TITLE: Immobilization of a multi-domain hydrocarbonoclastic consortium and its application in a marine oil spill mesocosm

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Despite their ecological importance, coral reefs, considered the most diverse marine ecosystems on the planet, are in decline due to natural and anthropogenic factors. Chronic or acute oil contamination can be highlighted as one of those factors. Among of proposed strategies for oil spills mitigation, bioremediation is gaining importance, due to its sustainable and environmental friendly appeal. One of the strategies that contributes to increase the effectiveness of bioremediation processes is the bioaugmentation with free or immobilized microbial cells. Immobilization consists in the incorporation of microorganisms into a support, which confers greater stability and better handling. This work aims to evaluate and compare the efficiency of the biodegradation of petroleum hydrocarbons by a multi-domain microbial consortium, applied in the immobilized form (in calcium alginate) and non-immobilized form (free consortium). The impact of different bioaugmentation approaches on the hydrocoral *Millepora alcicornis* was also addressed. The hydrocarbonoclastic consortium was composed of nine microrganisms (six bacteria, two fungi and one yeast strain), which were isolated from seawater and Brazilian endemic corals. The bioremediation experiment was conducted in a marine mesocosm, projected to mimic a large-scale oil spill. For this, eight different treatments were applied: A (Control – only seawater), B (Free Consortium), C (Immobilized Consortium), D (Calcium Alginate), E (Oil), F (Free Consortium + oil), G (Immobilized Consortium + oil) and H (Calcium Alginate + Oil). The biodegradation of n-Alkanes and PAH's was quantified by GC/FID and GC/MS analyses. The photosynthetic capacity of the symbiotic zooxanthellae (Fv/Fm) was determined as a proxy for coral health, as well as the holobiont morphological analysis. The evaluation of the shelf life of this inoculant was carried out using counting of colony forming units (CFU) and counting of conidia. The evaluation of the hydrocoral-associated microbial community profile was performed by sequencing the 16S region of the bacterial ribosomal RNA and the data analyses were performed in the Mothur, using the Greengenes database. The results of β-diversity showed that oil and alginate promoted a variation on hydrocoral microbial communities throughout the experiment. Regarding the relative abundance of OTUs, at the phylum level, Proteobacteria was dominant in all treatments; Bacteroidetes presented greater abundance in the treatments without oil, while the Firmicutes presented dominance in the treatments with oil. The immobilized consortium promoted higher hydrocarbon bioavailability when compared to the free consortium, as an important step for further biodegradation. In addition, the inoculants presented longer shelf life in the refrigerator condition when compared to the maintenance at room temperature. The results generated in this work allowed a better understanding of how calcium alginate can be used as a microbial support in oil spills bioremediation processes.

Key words: Oil spill, bioremediation, microbial consortium, immobilization, calcium alginate, impacts on corals.

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