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Bacterial community in semiarid hydrocarbon contaminated soils treated by aeration and organic amendments

Margarita Ros*, Irene Rodríguez, Carlos García, Maria Teresa Hernández

Department of Soil and Water Conservation and Organic Waste Management, CEBAS-CSIC, P.O. Box 164, 30100 Espinardo, Murcia, Spain

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ABSTRACT

Bioremediation of oil sludge contaminated soils may enhance the rate of petroleum hydrocarbons (PHs) biodegradation. Changes in bacterial community structure and functionality may be expected as a result of hydrocarbon contamination and bioremediation processes.

A 6 month microcosm experiment was performed in which an oil sludge contaminated semi-arid soil was treated by aeration (AE), and aeration plus organic amendment (dried biosolid, DB) and composted biosolid, CB)). Hydrocarbon degradation and functional and structural changes in microbial communities were examined after submitting the soil to these bioremediation treatments. AE reduced the amount of PHs in soil from the initial 5% to 2.7% whereas the amount of PHs left in the soil with the CB and DB treatments were 2.5% and 2.4%, respectively. Bacterial abundance, measured by real-time PCR, was significantly higher in bioremediated soils (AE, DB and CB) than in the non-contaminated soil (control soil). Functional and structural differences in bacterial community measured by BIOLOG and denaturing gradient gel electrophoresis (DGGE), respectively, were observed between bioremediated soils and control soil, both at the start and end of the bioremediation process. After 6 months of bioremediation, 16S rDNA clone libraries showed less phyla in bioremediated soils than in the control soil. The most dominant phylum observed in bioremediated soils was Actinobacteria whereas in the control soil Proteobacteria was the most dominant.

This study suggests that regardless of the type of bioremediation carried out and the degree of hydrocarbon degradation, the bioremediation process leads to an increase in soil bacterial abundance accompanied by a decrease in microbial diversity, as well as, to structural and functional changes in the bacterial community.

1. Introduction

Petroleum refining generates considerable volumes of oily sludge during oil production and processing activities. Petroleum hydrocarbons (PHs) are the most deleterious components in oily sludge, because of their potential hazard to human health and the environment (Marín et al., 2005). The periodic disposal of oily sludge on land near petroleum refineries is a common practice and can lead to hydrocarbon accumulation in soils, representing a risk of underground water and atmospheric contamination.

Natural PHs degradation by indigenous soil microbial communities represents one of the primary mechanisms by which PHs can be eliminated but it may be a slow process due to the lack of aeration in compacted soils, low microbial biomass and low substrate and nutrient availability (Leahy and Colwell, 1990; Scalenghe and Ferraris, 2009). Bioremediation techniques such as landfarming have shown promising results in the treatment of soils contaminated soils with PHs due to the stimulation they produce on microbial populations able to use PHs as carbon source (Garcia-Blanco et al., 2007; Muckian et al., 2009). Likewise, the incorporation of organic amendments to soils contaminated by PHs may enhance the rate of PHs biodegradation since the available substrates and nutrients added with the amendment stimulate the growth and activity of microbial populations (McBride, 2003; Gandolfi et al., 2010; Wallisch et al., 2014). The incorporation of organic amendments to the contaminated soil together with aeration has been used as a bioremediation treatment in order to encourage the activity and growth of PHs-degrading microorganisms, which is the key for a successful bioremediation (Gallego et al., 2001). In addition, organic amendments represent an

* Corresponding author. Tel.: +34 968396388; fax: +34 968396213.
E-mail address: margaros@cebas.csic.es (M. Ros).

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organic matter of low cost and high availability that increases the soil organic carbon content, fertility, and biological activity (Ros et al., 2003). However, some authors did not observe improvement of hydrocarbon degradation with organic amendments (Palmroth et al., 2002; Schaefer and Juliane, 2007).

Community level physiological profiles (CLPPs) measured by BIOLOG plates have been used to investigate the potential functional diversity of the microbial communities (e.g. Hueso et al., 2012), whereas molecular tools such as denaturing gradient gel electrophoresis (DGGE), cloning and sequencing or quantitative real-time PCR (qPCR) have proven invaluable for the qualitative and quantitative study of environmental microbial communities (e.g. Ros et al., 2009; Yergeau et al., 2014).

Many studies have been carried out on the bioremediation of light hydrocarbon contaminated soils or on the effects of compost stability or contaminant concentration on the bioremediation processes (e.g. Riffaldi et al., 2006; Sayara et al., 2010; Tyagi et al., 2011). However, studies of the changes of microbial communities during bioremediation, by organic amendment addition and aeration, of semiarid soils contaminated with recalcitrant hydrocarbons, are less abundant. Thus, Marin et al. (2005, 2006) established the efficiency of compost in degrading hydrocarbon in semiarid soils and in stimulating microbial activity. However, these studies did not contemplate the incidence of compost addition on the bacterial community composition. Other studies on the influence of compost (Gandolfi et al., 2010; Wallisch et al., 2014), soil organic matter content (Neumann et al., 2014) or nutrient disturbance (Bell et al., 2013) on the microbial community of hydrocarbon-contaminated soils have not been performed in semiarid soils. It is widely admitted that both the type of hydrocarbon contaminant and the environmental conditions influence changes in microbial community. Therefore we consider of interest the study of the changes induced in the microbial community of a semiarid soil by both oil contamination and bioremediation processes involving aeration and organic amendments.

A wider knowledge of the microorganisms involved in semiarid soil biodegradation processes and their dynamics will allow the design of suitable bioremediation strategies for a specific contaminated site by either, the addition of highly concentrated and specialised microorganisms consortia to the contaminated site, or/and adjustment of environmental factors that encourage the activity of the indigenous microorganisms (Tyagi et al., 2011).

The hypotheses investigated in this work were: i) recalcitrant hydrocarbon contamination will produce changes in soil microbial communities with respect to non-contaminated soil, ii) the incorporation of biosolid (fresh or composted) will produce changes in the microbial communities of PHs contaminated soils and iii) microbial communities undergo changes during bioremediation processes.

2. Materials and methods

2.1. Soil, refinery sludge and organic amendments

The soil used in this experiment was a non-contaminated agricultural soil from Santomera, Murcia (Spain) (Table 1). The climatic aridity index of this area ranged between 0.2 and 0.5. Refinery sludge proceeding from an oil refinery was used to contaminate the soil (Table 1).

The organic amendments used were a thermally-dried biosolid and a composted biosolid (Table 1). The biosolid was composted in static piles (1 m^3) for four months using sawdust as bulking agent (1:3 (w: w)). The compost piles were turned every week, and moisture content was maintained between 40 and 50%.

Table 1
Soil, refinery sludge and organic amendments characteristics.

	Soil (sandy loam soil)	Refinery sludge	Dried biosolid	Composted biosolid
pH water (1:5)	8.56	–	–	–
pH, water (1:10)	–	–	7.46	7.53
EC (1:10) μS cm^{-1}	325	–	2250	2800
Total organic carbon (%)	1.49	24.5	58.2	72.1
Total nitrogen (%)	0.14	–	4.53	2.70
Total phosphorous (%)	0.077	–	2.48	1.59
Total potassium (%)	0.85	–	0.61	0.44
Total hydrocarbons (%)	–	17.3	–	–
Cd (mg kg^{-1})	–	2.8	1.8	0.7
Cu (mg kg^{-1})	–	133.8	337.1	270.3
Cr (mg kg^{-1})	–	94.2	131.9	22.4
Ni (mg kg^{-1})	–	401.7	50.6	14.4
Pb (mg kg^{-1})	–	145.6	173.3	108.9
Zn (mg kg^{-1})	–	1204.2	1384.8	858.0

2.2. Soil microcosm setup and sampling

The Microcosm experiment was performed in 500 ml glass jars containing 500 g of amended or non-amended contaminated soil. Microcosms consisted of three different bioremediation treatments: (a) soil contaminated with refinery sludge to obtain 5% (w/w) of PHs in soil (AE) (non-amended contaminated soil); (b) soil contaminated with refinery sludge to obtain 5% (w/w) of PHs in soil and amended with 6% (w/w) of dried biosolid (DB); (c) Soil contaminated with refinery sludge to obtain 5% (w/w) of PHs in soil and amended with 6% (w/w) composted biosolid (CB). Non-amended soil was used as control (control soil). Treatments with biosolid and composted biosolid were also named amended soils. The oil and organic amendments were homogenized thoroughly with the soil.

Each treatment was set up in triplicate. Microcosms were randomly placed in an incubation chamber and incubated for 6 months under controlled conditions of temperature (25 °C) and humidity (60%) and with day night light cycles (16 h/8 h day/night). Soil moisture was maintained between 50 and 60% of soil water holding capacity. Each microcosm was aerated weekly by stirring and homogenising thoroughly the soil of each jar. Sterilized deionized water was added when necessary to replace moisture losses. Soils were sampled after one day (T1) and 6 months (T6) of incubation. Samples were stored at −20 °C for further analysis.

2.3. Detection of PHs

Total hydrocarbon content was determined by extraction of oil and grease with chloroform by soxhlet and subsequent treatment with silica gel to remove polar components following the method 5520-F (APHA, 1998).

2.4. Physico-chemical and chemical analysis

Electrical conductivity (EC) and pH were measured in a 1/5 and 1/10 (w/v) aqueous solution in a micro-conductivimeter CM2002 (Crison) and micro pHmeter pH2002 (Crison), respectively. Total organic carbon (TOC) was determined by the method of Yeomans and Bremner (1989). Nitrogen was determined by the Kjeldahl method. Total P and total K and heavy metal content were determined from a nitric-perchloric (1:1) digestion extract; P was determined by colorimetric method (Murphy and Riley, 1962), and K and heavy metals by Atomic Absorption Spectrometry (Perkin Elmer 5500).