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Research article

Large scale treatment of total petroleum-hydrocarbon contaminated groundwater using bioaugmentation

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ABSTRACT

Bioaugmentation or the addition of microbes to contaminated sites has been widely used to treat contaminated soil or water; however this approach is often limited to laboratory based studies. In the present study, large scale bioaugmentation has been applied to total petroleum hydrocarbons (TPH)-contaminated groundwater at a petroleum facility. Initial TPH concentrations of 1564 mg L^{-1} in the field were reduced to 89 mg L^{-1} over 32 days. This reduction was accompanied by improved ecotoxicity, as shown by *Brassica rapa* germination numbers that increased from 52 at day 0 to 82% by the end of the treatment. Metagenomic analysis indicated that there was a shift in the microbial community when compared to the beginning of the treatment. The microbial community was dominated by Proteobacteria and Bacteroidetes from day 0 to day 32, although differences at the genus level were observed. The predominant genera at the beginning of the treatment (day 0 just after inoculation) were *Cloacibacterium*, *Sediminibacterium* and *Brevundimonas* while at the end of the treatment members of *Flavobacterium* dominated, reaching almost half the population (41%), followed by *Pseudomonas* (6%) and *Limnobacter* (5.8%). To the author's knowledge, this is among the first studies to report the successful large scale biodegradation of TPH-contaminated groundwater (18,000 L per treatment session) at an offshore petrochemical facility.

1. Introduction

The rapid growth of petrochemical industries has resulted in toxic effluents contaminating the environment (Andreoni and Gianfreda, 2007). Total petroleum hydrocarbon (TPH) pollution from industrial sources poses a significant hazard to marine and terrestrial ecosystems. While the bulk of this pollution may be a consequence of oil refineries and petrochemical plants that discharge waste materials into the environment, some originate as fugitive materials from ships and leaking underground storage tanks (LUST). TPH contamination includes petrol, diesel, gasoline and other petrochemical products that contain monoaromatic compounds such as benzene, toluene, ethylbenzene and xylenes (BTEX) and other polycyclic aromatic compounds (PAH). In land based operations, these pollutants have resulted in substantial contamination of soil and groundwater (Kingston, 2002). The complex and diverse structural configurations of PAH, combined with their low bioavailability, hydrophobic nature and strong sorption phenomena makes the design of effective bioremediation methodologies a challenge (Macaulay and Rees, 2014).

One of those strategies is Monitored Natural Attenuation (MNA) and although it has been successful for the treatment of groundwater in several contaminated sites (Boonchan et al., 2000; Prince et al., 2003; van Hamme et al., 2003), it may not be the best method if the right conditions are not present (Andersson et al., 2006; Mao et al., 2012). Some of the important environmental parameters include temperature, salinity, microbial diversity and the C:N:P ratio, among others (Nikolopoulou et al., 2007).

Groundwater contamination usually occurs as a result of surface

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pollution as is the case when there is a leakage or a spill incident leading to the release of industrial chemicals into the environment (Boopathy, 2000). This unintentional release permeates into the surrounding soil and migrates into the water table threatening the groundwater and other water sources (Macaulay and Rees, 2014; USEPA, 2006). When such sites are investigated, suitable groundwater monitoring wells are constructed, the groundwater is evaluated and an assessment is made as to whether there is a need for remedial action.

Previous studies on the bioremediation of TPH-contaminated groundwater have stated that the success of engineered bioremediation systems depended largely on how effectively directions and rates of groundwater flow can be controlled and thus how efficiently oxidants and nutrients can be delivered to contaminated aquifer sediments. Thus, all these factors should be taken into account when setting up a bioremediation approach such as bioaugmentation; which involves the addition of adapted bacteria to the contaminated matrix for their treatment (Gavrilescu et al., 2014; Prince et al., 2003). The addition of specialised microbes to the contaminated site has different but complementary aims, namely the degradation of the contaminants by the inoculated microorganisms. However there may be other ways in which the augmented microorganisms can enhance the degradation. There may be ecosystem services other than biodegradation of the contaminant that can be a beneficial feature of bioaugmented organisms such as biofilm formation and surfactant generation.

Although there have been several successful bioaugmentation studies reported (Chen et al., 2013; Prince, 2010; Ron and Rosenberg, 2014) others have failed (Nikolopoulou et al., 2013). Most of the successful bioaugmentation cases have taken place in confined systems such as bioreactors where the conditions could be controlled to favour the survival and prolonged activity of the exogenous microbial population (Prince, 2010). Thus, successful bioaugmentation has been associated with a 'pump and treat' system. The integration of a fixed film microbial growth within such a system has been shown to be effective in the treatment of contaminated groundwater (Macaulay and Rees, 2014). In contrast, traditional pump and treat systems combined with non-biological methods such as sand and charcoal filtration have generally failed (Bao et al., 2012).

It is also critical to identify the predominant microorganisms within the bioreactors in order to have an idea of the processes occurring during the biodegradation. For example a recent study has found that the gene fusion of *alkB*, ferredoxin and ferredoxin reductases genes occurred in *Limnobacter* (Nikolopoulou et al., 2013). This is beneficial for electron transfer or for substrate-enzyme binding and suggests that members of this genus are involved in the degradation of hydrocarbons. The advent of metagenomics or next generation sequencing is a great aid in such a task and has definitely improved techniques such as DGGE which used previously to monitor microbial communities (Pradhan et al., 2016; Shahsavari et al., 2013). The use of next generation sequencing (NGS) provides more data than previous techniques and has been used for the elucidation of the microbial community in hydrocarbon-contaminated soils (Hassanshahian et al., 2014; Koshlaf et al., 2016), DDT-contaminated soils (Bao et al., 2012) among many other contaminated matrices as has been reviewed previously (Hassanshahian et al., 2014).

The aim of this study was to evaluate alternative technologies that could be implemented with minimal impact to the environment (small physical and energy footprint) that offered the flexibility of application at different sites based on the bioaugmentation approach and the pump and treat strategy (Afzal et al., 2007; Felföldi et al., 2010). In addition, the microbial community during the bioremediation study was assessed using 16 S rDNA sequencing.

2. Materials and methods

2.1. Consortium development

The bioaugmentation treatment consisted on the addition of 22 bacterial strains (Table 1) which were previously isolated and characterized (Poi et al., 2017). Briefly, each of the aerobic bacterial strains was systematically isolated from a biological trickling filter (biofilter) collected from an operational wastewater treatment plant (WWTP) located within a petroleum facility. The wastewater contained high phenol concentrations (>3000 mg L^{-1}), petroleum hydrocarbons and high chemical oxygen demand (COD) values (>10,000 mg L^{-1}). The strains were originally isolated to establish a collection of biofilm producing bacterial cultures capable of phenol and petroleum degradation (Macaulay and Rees, 2014; Zhao et al., 2011). These cultures were isolated using a positive end dilution approach to capture part of an ecological community that constituted the dominant indigenous microorganisms able to survive and persist in an environment with high concentrations of TPHs. These strains were tested for TPH degradation because they were adapted to high TPH concentrations but also because they were isolated from the original wastewater that was contaminated with a wide range of compounds such as phenol and TPHs.

2.2. Laboratory experiment

Bench scale experiments were performed in duplicate on TPH-contaminated groundwater sourced from the ISO Tank as described in our previous work (Poi et al., 2017). Briefly, custom-made polycarbonate cylinders housed in glass containers served as bioreactor vessels for the bench-scale bioreactor (Fig. 1a). In this experiment, 22 bioballs were used to make up the bulk of the matrix housing with 22 ceramic beads (placed in a plastic mesh, comprising 20% of the matrix volume). A peristaltic pump provided a flow rate of 450 mL per minute to ensure a continuous mixing and aeration. A total of 1500 mL per sample were treated in an aerated bioreactor mimicking the *in situ* conditions. The inoculum added in the bench scale experiment contained 3.0% (v/v) of the consortium (Treatment A). In addition, the control without addition of bioaugmnetion agent was used (Treatment C). The control was inoculated with 3.0% (v/v) of sterile water.

2.3. Field scale experiment

Translation and scale-up was performed with a portable bioreactor based on a modified aerated ISO tank with a holding capacity of 18 m^3 (Fig. 1B). After pumping the groundwater into the ISO Tank, the topmost layer of non-aqueous phase liquids (NAPL) was

Table 1
The list of bacteria used for this study.

No	Bacterial strain	No	Bacterial strain
1	*Bacillus lentus*	12	*Acinetobacter haemolyticus*
2	*Pseudomonas aeruginosa*	13	*Bacillus cereus*
3	*Pseudomonas stutzeri*	14	*Bacillus sphaericus*
4	*Pseudomonas stutzeri*	15	*Bacillus cereus*
5	*Bacillus megaterium*	16	*Bacillus megaterium*
6	*Pseudomonas stutzeri*	17	*Bacillus licheniformis*
7	*Arthrobacter* sp.	18	*Bacillus cereus*
8	*Bacillus pumilus*	19	*Acinetobacter baumannii*
9	*Bacillus cereus*	20	*Acinetobacter baumannii*
10	*Bacillus subtilis*	21	*Alcaligenes faecalis*
11	*Bacillus subtilis*	22	*Brevibacillus brevis*