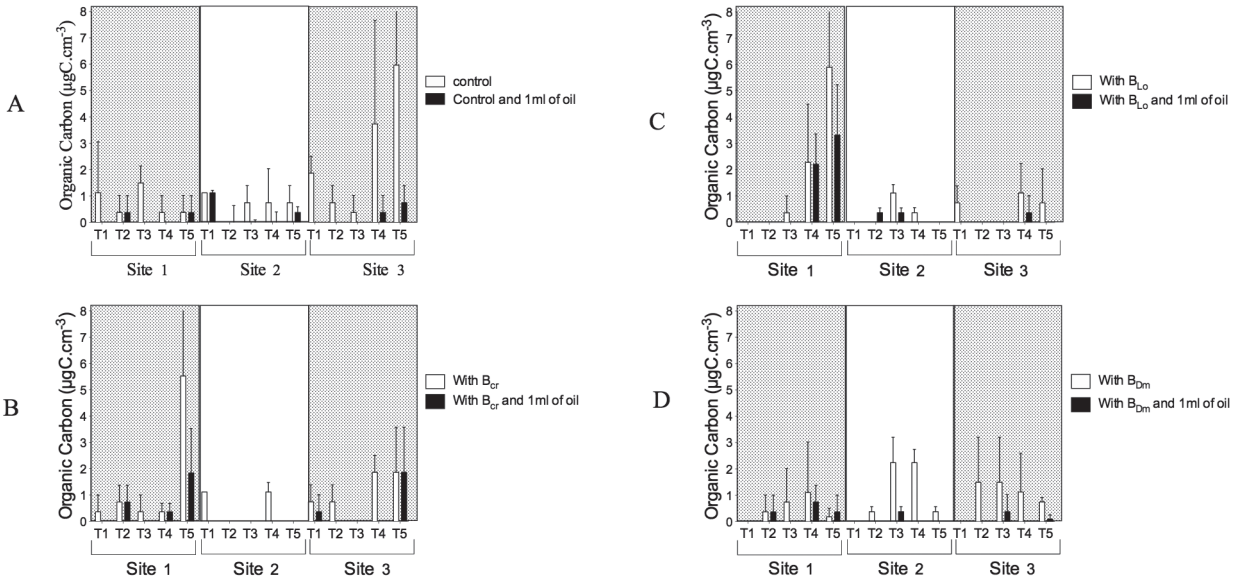

Figure 2 - Biomass from protists sampled from Guanabara Bay under influence of Bacterial consortia sampled from seaweed and oil. A-Control; B-With B_{Cr}; C-With B_{Dm}; D-With B_{Lo}.

At site 2, *Cryptomonadida* and *Scuticociliatida* in the presence of both B_{Cr} and B_{Lo} produced 1.2 µgC.cm^{-3} at 72 and 48 h. In the presence of B_{Dm}, *Cryptomonadida* and *Scuticociliatida* produced 2.2 µgC.cm^{-3} of biomass at 72 h. In the presence of oil, their biomass was reduced to 0.0 µgC.cm^{-3} in the presence of B_{Cr}, and 0.37 µgC.cm^{-3} in the presence of B_{Lo} after 48 h and B_{Dm} at 72 h (Fig. 2b, c, d).

At site 2, the bacterial organic carbon produced by B_{Cr} was 53.53 µgC.cm^{-3} of biomass at 72 h, and the carbon produced by B_{Lo} and B_{Dm} was 29.5 and 43.0 µgC.cm^{-3} of biomass after 48 h, respectively. However, in the presence of oil, B_{Cr}, B_{Lo} and B_{Dm} produced 10.57, 11.47 and 3.05 µgC.cm^{-3} at 72 h, respectively (Fig. 3b, c, d).

Cryptomonadida, *Euplotes sp.1* and *Euplotes sp.2* at site 3 in the presence of B_{Cr} or B_{Lo} produced a protist organic biomass of 1.9 and 1.1 µgC.cm^{-3} of biomass at 72 h, respectively. In the presence of B_{Dm}, *Cryptomonadida*, *Euplotes sp.1* and *Euplotes sp.2* produced 1.5 µgC.cm^{-3} of biomass at 24 h. In the presence of oil, they produced 1.9 and 0.4 µgC.cm^{-3} of biomass in the presence of B_{Cr} or B_{Lo} at 72 h, respectively, and 0.4 µgC.cm^{-3} after 48 h with B_{Dm} (Fig. 2b, c, d).

At site 3, the organic carbon produced by B_{Cr}, B_{Lo} and B_{Dm} was 11.35, 21.34 and 13.90 µgC.cm^{-3} of biomass at 96, 72 and 48 h, but in the presence of oil, B_{Cr}, B_{Lo} and B_{Dm} produced 10.78, 18.21 and 10.64 µgC.cm^{-3} at 96, 48 and 72 h, respectively (Fig. 3b, c, d).

At the end of the bioassay, the size of the B_{Cr}, B_{Lo} and B_{Dm} biofilm was 1.5 µm (48 h), 1.5 µm (72 h) and 1.5 µm (72 h), respectively; however, in the presence of oil, the capsule size was 1.7 µm (96 h), 1.6 µm (24 h) and 1.8 µm (24 h), respectively. The B_{Cr}, B_{Lo} and B_{Dm} cell size was 2.5 µm (96 h), 2.0 µm (72 h) and 2.0 µm (48 h), but in the presence of oil, the cell size was 2.3 µm (72 h), 2.2 µm (72 h) and 2.3 µm (96 h), respectively (Fig. 4a, b, c).

Statistical analyses showed significant differences between the biomass of protists and bacteria (ANOVA, $p \leq 0.05$), but their biomasses were significantly linked at 48 and 72 h of the bioassay (Tukey test, $p < 0.05$). The organic carbon produced by protists was not significantly affected in the bioassays by the presence of oil (Tukey test, $p > 0.05$).

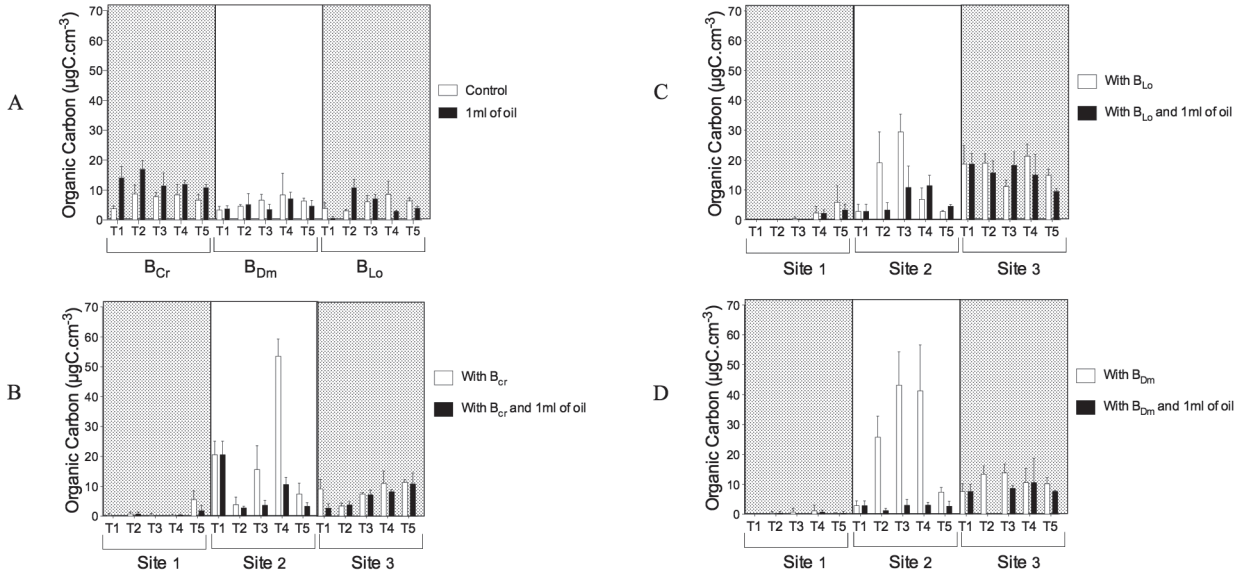

Figure 3 - Biomass from Bacterial consortia sampled from seaweed under influence of protists sampled from Guanabara Bay and oil. **A-**Control; **B-**With B_{Cr}; **C-**With B_{Dm}; D-With B_{Lo}.

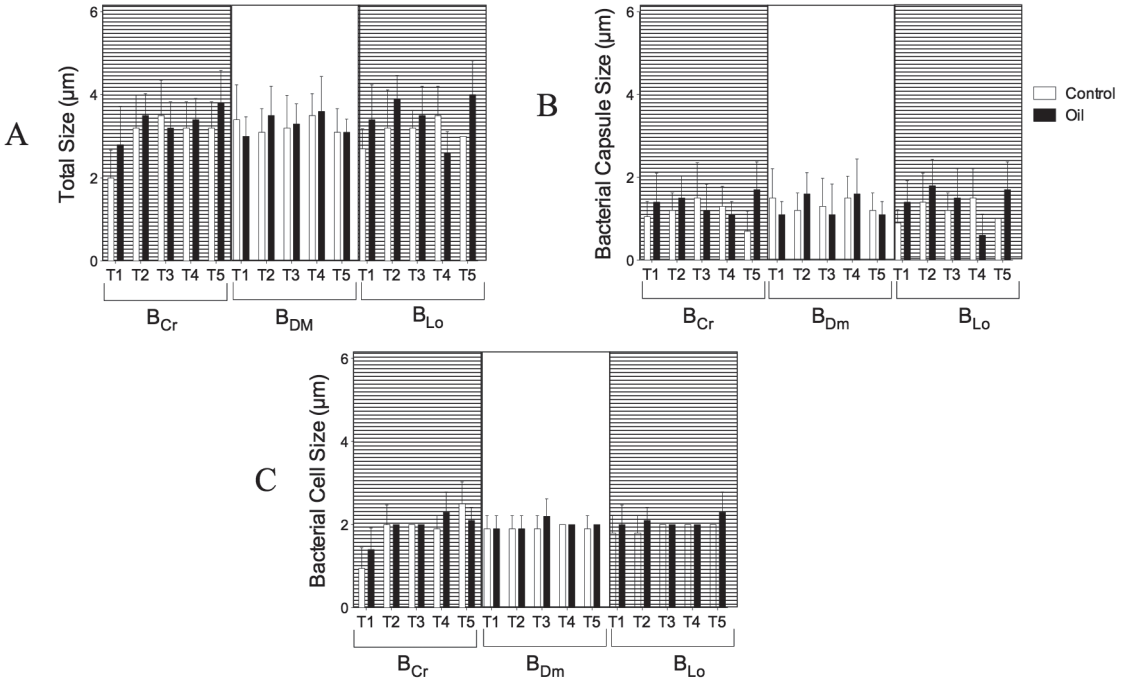

Figure 4 - Bacterial proportions measured during oil degradation. **A-**Total size; **B-**Bacterial capsula size; **C-**Bacterial cell size.

The quantity of organic carbon produced by the bacteria B_{Cr}, B_{Lo} and B_{Dm} was significantly different. The bacterial carbon from B_{Cr} and B_{Dm} showed significant differences in the bioassay in the presence of protists and oil (Tukey test, $p<0.05$). Oil also significantly affected the bacterial length in the bioassays (MANOVA, $p<0.05$).

DISCUSSION

Free-living protists are very common in bodies of water (Fenchel 1987, Sigee 2005). *Scuticociliatida*, *Cryptomonadida* and *Hipotrichea* are very common in eutrophized bodies of water, such as the Guanabara Bay (Slàdeček 1973, Foissner et al. 1995, Paiva and Silva-Neto 2004).

In the bioassay controls, protists showed a reduced ability to live in the presence of oil, and their associated bacteria did not utilize oil as a source of carbon and energy (Zarda et al. 1998, Mauclaire et al. 2003).

The hydrocarbon-degrading bacteria *C. racemosa* (B_{Cr}), *L. obtusa* (B_{Lo}) and *D. menstrualis* (B_{Dm}) emulsify hydrocarbon-water mixtures, which enable them to grow on the oil droplets (Crapez 2002, Krepsky et al. 2007, Rahman et al. 2003). These emulsification properties have also been demonstrated to enhance hydrocarbon degradation in the environment, making them potential tools for oil spill pollution control (Banat 1995, Krepsky et al. 2007).

The hydrocarbon-degrading bacteria are cosmopolitan, and seaweed offers a suitable place for bacterial establishment (Atlas 1995, Armstrong et al. 2000). Seaweeds offer a large surface for the settlement of bacteria, oxygen and dissolved organic matter (Armstrong et al. 2000, Sigee 2005). On the other hand, the bacteria consume the organic matter, releasing carbon, nitrogen, and phosphorus and provide defense against pollutants, such as oil and aromatic compounds, and epiphytic organisms (Bell et al. 1974, Atlas 1995, Sigee 2005).

Protists release some nitrogen and/or phosphorus-containing compounds when preying on inactive bacterial cells (Hahn and Höfle 2001, Kujawinski et al. 2002, Matz and Jürgens 2001). Protist feeding rates can be affected by characteristics of the prey particles, such as electrostatic charge (Hammer et al. 1999), cell shape (Kolaczyk and Wiackowski 1997) and exopolymer secretions (Liu and Buskey 1999).

During the bioassays, the hydrocarbon-degrading bacteria B_{Cr}, B_{Lo} and B_{Dm} showed significant size differences in the bioassays in the presence and absence of oil. These differences manifested in the increasing size of the biofilm. The biofilms contain bacteria depositing exopolysaccharides (EPS), which exhibit amphiphilic properties allowing these macromolecules to interface with hydrophobic substrates, such as hydrocarbons. These macromolecules effectively increase the solubility of aromatic hydrocarbons and enhance their biodegradation by the microbial community (Pacwa-Płociniczak et al. 2011, Gutierrez et al. 2013).

In the presence of oil, the production of a biofilm with surfactant activity (Krespsky et al. 2007) and an increase in cell size resulted in an increase in the biomass of the bacteria B_{Cr}, B_{Lo}, B_{Dm} and a decrease in the biomass of *Scuticociliatida*, *Cryptomonadida* (site 2), *Euplotes sp*. and *Cryptomonadida*, (site 3), suggesting a reduction in grazing and a decrease in the transfer of carbon to higher trophic levels. Site 2 had high concentrations of coprostanol due to the input of sewage from the city of São Gonçalo (Carreira et al. 2001), which selects the microorganisms capable of living in environments with high concentrations of organic matter.

In environments polluted by oil, such as site 1, hydrocarbon-degrading bacteria and their predators, such as *Cryptomonadida,* are selected (Crapez et al. 2001). The growth of the hydrocarbon-degrading bacteria B_{Cr}, B_{Lo} and B_{Dm} was followed by the growth of grazing *Cryptomonadida*. Site 1 is the nearest to the REDUC oil refinery, and sediment samples may be classified as moderately to highly contaminated (250 to 500 $\mu g.kg^{-1}$ total PAH) (Silva et al. 2007).

CONCLUSIONS

Protists were not found in a vegetative form, which is most likely linked to environmental pollution.

Oil pollution selects the hydrocarbon-degrading bacteria, and this resulted in an increase in the biomass of *Cryptomonadida* at site 1, suggesting a transfer of carbon to higher trophic levels.

Site 2 was polluted by sewage, which prevented *Cryptomonadida* survival in the presence of oil, suggesting a bottom-up effect.

Although hydrocarbon-degrading bacteria showed an increase in cell size and produced a biofilm with surfactant activity, the biomass of *Scuticociliatida*, *Cryptomonadida* (site 2), *Euplotes sp*. and *Cryptomonadida* (site 3) decreased. These sites showed no significant contribution of oil to select hydrocarbon-degrading microorganisms or oil-resistant protists.

ACKNOWLEDGMENTS

The authors wish to thank Antônio de Paula Filho from the IQ institute (UFRJ) and Camila M. da Silva, who mediated the transfer of Light Arabian oil from PETROBRAS to UFF. This study was supported by grants from the Brazilian Petroleum Agency (ANP) and its scholarship program (PRH-11), grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

RESUMO

Pouco se sabe sobre o papel dos protistas e as interações bacterianas durante a biodegradação de hidrocarbonetos. Este trabalho se concentrou no efeito do óleo sobre protistas de três localidades diferentes na Baía de Guanabara e bactérias de *Caulerpa racemosa* (B_{Cr}), *Dictyota menstrualis* (B_{Dm}) e *Laurencia obtusa* (B_{Lo}) durante 96 h de bioensaio. *Cryptomonadida* (locais 1 , 2 e 3), *Scuticociliatida* (local 2) e *Euplotes* sp.1 e *Euplotes* sp.2 (local 3) apareceram após incubação. As biomassas mais elevadas observadas nos controles foram como se segue: protista local 3 (6,0 μgC.cm^{-3}, 96 h) comparado com o local 3 com óleo (0,7 μgC.cm^{-3}, 96 h); para as bactérias, 8,6 μgC.cm^{-3} (B_{Dm}, 72 h) e 17,0 μgC.cm^{-3} (B_{Cr} com óleo, 24 h). Após o tratamento, as maiores biomassas foram como se seguem: protistas no local 1 e B_{Lo}, 6,0 μgC.cm^{-3} (96 h), em comparação com o local 1 e B_{Lo} com óleo, 3,31 μgC.cm^{-3} (96 h), a biomassa bacteriana foi de 43,1 μgC.cm^{-3} no local 2 e B_{Dm} (96 h). No local 3 e B_{Lo} com óleo, a biomassa foi 18,21 μgC.cm^{-3} (48 h). As maiores proporções de biofilme foram observadas de 1,7 μm B_{Cr} (96 h) a 1,8 μm B_{Lo} com óleo (24 h). B_{Cr}, B_{Lo} e B_{Dm} aumentaram o tamanho do biofilme e reduziram a capacidade dos protistas predarem.

Palavras-chave: consórcio bacteriano, biomassa, protista de vida livre, Baía de Guanabara, alça microbiana, petróleo.

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