MARINE BACTERIOPLANKTON DENSITY DYNAMICS IN MICROCOSMS SUPPLEMENTED WITH GASOLINE

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Dynamics of bacterial cell density in microcosms supplemented with gasoline evolved differently as compared with the control, emphasizing the role of gasoline on the dynamics of bacterial cell density. In supplemented microcosms, but not in the control, an association containing both organo- and phototrophic microorganisms developed that appear to be an important change in microbiota as a result of gasoline presence.

Key words: microcosms, bacterial density, cyanobacteria, epifluorescence.

INTRODUCTION

The role of prokaryotes in petroleum hydrocarbons consumption started to be an interesting field of Microbiology (*e.g.* ZoBell, 1946; Zarnea and Leu, 1967; Jones and Edington, 1968; Jones *et al*., 1970; Yoshida and Yamane, 1971; Atlas and Bartha, 1972), which has flourished in the past three decades (Atlas, 1981; Elsad, 1986; Leahy and Colwell, 1990; Head and Swannell, 1999; Lazar *et al*., 1999; Ramos *et al*., 2002; van Hamme *et al*., 2003; Voicu *et al*., 2003; de Oteyza *et al*., 2004; Harayama *et al*., 2004; Diestra *et al*., 2005; Head *et al*., 2006; Segura *et al*., 2007; Stefanescu *et al*., 2008; Nikolopoulou and Kalogerakis, 2009; Tanase 2009; Lazaroaie 2008, 2009).

Taking into account the advantages of microcosms (Iturbe *et al*. 2003; Molina-Barahona *et al*., 2004) we started research on marine microbiota able to tolerate/oxidize gasoline (Ardelean *et al*. 2009 a, b, c), a complex hydrocarbon mixture whose consumption by heterotrophic bacteria is under increased research (Jamison *et al*., 1975; Ridgway *et al*., 1990; Zhou and Crawford, 1995; Cunha and Leite, 1997; Solano-Serena, 1999, 2000; Röling *et al*., 2002, 2004; Sánchez *et al*., 2006; Teira *et al*., 2007; Genovese *et al*., 2008).

The aim of this paper is to compare the dynamics of marine bacterioplankton cell density in microcosms supplemented with gasoline and ammonium acetate, as experimental model systems for natural marine environments.

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MATERIAL AND METHODS

Samples. Water samples were collected from the Black Sea (0.5 m depth-Tomis harbor) and kept in polyethylene transparent bottles (Ardelean *et al*., 2009 a, b, c).

Microcosms construction was done as previously shown (Ardelean *et al*., 2009 a, c) as follows: Black Sea natural sample-control (M3); control supplemented with petroleum hydrocarbons (gasoline-0.25% v/w) (M2) and iii) control supplemented with petroleum hydrocarbons (gasoline-0.25% v/w) and nutrients (ammonium acetate 0.005% w/w) (M1) which were kept at ambient temperature and natural illumination for two months (from March 29 till May 30).

The disruption of planktonic cell aggregates and **Cell enumeration** were done as previously shown (Ardelean *et al*., 2009) adapted from literature (Sherr *et al*., 2001).

RESULTS AND DISCUSSION

BACTERIAL DENSITY DYNAMICS IN THE THREE TYPES OF MARINE MICROCOSMS

The original results obtained are presented in Figure 1 where one can see that the microcosms shows a in time (March the 29th till May the 30th) a dynamics of cell density from 2.9×10^3 to 15.2×10^3 cells/mL (M3 – control), from 3×10^3 to 64×10^3 in M1 and from 5.3×10^3 to 67×10^3 in M2.

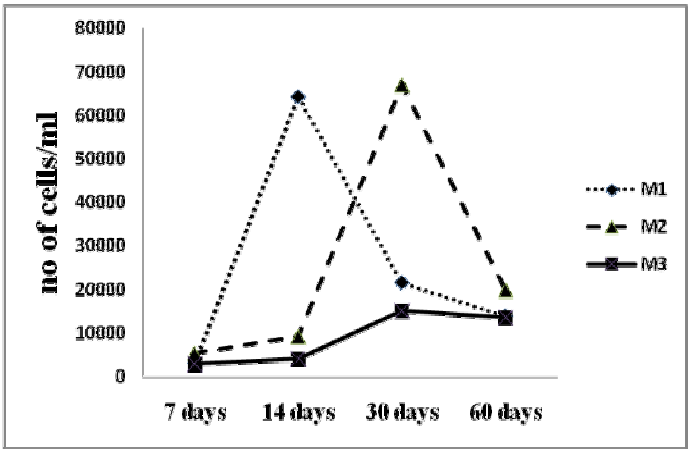

Fig. 1. Time evolution of cell density counted by epifluorescence microscopy (AO staining) in the three types of microcosms.

The experimental results show that the time evolution of cells densities in control (M3) is different from that in M2 and M1. The slow increase in control

could be explained by the slow increase in outdoor temperature from March till May, whereas the high increase cell densities in M1 and M2 could be further related to the presence of gasoline 0.25% v/w and ammonium acetate 0.005% in M1, and gasoline only in M2. The higher cell density is almost the same in both microcosms, the faster increase being counted in microcosms supplemented with both gasoline and ammonium acetate (M1), probably because of the nitrogen availability (and extra carbon source) in the form of ammonium acetate in M1 as compared with M2 where only gasoline was added.

As one can see in Figure 1, the true intriguing aspect concerns the diminution of total cell densities in M1 and M2 towards the end of this experiment. One possible explanation for this huge and sharp decrease in cell densities in microcosms could be the presence of bacteriovorous microorganisms (Vazquez-Dominguez *et al*., 2005). As one can see in Figure 2, there are visible eukaryotic microorganisms in samples from M1 and M2, but not in the control (not shown).

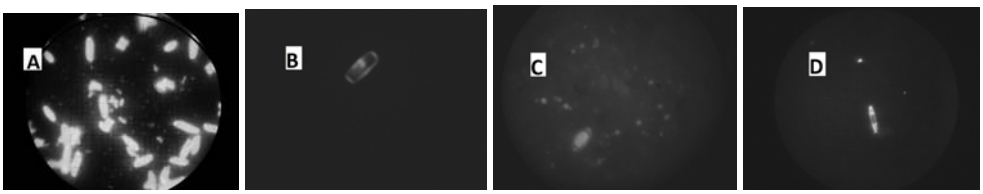

Fig. 2. The presence of eukaryotic microorganisms in microcosms. A – AO staining; B, C – natural chlorophyll fluorescence; D – DAPI colored samples.

To check this hypothesis/assumption there is the need to prepare microcosms by filtering the sea water through Millipore filter (0.45 μm) in order to remove eukaryotic microorganisms (*e.g.* flagellates) (Vazquez-Dominguez *et al.*, 2005), as previously indicated (Ardelean *et al*. 2009b).

Interestingly, in M1 and M2 but not in control (M3) within 3 months from the start of the experiment, a significant macroscopical layer of phototropic microorganisms has developed. This layer occurred mainly at the interface between the sediment and the water column as well as discrete macroscopic colonies on both sides of polyethylene transparent bottles. In Figure 3 there are presented images showing the natural fluorescence of chlorophyll, as an image of marine oxygenic gasoline tolerant/oxidant phototrophic microorganisms.

Interestingly, as one can see in Figure 4, in microcosms supplemented with gasoline, at the interface between the sediment and water column, after 11 months from the start of experiment, there are also filamentous cyanobacteria which differentiate heterocysts; up to our best knowledge this is the first image of a hydrocarbon/gasoline tolerant cyanobacterium which actually differentiates heterocysts, besides light dependent nitrogen fixation has been experimentally measured in oil contaminated sediments (Musat *et al*., 2006).

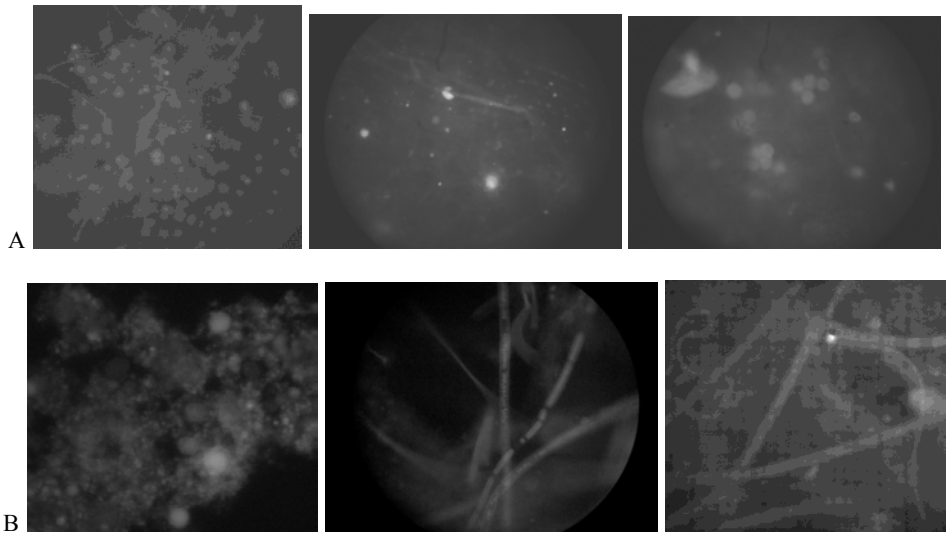

Fig. 3. Autofluorescence of chlorophyll from oxygenic photosynthetic microorganisms: microcosms 1 (A) and 2 (B), grown at the interface between the sediment and water column.

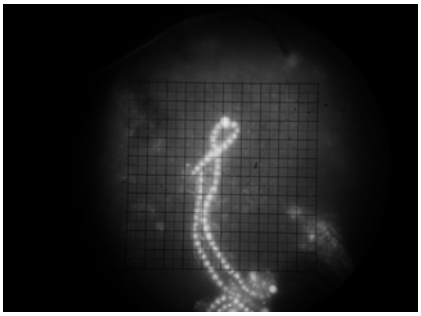

Fig. 4. Cyanobacteria with heterocyst presence in M2; AO staining.

These results show that photosynthetic microorganisms in these layers living in microcosms 1 and microcosms 2 (but not in M3, the control) are mainly filamentous, the unicellular ones being also present. The rationale of these differences is under study, as well as the attempts to isolate the photosynthetic microorganisms in axenic cultures.

These results argue that the relative abundance of oxygenic photosynthetic microorganisms at interfaces in M1 and M2 could be related to the possible involving of cyanobacteria in hydrocarbon oxidation, an increasing topic in petroleum microbiology (Al-Hasan *et al*., 1994*,* Raghukumar *et al.*, 2001*,* Röling *et al*., 2002, Harayama *et al.*, 2004, Head *et al*., 2006). These results indicate us to further investigate the density of oxygenic photosynthetic microorganisms, including cyanobacteria in the three microcosms.

CONCLUSIONS

The dynamics of marine bacterioplankton cell density measured by epifluorescence microscopy (acridine orange staining) showed that: there is a significant initial increase in cell densities in microcosms supplemented with gasoline 0.25% v/w and ammonium acetate 0.005% (M1) and gasoline alone (M2) as compared with the control (M3), followed by a sharp decrease produced, as suggested by indirect preliminary results, by the activity of bacteriovorous microorganisms.

In M1 and M2, but not in M3, oxygenic photosynthetic microorganisms, including cyanobacteria, developed macroscopic layers at the interface between sediment and water column and macroscopic colonies on the transparent walls of microcosms. These results suggest the enhancement of cyanobacterial growth in microcosms in the presence of gasoline, including the possibility for increased nitrogen input into the microcosm by photosynthetic nitrogen fixation.

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