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MICROCOSM EVALUATION OF AUTOCHTHONOUS BIOAUGMENTATION TO COMBAT MARINE OIL-SPILLS

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Abstract

Bioremediation through bioaugmentation (addition of oil-degrading bacteria) and/or biostimulation (addition of nutrients N&P) constitute a promising strategy for combating oil spills following first response actions. However, bioaugmentation is one of the most controversial issues of bioremediation since nutrient addition alone had a greater effect on oil biodegradation than the addition of microbial products that are highly dependent on environmental conditions. There is increasing evidence that the best way to overcome the above barriers is to use microorganisms from the polluted area, an approach proposed as autochthonous bioaugmentation (ABA) and defined as the bioaugmentation technology that uses exclusively microorganisms indigenous to the sites (soil, sand, and water) to be decontaminated. Isolated single strains or enriched cultures, which are obtained “before” or “after” the contamination of the target sites, are administered to the sites once contamination occurs. The key idea is to conduct the enrichment of contaminant-degrading bacteria under the same or very similar conditions to those where bioaugmentation will be performed. ABA as defined in this study uses indigenous microbial consortia or isolates that are highly enriched and much better adapted to the historically or artificially contaminated sites.

In this study we examined the effectiveness of a novel autochthonous bioaugmentation strategy for the successful remediation of polluted marine environments. Consortium was enriched from seawater samples taken from Elefsina Bay near the Hellenic Petroleum Refinery; a site exposed to chronic crude oil pollution. Duplicate microcosms were established in sterile 100 ml flasks containing 50 ml of seawater contaminated with 0.5% w/v weathered crude oil incubated under aerobic conditions at 20°C with continuous agitation. Three autochthonous bioaugmentation treatments were established as follows: (i) oiled seawater and pre-adapted consortium (ii) oiled seawater + KNO₃, K₂HPO₄ and pre-adapted consortium, and (iii) oiled seawater + KNO₃, K₂HPO₄, biosurfactant (rhamnolipids) and pre-adapted consortium. Nutrients were added to such amount to give a final concentration equivalent to a C:N:P molar ratio of 100:10:1. Growth of oil degraders was measured by most probable number (MPN) procedure and hydrocarbons were analysed with chromatographic techniques (solid-phase extraction, gas chromatography–mass spectrometry) after 0, 5, 15 and 30 days. After 30 days long experiment, MPN and plate counts showed highest growth during treatment (iii) with fertilizer and biosurfactant which correlate to chemical analysis that showed highest alkane and aromatics degradation. After 30 days treatments (ii) and (iii) were the most effective, C12-C35 n-alkanes fraction was degraded more than 70%, while in treatment (iii) degradation was 85% within 15 days. Previous work with lipophilic fertilizers (uric acid and lecithin) and biosurfactants (rhamnolipids) resulted in increased removal of petroleum hydrocarbons as well as in a reduction of hydrocarbon degraders’ lag phase. Considering the above, the use of biostimulation additives in combination with naturally pre-adapted hydrocarbon degrading consortia has proved to be the most effective treatment and is a promising strategy in the future especially when combined with lipophilic fertilizers instead of inorganic nutrients. Such an approach becomes more pertinent when the oil spill approaches near the shore line and immediate hydrocarbon degradation is needed.
