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Effect of biostimulation, temperature and salinity on respiration activities and bacterial community composition in an oil polluted desert soil

Raeid M.M. Abed*, Samiha Al-Kharusi, Manal Al-Hinai

Biology Department, College of Science, Sultan Qaboos University, P. O. Box: 36, PC 123 Al Khoud, Sultanate of Oman

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ABSTRACT

Bioremediation of oil-polluted soils under the extreme environmental conditions of deserts is challenging. Lack of nutrients, elevated temperatures and high salinities, caused by continuous evaporation of irrigation water/seawater, are among the major environmental factors that influence the diversity and activity of soil microorganisms. We compared, using microcosms, the effect of different temperatures and salinities on respiration activities, oil mineralization and bacterial community composition in desert soils with and without the addition of inorganic nutrients and exogenous bacteria. The addition of nutrients resulted in ca. 20% increase in oil mineralization rates, however additional exogenous bacteria lead to a further increase of only 1%. While the evolved CO_2 increased with increasing temperatures, it decreased with increasing salinity in both untreated and biostimulated soils. The maximum amount of evolved CO_2 reached 18.4 ± 1.1 mg-CO_2 g^{-1} in the biostimulated soil at 50 °C and this corresponded to ca. 10% oil mineralization. Bacterial communities exhibited shifts at different temperatures and salinities in the favor of genera that contain potential oil-degrading aerobic and anaerobic bacteria. We conclude that fluctuations in temperature and salinity of desert soils will directly influence the activity and diversity of microorganisms therein and consequently affect the efficiency of applied bioremediation treatments.

Introduction

Deserts contribute to more than 50% of the world oil production and contain approximately 75% of oil reserves (Ezcurra, 2006). The Arabian Peninsula is considered as a world major producer of oil and has the largest oil field in the world (i.e. Ghawar in Saudi Arabia). Therefore, deserts in this region are highly vulnerable to small and large scale oil spills during oil exploration, extraction and transportation. The removal of pollutants from desert soils in the Arabian Peninsula poses a serious challenge, mainly because of the very harsh environmental conditions. Temperature in the Arabian Desert can be very extreme with values exceeding 60 °C during hot summer days and falling down below 10 °C during winter nights (Stoppato and Bini, 2003; Allaby, 2006; Julie, 2009). Temperature has been shown to influence the physical and chemical properties of oil as well as the diversity and activity of oil-degrading microorganisms, which play a vital role in bioremediation of these soils. Although the effect of temperature on natural degradation rates of hydrocarbons has been studied (Wu et al., 1996; Annweiler et al., 2000; Abed et al., 2006), few reports investigated its effect on the efficiency of applied bioremediation treatments.

Various bioremediation treatments in the field, including irrigation with water mixed with nutrients and saturated with dissolved oxygen, were used to decontaminate oil-polluted arid desert soils (Balba et al., 1998; Margesin and Schinner, 2001). While the use of large volumes of freshwater may lead to the leaching of salts and the reduction of soil salinity, continued irrigation and high evaporation rates in arid deserts results in the accumulation of high quantities of salts in the soil (Balba et al., 1998). In some cases, seawater is used for irrigation due to logistical reasons and lower costs. Saltwater accelerates the soil water evaporation and as a result salts accumulate at the soil surface (Xu et al., 2003). High salinities have been shown to affect rates of hydrocarbon degradation (Ward and Brock, 1987; Rhykerd et al., 1995; Foght and McFarlane, 1999; Margesin and Schinner, 2001) and to decrease bacterial activities (Walker and Calwell, 1975; Riis et al., 2003; Minai-Tehrani et al., 2009). Therefore, increased salinities in desert soils upon irrigation treatments is a crucial factor that could hamper the efficiency of bioremediation processes.

* Corresponding author. Tel.: +968 92867442; fax: +968 24141437.
E-mail address: rabed@mpi-bremen.de (R.M.M. Abed).

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This study was undertaken to compare, using microcosms, the changes in respiration activities and bacterial community structure of an oil-polluted desert soil when subjected to biostimulation with inorganic nutrients with and without the addition of exogenous oil-degrading bacteria at different temperatures (10 °C, 30 °C and 50 °C) and salinities (2%, 4% and 7%). Although desert soils are expected to harbor extremophilic microorganisms, we hypothesize that elevated temperatures and salinities will still affect the efficiency of the applied bioremediation treatment. The extent of oil mineralization at each treatment was calculated from the amount of evolved CO_2. Pyrosequencing of 16S rRNA genes was carried out to follow the response of bacterial communities in the untreated and biostimulated soils at the different incubation temperatures and salinities.

Materials and methods

Sample collection and sampling site

Samples from an oil-contaminated desert soil were collected in sterile boxes in May, 2011 from a sludge land farm, north of Oman (Table 1). Electrical conductivity (EC) and pH of the soil were 9.5 mS/cm and 7.3, respectively (Table 1), as measured using calibrated YSI instruments after mixing ten grams of soil with 50 ml of deionized water and allowed to settle for 20 min. Anions were identified using ion chromatography (IC, Metrohm AG, Herisau, Switzerland) as described before (Abed et al., 2014). The highest detected anions were chloride and sulphate (28 and 12 g kg^{-1}, respectively), followed by nitrate, fluoride and bromide (2–97 mg kg^{-1}). Phosphate and nitrite were not detected (Table 1). A standard hydrometer method was used to determine the percent sand, silt and clay (Brady, 1984; Klute, 1986) and was found to be 31%, 58% and 11%, respectively (Table 1). Total petroleum hydrocarbons (TPH) reached 55.2 ppm in the soil, extracted and analysed using previously described protocols (Weisman, 1998).

Experimental setup

Ten grams of soil were mixed with 20 ml of carbon- and nutrient-free (i.e. without KH_2PO_4 and NH_4Cl) minimal salt medium in a 165 ml serum bottle. The medium contained $MgCl_2.6H_2O$ (5.6 gl^{-1}), $MgSO_4.7H_2O$ (6.8 gl^{-1}), $CaCl_2.2H_2O$ (1.47 gl^{-1}), KCl (0.66 gl^{-1}), KBr (0.09 gl^{-1}) and was supplemented with trace elements mixtures (Widdel and Bak, 1992) and vitamins (Heijthuijsen and Hansen, 1986). The following treatments were maintained, each in triplicate: 1) biostimulation (BS), performed by adding NH_4Cl and NaH_2PO_4 as N and P sources at a final concentration of 2 and 0.32 g l^{-1}, respectively; 2) biostimulation and bioaugmentation (BSA) where a mixture of different bacterial strains (see below) and NH_4Cl and NaH_2PO_4 were added and 3) untreated soil where neither bacteria nor nutrients were added. The bacterial mixture for the BSA treatment was prepared by mixing equal proportions of pure cultures of the oil-degrading bacterial strains MH2, MH3, MH21, MH22, AH2, AH3, AH6, AH8 and AH23 (detailed phylogenetic and physiological characterization of the strains can be found in Abed et al., 2014), pre-grown in acetate-containing medium. These strains were selected based on their growth patterns on selected alkanes and to represent different bacterial genera.

The bottles were divided into two sets. In the first set, salinity of the medium was fixed at 2% and triplicate of each treatment (i.e. BS, BSA and the untreated soil) were incubated at 10 °C, 30 °C and 50 °C. In the second set, the salinity of the medium was adjusted to 2%, 4% and 7% by addition of NaCl and all treatments were incubated at 30 °C. All bottles were then closed and sealed immediately with thick, black rubber stoppers, which were sealed with glue and screw caps to ensure no gas leakage. The bottles were incubated without shaking in order to mimic the situation that occurs in nature following an oil spill. CO_2 measurements were performed weekly using GC for 63 days.

Table 1
Physical and chemical characteristics of the studied desert soil.

Parameter	Unit	Desert soil
GPS		22° 19′ 0″ N 56° 33′ 0″ E
TPH[$]	ppm	55.2
pH		7.3
EC	mS/cm	9.5
Anions		
Fluoride	mg/kg	2.1
Chloride	g/kg	28.3
Bromide	mg/kg	97.7
Sulphate	g/kg	12.3
Nitrate	mg/kg	52
Phosphate		ND*
Nitrite		ND*
Soil texture		
Sand	%	31
Clay	%	58
Silt	%	11
Organic matter	%	5.2
Carbon	%	1.8

* ND: not detected; [$]TPH: Total petroleum hydrocarbons.

Respiration activities and oil mineralization rates

CO_2 in the headspace was measured by withdrawing 250 μl at different time intervals using a gas-tight glass syringe and injecting it manually into GC (GC, Agilent model 6890N). The GC was equipped with thermal conductivity detector and a 30 m × 250 μm capillary column (HP-PLOT Q). Helium was used as a carrier gas at a flow rate of 4 ml min^{-1} and the injector and detector temperatures were maintained at 200 °C and 210 °C, respectively. The oven temperature was programmed from 50 °C to 80 °C (final hold time 3 min) at a rate of 20 °C min^{-1}. CO_2 evolution data were statistically analyzed by one-way ANOVA using the SPSS software (10th edition, Chicago, USA). *P*-values were adjusted using the sequential Bonferroni (Quinn and Keough, 2002) and Tukey's test was used to determine differences between individual means. The extent of oil mineralization was calculated from the CO_2 evolution data by comparing the experimentally quantified CO_2 with the theoretical amount of CO_2 that would be formed by complete oxidation of the present oil after subtraction of the CO_2 evolved from an oil-free sediment (Abed et al., 2014).

Pyrosequencing and sequence analyses

Pyrosequencing was performed only on the untreated and BS soils, but not on BSA soils, since the CO_2 evolution experiments did not show a dramatic difference between the BS and BSA soils at each temperature (see result Section 3.1 and 3.2). DNA was extracted from triplicate soil samples at the end of the experiments using skim milk protocol (Volossiouk et al., 1995). Purified DNA extracts from the triplicates of each soil were pooled together and submitted to Molecular Research MR DNA Laboratory (Shallowater, TX, USA) for tag-pyrosequencing. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed as described before (Dowd et al., 2008a,b) using the GS FLX titanium sequencing kit XLR70. One-step PCR was performed using a mixture of hot start and hot start high fidelity taq polymerases resulting in amplicons