

ENRICHMENT OF PIEZOTOLERANT HYDROCARBON DEGRADERS FROM DEEP WATER COMMUNITIES OF THE EASTERN MEDITERRANEAN SEA

Georgia Charalampous¹, Efsevia Fragkou¹, Nicolas Kalogerakis^{1,2}, Eleftheria Antoniou^{1,3}, Evangelia Gontikaki^{1,2}

¹ School of Chemical and Environmental Engineering, Technical University of Crete, 73100 Chania, Greece

² Institute of Geoenergy, Foundation for Research and Technology Hellas, 73100 Chania, Greece

³ School of Mineral Resources Engineering, Technical University of Crete, 73100 Chania, Greece

Corresponding author email: geoch1990@gmail.com (G.C), egontikaki@ipr.forth.gr (E.G)

ABSTRACT

Background information

Oil and gas exploration activities in the Eastern Mediterranean Sea (EMS) are currently in progress at depths similar or greater than those where the Deepwater Horizon blowout occurred. The vast majority of studies addressing the microbial community response to oil contamination in the deep sea have been conducted at atmospheric pressure or at *in situ* pressure following decompression during sample retrieval, despite that pressure has been acknowledged as a critical factor affecting the function of microbial communities. While site-specific research is required in order to develop effective bioremediation protocols, the self-healing ability of the EMS through biodegradation after a potential deep oil spill has not been addressed so far under *in situ* pressure conditions. In this study, the effect of pressure, crude oil and dispersant on deep EMS microbial communities was studied using un-decompressed (HP treatment) and depressurized (REPRESS treatment) deep-sea water.

Methodology

Seawater for the HP treatment was collected with a high-pressure sampling unit from a depth range of 600-1000 m and was retrieved to the surface without pressure disruption. Seawater was also collected from 1000 m using Niskin bottles (REPRESS treatment). HP and REPRESS microbial communities were acclimatized in HP-vessels in the presence of crude oil (WAF) or dispersed-crude oil (CEWAF; 1:25 COREXIT 9500A) for 7 days at 10MPa. The HP and REPRESS acclimatized communities were used as inocula for the sequential enrichment of piezotolerant hydrocarbon-degrading bacteria. The HP WAF and CEWAF inocula were transferred via a HP-piston pump in HP-vessels containing the medium ONR7 and crude oil with or without dispersant for the enrichment. Three sequential transfers without depressurization were performed for the WAF and CEWAF, each incubated for 10 days at 14°C and 10MPa pressure (HP treatment). A second set of HP-vessels were prepared and incubated as above, the difference being that these were decompressed and re-compressed at every transfer step to assess the robustness of hydrocarbon-degraders to pressure changes (REPRESS treatment). Finally, hydrocarbon degrading strains from all treatments were isolated at the end of the enrichment on solid media.

Main results

Our results highlighted the pivotal role of pressure on the natural microbial community diversity but not on the enriched hydrocarbon degraders. Alpha diversity levels were significantly higher for the HP compared to the respective REPRESS inocula possibly due to decompression effect during sample retrieval. *Pseudoalteromonas*, *Halomonas* and *Alcanivorax* dominated the enriched communities under both HP and REPRESS conditions. GC-MS data revealed a high capacity of the HP-CEWAF community, during the 1st incubation period, to degrade crude oil including the recalcitrant compounds (PAHs, Heavy alkanes). A decrease in biodegradation capacity, especially for the heavier oil substances, was observed between the 1st and the subsequent periods of HP-CEWAF treatment which was accompanied by a decrease in the relative abundance of *Halomonas* in favour of *Alcanivorax*.

******* Abstract should not exceed TWO pages *******