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Potential of bioremediation for buried oil removal in beaches after an oil spill

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ABSTRACT

Bioremediation potential for buried oil removal, an application still lacking thorough research, was assessed in a specifically designed system in which an artificially contaminated oil layer of sand was buried in a sand column subjected to tidal simulation. The efficiency of biostimulation (BS, fertilizer addition) and bioaugmentation (BA, inoculation of pre-stimulated indigenous hydrocarbon-degrading microorganisms plus fertilizer) compared to natural attenuation was tested during a 180-day experimental period. The effect of BA was evident after 60 days (degradation of hydrocarbons reached 80%). BS efficacy was revealed only after 120 days. Microorganisms and nutrients added at the top of the sand column were able to reach the buried oil layer and contributed to faster oil elimination, an important feature for effective bioremediation treatments. Therefore, autochthonous BA with suitable nutritive conditions results in faster oil-biodegradation, appears to be a cost-effective methodology for buried oil remediation and contributes to the recovery of oil-impacted areas.

1. Introduction

Large quantities of petroleum enter the environment every year through leakage from storage tanks and pipelines or by release in accidental spills, such as that of the *Prestige* tanker (Albaigés et al., 2006). Recent research on sandy beaches affected by this oil spill revealed the persistence of oil in the sand at depths up to 4 m, a much greater depth than previously expected (Bernabeu et al., 2009). Although the buried oil from this spill is considered very persistent, oil degradation has been observed, as different morphologies of the buried oil were found: tar-balls, particles, oil coatings and emulsions (Bernabeu et al., 2006). The biodegradation of oil, however, is a slow process that requires weeks, months or years (Atlas and Hazen, 2011; Zhu et al., 2001). It is important to study approaches that may accelerate the existing natural attenuation of oil, such as bioremediation.

Bioremediation might be a cost-effective treatment tool for accelerating oil removal from contaminated environments. This treatment can be applied by stimulating indigenous microbial assemblages (biostimulation) or by increasing microbial assemblages capable of degrading the oil (bioaugmentation). In fact, the growth of hydrocarbon-degrading bacteria and hydrocarbon degradation can be strongly enhanced by fertilization and biostimulation and has proven to be an effective bioremediation treatment on several types of shorelines (Röling et al., 2002). However, the amendment of nutrients to open beach environments is often impractical because water soluble nutrients can be rapidly diluted and leached out of the sediment (Nikolopoulou and Kalogerakis, 2009). An option that avoids these adverse impacts is the use of oleophilic fertilizers, such as Inipol EAP 22 or S200, that are designed to release nutrients continually or intermittently over a period of time on contact with water (Xu et al., 2004). Although biostimulation is considered to be effective, it may still require time to be successful because of the scarcity of indigenous microbes capable of degrading hydrocarbons (Hosokawa et al., 2009). This can be overcome by bioaugmentation. In general, bioaugmentation can be conducted by adding allochtonous microbial assemblages with a known capability of degrading the oil to the impacted beach environment. However, this option can be quite uncertain because it implies the addition of exogenous microorganisms to the environment with unknown effects on the microbial natural diversity and on the environment. In addition, several studies have demonstrated that this type of bioaugmentation is not successful in most cases because exogenous microorganisms are not able to compete with the indigenous ones

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(Hosokawa et al., 2009). Therefore, another option is bioaugmentation with indigenous microbial assemblages, a protocol that has only been explored in a few reported studies (Hosokawa et al., 2009).

Bioremediation is a proven technique for the cleaning and restoration of sites that have been superficially contaminated by petroleum products (Gallego et al., 2007), including marine habitats of a shoreline contaminated by marine oil spills (Swannell et al., 1996; Head and Swannell, 1999). Although bioremediation has been applied to groundwater in terrestrial ecosystems (e.g., Farhadian et al., 2008; Van Stempvoort and Biggar, 2008), to our knowledge, it has still not been applied to the remediation of buried oil in coastal ecosystems. Accordingly, research on this topic is imperative. The problem with buried oil is completely different from that of superficial oil. When bioremediation techniques are applied to superficial oil, the treatments are added directly to the layer of oil. In the case of buried oil, these treatment approaches may not be successful. One must ensure that the bioremediation treatment (i.e., the solution added that contains either nutrients or microorganisms) reaches the buried oil.

In this study, bioremediation efficiency for buried oil removal was assessed in controlled laboratory conditions. Two treatments, biostimulation (addition of the oleophilic fertilizer S200) and bioaugmentation (inoculation of an indigenous oil-degrader consortium pre-stimulated in the laboratory combined with the addition of S200 fertilizer), were tested and compared with natural attenuation (oil degradation by indigenous microorganisms present naturally in the sand). Experiments were conducted in a system designed specifically for this study, with a tide regime simulation to mimic the natural conditions of the intertidal area of sandy beaches and to better reproduce the buried oil conditions.

2. Materials and methods

2.1. Sampling

Sandy sediment (between 2 and 20 cm) was collected (in black plastic bags) in August 2011 in the middle tide zone of the *São Pedro de Maceda* beach (NW of Portugal) at low tide to retrieve wet, but not submerged, sediment. The beach is protected from direct industrial and human contamination and, to our knowledge, has not been affected by any major oil spills.

In the laboratory, the collected sediment was carefully combined and homogenized to avoid small-scale variations in its composition that could influence the experiments. A small portion of the homogenized sediment was separated for microbial and chemical characterization. The sediment was also characterized in terms of organic matter content and grain size (as described in Ribeiro et al. (2011)).

The natural seawater used was also obtained from the NW Portuguese coast and filtered through activated carbon before entering the system to prevent the input of impurities. The salinity, pH and nutrient levels were also assessed (as described in Almeida et al., 2012).

2.2. Experimental design

The experiments were conducted in a system composed of 9 columns divided into 3 sets of 3 columns each (Fig. 1). Each set was individually connected to an automatic pump system, which was connected to a container filled with seawater, working independently of the other sets. At the base of each column, an inlet was connected to an electric water pump, and an outlet was connected to an electrovalve. The inlet and outlet were controlled by a timer (activated independently every 12 h) to induce the water entrance and exit from the columns' bottom. Filling the columns took ca. 45 min, allowing the water to remain slightly above the sand top. At this stage, each column was fully flooded with seawater and remained flooded for ca. 5 h. Afterwards, the water drained out slowly (ca. 2 h), leaving the column dry for ca. 4 h. The water residence time was selected to mimic the period of sediment inundation in the beach intertidal zone.

To reproduce the buried oil, a portion of sandy sediment (1.6 L per column) was mixed with crude oil supplied by a refinery (80 mL per column). The mixture was then left to age in a hood for 3 days to allow the physical adsorption of petroleum hydrocarbons to sand particulates and to eliminate the more volatile petroleum fractions, simulating natural weathering. This contaminated sand was mixed daily to maintain aerobic conditions. Then, it was buried at approximately 30 cm from the surface in each column. The contaminated layer (ca. 5 cm height) had approximately 20 cm of clean sand below and ca. 30 cm of clean sand above (each column contained approximately 17.5 L of sediment) (Fig. 1 B). After the completion of column assemblage, the columns were left undisturbed without any physical shaking, except for the water flow movement. The grain size of the sediment and the flow of water going in and out resulted in oxic conditions, under the assumption that the hydrocarbon degradation processes occurred in oxic conditions.

The column system was placed indoors in a dark room under a constant room temperature (at 19 °C, the average temperature expected to be found at the sub-surface sediment layer). In addition, all columns were covered with a black cover, and a low intensity red light was used during column manipulation to prevent photo-oxidation.

2.3. Treatments performed

Following the assembly of the sand columns, the system was left to equilibrate, with tide simulation, for 3 days. After that period, the treatments (performed in triplicate) began. Accounting for the possible variability among the 3 sets of columns from the filling and draining of each set, each replicate of each treatment was placed in a different set (Fig. 1). The treatment solutions were added when the sand columns were void of water.

Three sets were assembled as follows:

(NA) *Natural attenuation*: action of indigenous microorganisms naturally present in the sediment;
(BS) *Biostimulation treatment*: addition of the oleophilic fertilizer S200 to enhance the activity of indigenous oil-microorganisms degraders; and
(BA) *Bioaugmentation treatment*: inoculation of an indigenous oil-degrader consortium pre-stimulated in the laboratory, plus S200.

Abiotic losses were not controlled and were assumed to be identical in all treatments. The results were interpreted using comparisons of the two tested treatments with natural attenuation.

The commercial fertilizer S200 is a widely used bioremediation agent designed to adhere to oil (Díez et al., 2005; Gallego et al., 2006; Jimenez et al., 2006). It contains a saturated solution of urea (nitrogen source) in oleic acid with phosphate esters (phosphorous source) (Díez et al., 2005). The fertilizer solution was applied according to instructions from the manufacturer to attain a final molar ratio of C:N:P equivalent to 120:10:1 in each column. Assuming that the only source of carbon was provided by the addition of crude oil, the ratio of carbon:S200 was 10:1.

For the BA treatment, an inoculum of an indigenous oil-degrader consortium pre-stimulated in the laboratory, plus S200, was added at the beginning of the experiment. In fact, the BA