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Bioremediation of hydrocarbon-contaminated soils in cold regions: Development of a pre-optimized biostimulation biopile-scale field assay in Antarctica

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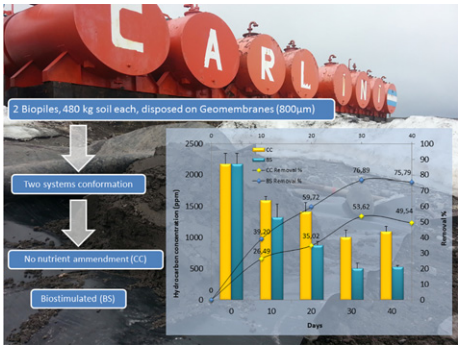
HIGHLIGHTS

- Geomembrane-covered 0.4 ton biopiles were chosen as experimental systems.
- RSM-optimized levels of N and P significantly enhanced diesel fuel biodegradation.
- Average temperature was higher in covered systems than in the non-covered ones.
- In situ bioremediation of Antarctic soils is a useful tool for hydrocarbon removal.
- High removal (75%) of fuels from amended Antarctic soils was achieved in 40 days.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioremediation proved to be an effective approach to deal with soil contamination, especially in isolated, cold environments such as Antarctica. Biostimulation, involving the addition of macronutrients -mainly nitrogen and phosphorous- is considered the simplest and cheapest bioremediation process. Optimizing the levels of these nutrients is a key step prior to the application of a biostimulation strategy. In this work, N and P levels, optimized by Response Surface Methodology (RSM) at lab-scale, were applied to an Antarctic hydrocarbon contaminated soil. The process was performed *on-site*, using high density polyethylene geomembranes (800 µm) to isolate treated soil from the surroundings and under environmental conditions at Carlini station (Antarctica) during 50 days. Two 0.5 ton biopiles were used as experimental units; a control biopile (CC), and a biostimulated system (BS), amended with N and P. At the end of the assay, hydrocarbon removal was significantly higher in BS system compared to CC (75.79% and 49.54% respectively), showing that the applied strategy was effective enough to perform a field-assay in Antarctica that significantly reduce soil contamination levels; and proving that RSM represents a fundamental tool for the optimization of nutrient levels to apply during bioremediation of fuel contaminated cold soils.

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1. Introduction

Contamination with organic compounds is an issue of general concern around the world. A myriad of hydrocarbon polluted sites can be found worldwide (Sharma et al., 2000; Konečný et al., 2003; Shi et al., 2008; Panagos et al., 2013). This environmental problem occurs even in cold isolated regions such as the Arctic and Antarctica (Aislabie et al., 2006; Gomez and Sartaj, 2013) due to the hydrocarbon-based fuels which are used as energy source in these locations (Aislabie et al., 2004). In Antarctic stations, fuel management has caused both minor and major spills, deeply affecting the surrounding soils, waters and sediments. Given the remoteness from suitable infrastructure for remediation of the Antarctic affected environment, bioremediation is considered the most adequate approach to recover these soils from contamination (Ruberto et al., 2003; de Jesus et al., 2015). In this frozen continent, environmental liabilities dating back to times when fuel management was environmentally inattentive, as well as new contamination events require the development of simple bioremediation protocols, able to be applied in locations where the availability of machinery and facilities is scarce.

From a biological point of view, soil is a substantially complex matrix, particularly those contaminated with organic compounds. In these soils, natural carbon and energy sources coexist with several kinds of xenobiotic compounds, each one with its own chemical structure, individual concentrations and toxic effects on the soil biota. It is estimated that 1 g of soil could contain as much as 10^{10}–10^{11} bacteria (Horner-Devine et al., 2004), comprising 6000 to 40,000 different species (Curtis et al., 2002). These huge biomass values refer also to fungi, which could reach up to 200 m of hyphae per gram of soil (Leake, 2004). Although Antarctic soils present lower biomass and microbial diversity values than tropical ones, these numbers give an idea of the biological potential of a soil and reflect a wide catabolic capability to support a bioremediation process. In this regard, even the Antarctic soils have proved to contain a diverse microbiota capable of reaching satisfactory levels of contaminant removal under biostimulation processes (Coulon and Delille, 2003; Ruberto et al., 2008).

Due to its biological nature, bioremediation is dependent on several physicochemical factors (Boopathy, 2000). Thus, the development of bioremediation processes should consider the features of the treatment site. These include soil texture, air temperature and other climate variables, water availability, existing vegetation and terrain topology (Ruberto et al., 2013). Concentration of nutrients (such as bioavailable Nitrogen and Phosphorus, total organic matter and O_2 levels) and the thermal amplitude to which each soil is exposed to, can also be considerably diverse in different soils. All these physicochemical variables should be taken into consideration when a biostimulation approach is intended.

Different C:N:P ratios were considered as reference for nutrient addition in biostimulation approaches over the years (Waksman, 1927, Redfield et al., 1963, Brown et al., 1983, Morgan and Watkinson, 1989, Zhou and Crawford, 1995, Dong et al., 2015). Considering the stoichiometry of microbial growth, a C:N:P ratio of 100:10:1 has been reported as the optimum choice (Cheng and Mulla, 1999; Dibble and Bartha, 1979). However, in order to get optimal results, this established ratio should be tested whenever a bioremediation process is considered, since each soil has its own biological diversity and requirements. These considerations are clearly visible when an excessive addition of nutrients is provided, resulting in the inhibition of biological activity (Liu et al., 2011). For this reason, the optimization of this strategy is a key step prior to field application.

Landfarming is not suitable for a large scale process with Antarctic soils, because water content is considerably altered by snow and wind. In addition, wind can spread contaminated soil particles to a larger area or even to the sea. On the other hand, indoor processes present the advantage of enabling temperature regulation and avoiding sharper modifications in water content. However, these kinds of processes require adequate facilities and entail costs associated to heating. For these reasons, geomembrane covered biopiles are a suitable alternative, combining smaller surface requirements than landfarming and the protective effect of the membrane, avoiding abrupt water content changes as well as wind drag and lixiviation. Coverage of soil with coatings has also been reported as a way to enhance soil average temperature and to reduce thermal amplitude inside biopiles, benefiting the degrading potential of the microbial community and achieving a higher biological hydrocarbon removal (Mohn, 2001; Delille et al., 2004, 2008; Gomez and Sartaj, 2014).

As information about "in situ" bioremediation in Antarctica is scarce, and based on the priority that environmental restoration has for our country and the rest of the Antarctic Treaty members, in this work we report the results obtained from a field assay consisting on biostimulation of a biopile arranged with a diesel fuel contaminated soil from Carlini Station (25 de Mayo Island, South Shetlands, Antarctica) with levels of N and P previously optimized using Response Surface Methodology (RSM) (Martinez Alvarez et al., 2015). As far as we know, this experiment constitutes the first report of a rational bioremediation design based on such an optimization for Antarctic gasoil contaminated soil.

2. Materials and methods

2.1. Soil analysis and characterization

Soil for this field assay was gathered in December 2013, during 2013–2014 Argentinian Antarctic expedition from the diesel fuel storage tanks surrounding area at Carlini scientific station, Isla 25 de Mayo (King George Island), South Shetlands, Antarctica (62°14′18″S 58°40′00″W). Contaminated soil containing an average hydrocarbon concentration of 2180 mg kg^{-1} was sieved (10 mm mesh) to remove stones, concrete, large paint residues and any other rough material. Sieved soil was divided in two fractions. One of them was used for the field assay and the other one stored at −20 °C for further studies. The soil was also analyzed for texture by the pipette method (Gee and Bauder, 1986), organic carbon (Walkley and Black, 1934), extractable phosphorous (Bray and Kurtz, 1945) and total Kjeldhal nitrogen. Water content was determined gravimetrically by drying samples at 105 °C during 24 h. For pH measurements, 10 ml of sterile saline solution (NaCl 8.9 g/L) were added to 1 g of soil and vortexed for 1 min. The pH of the resulting suspension was measured using a Docu pH+ meter probe (Sartorius). All soil samplings were randomly gathered from the biopile after proper homogenization and mixture in order to assure representative samples.

2.2. Biopiles design

For the field assay, approximately 830 kg of sieved contaminated soil were homogenized using a rotating drum, and then divided into two equivalent fractions in order to set up the community control biopile (CC) and the biostimulated biopile (BS). CC experimental unit consisted in contaminated soil with periodical mixing and water addition to maintain moisture target level (15% w/w). The BS experimental unit was similar to CC, but with the addition of nutrients to reach the previously optimized C:N:P ratio of 100:17.6:1.73 (Martinez Alvarez et al., 2015). Water content and mixing frequency were the same in both units. Both systems were arranged to define a truncated pyramid or pyramidal frustum (210 cm × 140 cm, 200 cm × 130 cm, h = 20 cm), holding 0.53 m^3 of soil (Fig. 1). This arrangement provides a trade-off between surface and volume, allowing the treatment of a large soil volume using a moderate surface size of terrain, minimizing the contaminant volatilization as well as the geomembrane requirements. In addition, it represents an adequate configuration to withstand strong winds.

Soil biopiles were isolated from the surroundings using a 3 × 5 m high-density polyethylene geomembrane (800 μm). This conformation