Chemosphere 147 (2016) 279–286

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# EDTA addition enhances bacterial respiration activities and hydrocarbon degradation in bioaugmented and non-bioaugmented oil-contaminated desert soils

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## HIGHLIGHTS

- EDTA addition enhances bacterial respiration rates and oil biodegradation.
- EDTA enhances oil degradation by increasing the bioavailability of hydrocarbons.
- Desert soils can be efficiently bioremediated using EDTA and exogenous bacteria.
- Some bacteria can grow on EDTA as a carbon source.
- *Alcanivorax* sp. and *Parvibaculum* sp. can be ideally used in bioaugmentation.

## ARTICLE INFO

*Article history:*
Received 16 July 2015
Received in revised form
2 November 2015
Accepted 25 December 2015
Available online 14 January 2016

Handling Editor: Chang-Ping Yu

*Keywords:*
Oil
Ethylenediaminetetraacetic acid (EDTA)
MiSeq
Bioaugmentation
*Alcanivorax*
GC–MS

## ABSTRACT

The low number and activity of hydrocarbon-degrading bacteria and the low solubility and availability of hydrocarbons hamper bioremediation of oil-contaminated soils in arid deserts, thus bioremediation treatments that circumvent these limitations are required. We tested the effect of Ethylenediaminetetraacetic acid (EDTA) addition, at different concentrations (i.e. 0.1, 1 and 10 mM), on bacterial respiration and biodegradation of Arabian light oil in bioaugmented (i.e. with the addition of exogenous alkane-degrading consortium) and non-bioaugmented oil-contaminated desert soils. Post-treatment shifts in the soils' bacterial community structure were monitored using MiSeq sequencing. Bacterial respiration, indicated by the amount of evolved $CO_2$, was highest at 10 mM EDTA in bioaugmented and non-bioaugmented soils, reaching an amount of $2.2 \pm 0.08$ and $1.6 \pm 0.02$ mg-$CO_2$ $g^{-1}$ after 14 days of incubation, respectively. GC–MS revealed that 91.5% of the $C_{14}$–$C_{30}$ alkanes were degraded after 42 days when 10 mM EDTA and the bacterial consortium were added together. MiSeq sequencing showed that 78–91% of retrieved sequences in the original soil belonged to *Deinococci*, *Alphaproteobacteria*, *Gammaproteobacteia* and *Bacilli*. The same bacterial classes were detected in the 10 mM EDTA-treated soils, however with slight differences in their relative abundances. In the bioaugmented soils, only *Alcanivorax* sp. MH3 and *Parvibaculum* sp. MH21 from the exogenous bacterial consortium could survive until the end of the experiment. We conclude that the addition of EDTA at appropriate concentrations could facilitate biodegradation processes by increasing hydrocarbon availability to microbes. The addition of exogenous oil-degrading bacteria along with EDTA could serve as an ideal solution for the decontamination of oil-contaminated desert soils.

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## 1. Introduction

Human activities rely on the use of crude oil which potentially leads to environmental contamination (Macaulay and Rees, 2014). Many attempts have been made to remove oil pollutants from the environment; however, the success of these remediation processes, in many cases, is hindered by the existence of a low number of oil-

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http://dx.doi.org/10.1016/j.chemosphere.2015.12.114
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degrading microorganisms and the low solubility and availability of hydrocarbons (Whitehouse, 1984; Jain et al., 1992; Northcott and Jones, 2000; Millioli et al., 2009). To circumvent these problems, several bioremediation approaches have been designed that involved the addition of exogenous oil-degrading bacteria (known as bioaugmentation) or the addition of chemicals such as surfactants to emulsify oil and increase its bioavailability (Volkering et al., 1995; Li and Chen, 2009; Tyagi et al., 2011; Chen et al., 2015). The oil-degrading bacteria will then utilize hydrocarbons either by directly uptaking the dissolved fraction from the aqueous medium or by adhesion to the small-emulsified oil droplets (Beal and Betts, 2000; Hua et al., 2007; Mishra and Singh, 2012; Hua and Wang, 2013; Abdel-Megeed et al., 2014). So far, most bioremediation studies have been performed either by the addition of exogenous bacteria or chemicals to change the physical properties of oil but never both together. The major concern in this case is that the added chemicals would have a negative effect on growth of the introduced exogenous oil-degrading bacteria.

Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), have been previously used in oil fields in a mixture with other chemicals to increase oil production, to make drilling more efficient and to inhibit scale formation (Cikes et al., 1990; Crabtree et al., 1999). Also, EDTA has been reported to have the ability to remove adsorbed hydrocarbons from soil particles, thus rendering them available to hydrocarbon-degrading bacteria (Hua et al., 2007; Han et al., 2009; Baziar et al., 2013). In spite of that, the use of EDTA in bioremediation has been very limited, which could be attributed to its adverse effect on microorganisms. For instance, EDTA was found to increase dispersal of some bacterial biofilms (Banin et al., 2006; Ramage et al., 2007; Robertson et al., 2012; Meng et al., 2013; Saadat et al., 2013) and to strongly suppress the catalytic properties of microbes (Meng et al., 2013). The strong chelating binding affinity of EDTA to different metal ions such as $Ca^{2+}$, $Mg^{2+}$ and $Fe^{3+}$ destabilizes the microbial extrapolymeric substances (EPS) matrix and causes cells to separate from biofilms. The divalent chelating capacity of EDTA causes lipopolysaccharides to separate from the outer membrane of microbial cells, thus increasing the membrane permeability and subsequently cell death (Gray and Wilkinson, 1965; Banin et al., 2006; Yakandawala et al., 2007; Saadat et al., 2013). For these reasons, EDTA has been used in food and therapeutic industry to eradicate microbes and their biofilm formation (Ramage et al., 2007; Juda et al., 2008; Chauhan et al., 2012; Shaikh and Musaddiq, 2012).

In this study, we tested the role of EDTA in stimulating bacterial respiration and oil biodegradation in an oil-polluted desert soil subjected to a bioaugmentation treatment. Our hypothesis is that EDTA will increase the bioavailability of hydrocarbons to the oil-utilizing bacteria, hence facilitating the degradation process. Moreover, the effect of EDTA on the fate of the introduced oil-degrading bacterial consortium as well as on the soil's bacterial community composition was investigated.

## 2. Material and methods

### 2.1. Sample collection and characterization

Oil contaminated soils were collected from an area close to an oil production facility in Mina Al-Fahal, a coastal area in Muscat, Oman (23° 37′ 49.92″N; 58° 31′ 23.84″E). Soils from this area have been previously investigated for their physical and chemical properties as well as for their response to different bioremediation treatments including the addition of inorganic nutrients and exogenous microorganisms (Abed et al., 2014a). Soils were collected in sterile plastic boxes and brought back to the laboratory, where all incubations were performed.

### 2.2. Effect of EDTA on respiration activities and oil degradation

Five alkane-degrading bacterial strains (i.e. *Alcanivorax* sp. MH3, *Parvibaculum* MH21, *Azospirillum* sp. AH2, *Marinobacter* sp. AH3 and *Marinobacter* sp. AH6) were cultivated in a minimal salt medium (see below) at 30 °C using acetate as a carbon source. Detailed biochemical, physiological and phylogenetic characterization of the strains can be found in Abed et al. (2014a,b). In brief, the strains were Gram negative, rod in shape and were phylogenetically affiliated to the classes *Alphaproteobacteria* (i.e. *Parvibaculum* MH21, *Azospirillum* sp. AH2) and *Gammaproteobacteria* (i.e. *Alcanivorax* sp. MH3, *Marinobacter* sp. AH3 and *Marinobacter* sp. AH6). They grew well at 2–7% salinity and between 20 and 60 °C. All strains exhibited a better growth on long chain than on short chain alkanes. The selection of these strains was based on their utilization of different alkanes and representation of different bacterial genera. The cells were concentrated by centrifugation, washed with sterile water and then re-suspended in carbon- and nutrient-free (i.e. without $KH_2PO_4$ and $NH_4Cl$) minimal salt medium. The medium contained $MgCl_2.6{\cdot}H_2O$ (5.6 $gl^{-1}$), $MgSO_4.7{\cdot}H_2O$ (6.8 $gl^{-1}$), $CaCl_2.2{\cdot}H_2O$ (1.47 $gl^{-1}$), KCl (0.66 $gl^{-1}$), KBr (0.09 $gl^{-1}$) and was supplemented with trace elements mixture (Widdel and Bak, 1992) and vitamins (Heijthuijsen and Hansen, 1986). A bacterial consortium was prepared by mixing equal volumes of each bacterial culture ($OD_{600} = 0.2$) and 1 ml of this mixture was added to 19 ml medium for each treatment (see below).

The experiment was performed in glass bottles (volume 165 ml). Each bottle received 10 g of the oil-polluted soil mixed with 20 ml of carbon- and nutrient-free minimal salt medium. Soils with and without the addition of exogenous bacterial consortium were incubated in the presence of different concentrations (i.e. 0.1, 1 and 10 mM) of EDTA (Sigma Aldrich, USA) in the medium. The same concentrations of EDTA were also added to the bacterial consortium alone without soil. Incubations (i.e. bioaugmented soil, non-bioaugmented soil and the bacterial consortium without soil) in the absence of EDTA served as controls. All treatments and controls were maintained in triplicates. The bottles were sealed with thick, black rubber stoppers to ensure no gas leakage and incubated at 30 °C for 42 days.

Respiration activities were measured in all treatments by following evolved $CO_2$ at different time intervals using gas chromatography (GC). From the headspace, 250 μl of gas was withdrawn using a gas-tight syringe and then manually injected into the GC (GC, Agilent model 6890N). The GC used helium gas as a carrier gas at a flow rate of 4 ml $min^{-1}$ in the 30 m × 250 μm capillary column (HP-PLOT Q). The equipped thermal conductivity detector was maintained at 200 °C while the injector temperature was 210 °C. The oven temperature was programmed from 50 °C to 80 °C with a final hold time 3 min at a rate of 20 °C $min^{-1}$. Actual oil mineralization rates were calculated from the evolved $CO_2$, assuming that oil was the only carbon source available for microbes. These rates were estimated by comparing the experimentally quantified $CO_2$ with the theoretical amount of $CO_2$ that would be produced by complete oxidation of the present oil (Abed et al., 2015).

Alkane degradation at the end of all treatments was evaluated using gas chromatography-mass spectrometry (GC–MS). One gram of each soil sample was extracted 2–3 times in 5 ml dichloromethane (DCM, Sigma–Aldrich, Germany) and then sonicated for 25 min at 10 °C. The extracted supernatant was mixed with sodium sulfate and was filtered with non-absorbent cotton to remove solid particles. The filtrate was then evaporated using a rotary evaporator. The dry extract was re-dissolved into DCM and passed through silica gel to remove any solid particles prior to injection. Individual alkanes were quantified after injecting the extract into

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