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Oily sludge stimulates microbial activity and changes microbial structure in a landfarming soil

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ABSTRACT

We aimed to evaluate the microbial community structure in microcosms containing a soil collected at a landfarming (LF) site and which was artificially contaminated with oily sludge from the same site. In addition we have examined the hydrocarbons degradation under different bioremediation conditions. The experiment was conducted during 60 days in microcosms containing 300 g of LF soil and 18 g of oily sludge. The treatments employed were: a) LF soil without oily sludge addition (control); b) minimal management (LF soil + oily sludge adjusted to pH 7.0 and aerated); c) bioaugmentation (LF soil + oily sludge + pH 7.0 + aeration + inoculation of an adapted bacterial consortium); d) Biostimulation (LF soil + oily sludge + pH 7.0 + aeration + addition of N and P); e) native forest soil (to compare with the microbial community from LF soil). CO_2 released from the microcosms was captured and quantified. Fifteen aliphatic hydrocarbons (C15-C29) and 17 PAHs were quantified by gas chromatography mass spectrometry. Microbial communities were analyzed using next generation sequencing and the results were correlated with the hydrocarbon degradation rates. Bacterial families *Sphingomonadaceae*, *Xanthobacteraceae*, *Pseudomonadaceae*, *Ectothiorhodospiraceae*, *Xanthomonadaceae*, and *Comamonadaceae* were the most abundant in all microcosms. The treatments involving biostimulation and minimal management removed 40% and 60% of the aliphatic hydrocarbons in the respective microcosms, and the those involving minimal management and bioaugmentation removed 50 and 40% of [PAHs] at the end of the respective treatments. In all treatments, phenanthrene, methylphenantrene, and pyrene were not removed.

1. Introduction

Oily sludge is one of the most substantial solid wastes produced by the petroleum industry (Hu et al., 2013). Oil refineries and petrochemical industries produce tons of this waste per year, which represents a serious environmental concern (Srinivasarao et al., 2011).

Oily sludge is the final residue of the petroleum refining process and corresponds to 1/3 to 1/4 of the initial volume of the crude oil used. The waste is classified as hazardous and it is characterized as an emulsion formed of water, sediment, aliphatic and aromatic hydrocarbons, resins and asphaltenes (Lima et al., 2014). Generally, these wastes contain 30–90% oil, 10–50% water and 5–40% of mineral particles (Francis and Stehmeier, 1991; Zhang et al., 2012; Vdovenko et al., 2015), and also inorganic compounds, metals, oils and greases, microorganisms, nutrients (nitrogen and phosphorus), hydrocarbons (benzene, xylene and toluene), and many other potentially toxic compounds, as well as inorganic compounds and metallic species (Shie et al., 2000). Metals like Pb, Mn, Cu, Zn, As, Bi, Cd, Cr, Co, and Ni were found related with the oily sludge

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(Jasmine and Mukherji, 2015). Polyaromatic hydrocarbons (PAHs), oily sludge constituents, have been classified as pollutants due their toxicity, mutagenicity and carcinogenicity (Guazzaroni et al., 2013). As petroleum compounds are highly hydrophobic (Bezza et al., 2015), they persist in the ecosystem. The disposal of oily sludge in nature is a potential source of soil and water pollution.

Landfarming (LF) is a bioremediation process performed under controlled conditions to promote degradation and immobilization of pollutants through micobial metabolism. It is well known that fungi and bacteria are able to degrade aliphatic and aromatic hydrocarbons and to produce biosurfactants that help in hydrocarbon emulsification and its bioavailability to the microbial community (Jasmine and Mukherji, 2014; Bezza et al., 2015; Viana et al., 2015; Marco-Urrea et al., 2015), and the biodegradation of these hydrocarbons (Ward et al., 2003; Cerqueira et al., 2011; Thavamani et al., 2012; Hu et al., 2013; Cerqueira et al., 2014). Factors such as aeration, use of inorganic fertilizers or nutriments, and inoculation of microbial species with degrading capabilities play an important role in the remediation of sites contaminated with oil (Vasudevan and Rajaram, 2001; Cerqueira et al., 2012; Moussavi and Ghorbanian, 2015). Several prokaryotes have been detected in soil and water contaminated with hydrocarbons (Delille et al., 2002; Bento et al., 2005; Reunamo et al., 2013; Gao et al., 2014; Deng et al., 2014; Dellagnezze et al., 2014; Moussavi and Ghorbanian, 2015; Moubasher et al., 2015), as well as in areas of bioremediation (Mishra et al., 2001; Frank and Castaldi, 2003; Hejazi et al., 2003; Marin et al., 2005; Kriipsalu et al., 2007; Delille et al., 2009), and composting (Marin et al., 2006), showing reduction in the hydrocarbons concentration or even its mineralization (Bauer and Capone, 1985; Boonchan et al., 2000). Also, bioaugmentation might be favored by autochthonous microorganisms in the bioaugmentation process (Mishra et al., 2001; Gallego et al., 2007; Hosokawa et al., 2009; Rojo, 2009).

The DNA high throughput sequencing of microbial communities allows detecting culturable and unculturable microorganisms, making possible the study of ecological richness and diversity indexes. The understanding of soil microbial structure during the landfarming process is essential to set the best environmental conditions to enhance oily sludge biodegradation. Our objective was to examine the microbial community structure and the hydrocarbons biodegrading abilities of the microbial populations in microcosms set up with a landfarming soil which was artificially contaminated with oily sludge and incubated during 60 days under conditions mimicking various landfarming strategies including biostimulation and bioaugmentation.

2. Material and methods

2.1. Sample collection

Fifty soil sub-samples were collected from a landfarming site at SICECORS (Centralized South Petrochemical Pole of Solid Waste Control System, RS, Brazil) at a depth of 0–20 cm, which were placed in plastic bags. This geographic region has a subtropical climate where the local annual mean temperature is 19.4 °C, and the average rainfall is 1440 mm. The soil is classified as Acrisol according to the FAO classification system and as Typic Paleudult by US taxonomy, having 12% of clay. Samples were transported to the laboratory and kept at room temperature. Soil samples were homogenized and sieved on a 4 mm sieve. Soil physical-chemical analysis was performed according to the methodology described by Tedesco et al. (1995). The remaining soil was sieved, packed in plastic bags, and stored at 4 °C for 24 h before the microcosms assemblies. Approximately 1L of oily sludge was also collected in the oily sludge embankments of SICECORS and stored at 4 °C for subsequent contamination of the collected landfarming soil.

2.2. Bioremediation experiments

The microcosms were prepared in hermetic glass flasks with 1L capacity containing 300 g of soil each. The soil was contaminated with 6% (18 g) of oily sludge (Cerqueira et al., 2012). The soil pH was adjusted to pH 7 using calcium carbonate, the soil moisture was standardized to the field capacity (around 70%) for all treatments, and every three days the microcosms soil was returned for aeration. The microcosms were incubated at 28 °C for 60 days.

The experiment was performed using six replicates of each of the following treatments:

a) Control (LF soil without addition of 6% of oily sludge);
b) Minimal management (LF soil contaminated with 6% of oily sludge, which was only aerated and the pH and moisture were corrected);
c) Bioaugmentation (LF soil contaminated with 6% of oily sludge, inoculated with a consortium of five bacteria),
d) Biostimulation (LF soil contaminated with 6% of oily sludge, with C:N:P ratio adjusted to 100:6:1.5);
e) Native forest soil (to compare its microbial community with the LF soil microbial community).

2.2.1. Preparation of the microbial consortium for inoculation

The bacteria used in this study were previously isolated from oily sludge and landfarming soil by Cerqueira et al. (2011). Bacterial isolates *Stenotrophomonas acidaminiphila, Bacillus megaterium*, and *Bacillus cibi* were obtained from the oily sludge, and *Pseudomonas aeruginosa* and *Bacillus cereus* were isolated from a landfarming soil. To prepare the inoculum, a pure colony of each of the five isolates was inoculated into 20 mL nutrient broth. Each flask was incubated in a shaker at 28 °C for 48 h and the cultures were centrifuged at 4 °C for 10 min at 10,000 RPM and washed with sterile saline solution (0.85% NaCl). For inoculum standardization, each pellet was suspended in sufficient saline solution to achieve a final concentration of 10^8 bacterial cells g $soil^{-1}$.

2.2.2. Preparation of nutrients for the biostimulation treatment

Two solutions of $(NH_4)_2SO_4$, and KH_2PO_4 were prepared separately and used in order to adjust the C: N: P to 100: 6: 1.5. An aliquot of 2 mL of each solution was added to microcosms under the biostimulation treatment.

2.2.3. Microbial CO_2 production

The carbon dioxide (CO_2) released was monitored to determine the soil's respiratory activity generated by the microcosms microbial community during 60 days. The procedure was as described by Moreira and Siqueira (2006). Each microcosm was equipped with a device containing 0.5 M NaOH to CO_2 capture. The gas release was monitored periodically. The flasks were hermetically sealed and were kept open only for determination of basal respiration. For each periodical analysis, 2 mL of BaCl 30% were added to the NaOH solution (to stop the reaction), and 6 drops of phenolphthalein 1%. The amount of residual NaOH in the solution was titrated with 1 M HCl and production of C-CO_2 (mg kg^{-1} soil) was determined according to the formula described by Stotzky (1965).

2.3. Estimating the total population of culturable heterotrophic and degrading microorganisms

The culturable microbial population was estimated according to Braddock and Catterall (1999), on the first and the 60th days of