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Biofilm communities and biodegradation within permeable reactive barriers at fuel spill sites in Antarctica

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ABSTRACT

Determining the composition of the complex microbial communities within biofilms can aid us in understanding the potential for biodegradation on different permeable reactive barrier materials. Microbial communities were examined under Antarctic conditions across laboratory and field studies using amplicon pyrosequencing, and the reaction of these communities to petroleum hydrocarbon contamination and NH_4^+ supplementation was assessed. Putative hydrocarbon degrading bacteria in the orders *Actinomycetales* and *Burkholderiales* were identified in laboratory flow cells and within the upper PRB and lower PRB at Casey Station, Antarctica. The release of NH_4^+ from ammonium exchanged zeolite was shown to reduce the microbial diversity of biofilms, when compared to natural zeolite. Bacteria from the orders *Sphingomonadales*, *Rhodocyclales*, *Pseudomonadales* and *Xanthomonadales* were also observed across the Zeopro™/granular activated carbon permeable reactive barrier mixtures. The bacterial species were observed within a uniform microbial biofilm layer, embedded within extracellular polymeric substances on the surface of the materials. This study demonstrates that petroleum hydrocarbon contamination can contribute to the enrichment of certain hydrocarbon degrading species on permeable reactive barrier materials.

1. Introduction

Several studies have reported the positive implications of reactive materials, such as granular activated carbon (GAC) and zeolites, for biofilm formation and biodegradation of petroleum hydrocarbons in a number of water treatment configurations (Guerin et al., 2002; Kalmykova et al., 2014). A commonly applied configuration is a permeable reactive barrier (PRB); constructed by excavating a section of earth and replacing this with a filling material, such as GAC or zeolite, through which flows petroleum hydrocarbon contaminated groundwater (Mumford et al., 2013). Soil water flows through a single material PRB bed, or a PRB bed containing a sequence of materials under natural hydraulic gradients wherein the contaminants are physically adsorbed and chemically or biologically degraded (Mumford et al., 2015). Where petroleum hydrocarbon and nutrient delivery in the subsurface are conducive to growth, the metabolic activity of the microbial biofilms attached onto GAC and zeolites can be increased significantly, resulting in the formation of a bio-reactive barrier (Herzberg et al., 2003; Simpson, 2008).

An advantage of a bio-reactive barrier is that more reliable and effective soil nutrient supply and hydraulic conductivity can be achieved, compared to that possible using the resident regolith materials (Mumford et al., 2013). Furthermore, the use of a homogenous PRB material can facilitate the study and understanding of the microbial community and biodegradation processes occurring across a range of environmental conditions. The application of zeolites, as a bio-reactive material for use in PRBs, is due to their ability to provide controlled nutrient release for bioremediation (Leggo, 2000). When natural zeolite is 'loaded' with ammonium (NH_4^+) ions and used as a PRB material, NH_4^+ will exchange with

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cations in the soil water, releasing NH_4^+ and making this nutrient available for uptake by the resident microbial community (Freidman et al., 2016). Leggo (2000) and Freidman et al. (2016) report that this ion exchange process significantly enhances the microbial population on the surface of nutrient amended zeolites. Furthermore, nitrogen has also been shown to be the primary limiting factor to bioremediation in Antarctica (Freidman et al., 2016), illustrating the importance of NH_4^+ amendments in PRBs.

Regeneration of the adsorption capacity of GAC can be achieved by stimulating or promoting biofilm development. Biofilm activity on GAC can lead to an increase in desorption and biodegradation rates, and extend the lifetime of GAC materials within water treatment settings (Aktas and Cecen, 2007). This process is known as bioregeneration. The ability to replicate the conditions necessary for enhancing biofilm growth and therefore bioregeneration of PRB materials, offers the potential to lower the inflated costs associated with hydrocarbon contaminated groundwater treatment in Antarctica. Similarly, bioregeneration serves to lower the *ex-situ* costs accrued from PRB material replacement, transport and disposal in authorised landfills (Northcott et al., 2016; Freidman et al., 2017, 2017a).

Petroleum hydrocarbon contamination has been shown to reduce microbial biodiversity in soils, as these compounds are toxic to many microbes, adversely affecting cell membrane integrity (Saul et al., 2005; Vazquez et al., 2013; Jung et al., 2016). Some bacterial species however can utilise petroleum hydrocarbons as carbon sources (Bell et al., 2013). Contaminated soil water therefore has the potential to enrich certain microbial populations transported to PRBs (Jung et al., 2016). Unfortunately, not all microbes that tolerate heightened concentrations of petroleum hydrocarbon contamination will contribute directly to its degradation (Bell et al., 2013). Care has to be taken to differentiate between those bacterial groups that directly utilise and degrade petroleum hydrocarbons, those that may assist key degrading groups/species through commensal or symbiotic interactions, and those that make little or no contribution to degradation processes.

Bell et al. (2013) observed a heightened abundance of *Nocardioidaceae* (*Actinobacteria*) and *Burkholderiales inc. sed* (*Betaproteobacteria*) in Arctic soils treated with diesel, and in the presence and absence of nutrient supplements (including ammonium). Furthermore, fluctuations in *Actinobacteria* and *Betaproteobacteria* populations correlated well with hydrocarbon degradation rates in soils containing low-organic matter and high-organic matter respectively (Bell et al., 2013). *Rhodococcus*, *Pseudomonas*, *Sphingomonas*, *Stenotrophomonas* and *Brevundimonas* are thought to be especially important for the bioremediation of petroleum hydrocarbon contaminated Antarctic soils (Ma et al., 2006; Jesus et al., 2015). At present the assessment of bacterial diversity on GAC at low temperatures has been restricted to municipal water treatment studies (Kaarela et al., 2015), with little evidence of extension to bio-reactive PRB materials applied for remediation in remote polar regions.

Five PRBs are installed at Australia's Casey Station, Antarctica to address petroleum hydrocarbon spills, providing an opportunity to further characterise these bacterial communities (Filler et al., 2008). Two of these barriers are installed at the Main Power House (MPH) to address a petroleum hydrocarbon spill that occurred in 1999 (Mumford et al., 2013; McWatters et al., 2016). The spill was diesel range fuel, comprising a mixture of marine diesel from Bergen, Norway (80%) and Aviation Turbine Kerosene (20%). The two PRBs comprise zeolite and GAC materials in a ‘funnel and gate’ configuration to capture and biodegrade petroleum hydrocarbons (Mumford et al., 2013). The advantage of the funnel and gate system is that a wide catchment area can be treated and a smaller reactive region is required, resulting in lower construction and ongoing monitoring costs (Careghini et al., 2013). Reports of microbial growth on zeolite and GAC materials under Antarctic conditions has revealed a positive correlation between controlled nutrient release and petroleum hydrocarbon degradation rates (Mumford et al., 2015; Freidman et al., 2016).

This study reports the bacterial diversity of biofilm communities attached to zeolite and GAC materials, comparing data from laboratory flow cell experiments and the two PRBs installed at the MPH, Casey Station. Identifying the bacterial groups responsible for biodegradation, shifts in bacterial diversity following exposure to petroleum hydrocarbon contamination and nutrient delivery in a PRB setting will facilitate a better understanding of the performance of these systems (Bell et al., 2013; Mumford et al., 2015).

2. Materials and methods

2.1. PRB design

The laboratory flow cell design is presented in Fig. 1. Operating conditions are reported in detail in Freidman et al. (2016). The laboratory flow cells contained Antarctic soil spiked with 5000 mg/kg Special Antarctic Blend (SAB) diesel prior to commencing flow at 23 ± 2.0 °C. The advantage of using laboratory scale PRBs is that individual bio-reactive materials can be more reliably assessed for microbial diversity under specific temperature and flow conditions.

The two MPH PRBs are designated ‘upper PRB’ and ‘lower PRB’, as described by Mumford et al. (2013). The location, design and construction of the upper PRB (Fig. 2a) and lower PRB (Fig. 2b) at Casey Station have been reported previously (Mumford et al., 2013; McWatters et al., 2016). The summer temperature (December–February), obtained using temperature sensors, within the lower PRB has been reported between 2 and 5 °C at the base of the PRB (Mumford et al., 2013). Winter conditions (March–November)

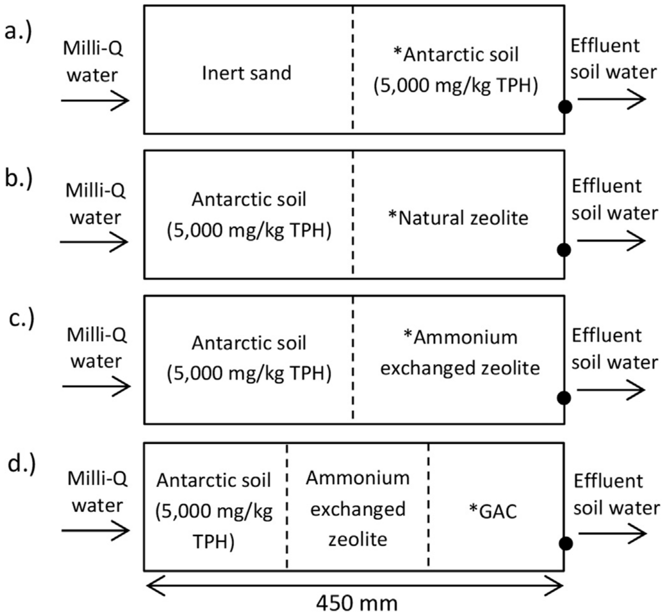

Fig. 1. The composition of laboratory flow cells containing a contaminated Antarctic soil control (a.), natural zeolite (b.), ammonium exchanged zeolite (c.) and a sequenced bed of ammonium exchanged zeolite and GAC (d.). The symbol * represent the soil and PRB materials targeted for amplicon pyrosequencing. The symbol ● represent the sampling location for the effluent soil water (Table 1). The flow cells had a depth of 40 mm and a width of 50 mm. These flow cells comprise soils that have been spiked with 5000 mg/kg SAB diesel prior to commencing flow. Additional information surrounding the laboratory flow cells can be found in Freidman et al. (2016).