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Exploiting the intrinsic microbial degradative potential for field-based *in situ* dechlorination of trichloroethene contaminated groundwater

Eric M. Adetutu[a,1], Taylor D. Gundry[a,*,1], Sayali S. Patil[a,b], Aida Golneshin[a], Joy Adigun[c], Vijay Bhaskarla[a], Samuel Aleer[a], Esmaeil Shahsavari[a], Elizabeth Ross[d], Andrew S. Ball[a]

[a] School of Applied Sciences, Royal Melbourne Institute of Technology, Bundoora, Melbourne, VIC 3083, Australia
[b] School of Biological Sciences, Flinders University of South Australia, Adelaide, SA 5042, Australia
[c] GeneDX, 207 Perry Parkway, Gaithersburg, MD 20877, USA
[d] The University of Melbourne, Faculty of Veterinary and Agricultural Sciences, Parkville, VIC, Australia

HIGHLIGHTS

- Cutting edge next generation sequencing tool applied to in situ TCE bioremediation.
- Metagenomics showed that Epsilon- and Deltaproteobacteria were key dechlorinators.
- Gammaproteobacterial groups associated with dechlorination only in untreated wells.
- Accelerated TCE removal in biostimulated and bioaugmented wells compared to control.
- Indigenous dechlorination potential and microbial functional redundancy observed.

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ABSTRACT

Bioremediation of trichloroethene (TCE) polluted groundwater is challenging, with limited next generation sequencing (NGS) derived information available on microbial community dynamics associated with dechlorination. Understanding these dynamics is important for designing and improving TCE bioremediation. In this study, biostimulation (BS), biostimulation–bioaugmentation (BS–BA) and monitored natural attenuation (MNA) approaches were applied to contaminated groundwater wells resulted in ≥95% dechlorination within 7 months. Vinyl chloride's final concentrations in stimulated wells were between 1.84 and 1.87 μg L^{-1}, below the US EPA limit of 2.0 μg L^{-1}, compared to MNA (4.3 μg L^{-1}). Assessment of the groundwater microbial community with qPCR showed up to ~50-fold increase in the classical dechlorinators' (*Geobacter* and *Dehalococcoides* sp.) population post-treatment. Metagenomic assays revealed shifts from Gammaproteobacteria (pre-treatment) to Epsilonproteobacteria and Deltaproteobacteria (post-treatment) only in stimulated wells. Although stimulated wells were functionally distinct from MNA wells post-treatment, substantial dechlorination in all the wells implied some measure of redundancy. This study, one of the few NGS-based field studies on TCE bioremediation, provides greater insights into dechlorinating microbial community dynamics which should be useful for future field-based studies.

1. Introduction

Chlorinated hydrocarbons are one of the most environmentally persistent pollutants due to their strong C—Cl bonds. They have been extensively used in agriculture and industrial applications [1] with the dry cleaning agent tetrachloroethene (or perchloroethene (PCE)) and the solvent trichloroethene (TCE) being recalcitrant under oxic conditions. These compounds are carcinogenic and pose significant health risks to humans and should be removed from contaminated environments.

For TCE remediation, a number of physical and chemical approaches can be used for the removal of chlorinated hydrocarbons from groundwater. These include pump-and-treat, surfactant flushing, electrokinetics and *in situ* chemical oxidation/reduction or a combination of these remedies [2,3]. Chlorine atoms on the contaminant can be decoupled by various mechanisms such as hydrolysis, dehydrochlorination, hydrogenolysis and dichloroelimination [4]. Biological methods or bioremediation can also be effectively used for treating TCE contaminated environments

* Corresponding author. Fax: +61 3 99257110.
E-mail address: taylor.gundry@rmit.edu.au (T.D. Gundry).
[1] Authors contributed equally to the research.

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with reductive dechlorination being largely facilitated by microbial dehalogenases such as PceA, TceA, VcrA and BvcA enzymes [5]. Although the use of any of these methods depends upon economical, legislative and site-specific technical factors, biological approaches are sometimes preferred due to their cost effectiveness and environmental friendly way of reductive dechlorination.

The bioremediation process can involve the active introduction of dechlorinating organisms (bioaugmentation) [6]. For example, Okutsu et al. showed that the addition of "bioaugmentation agents contributed to shortening the clean-up time better than biostimulation by increasing the initial *Dehalococcoides* sp. (*Dhc*) population in the groundwater" in a pilot study of TCE contaminated groundwater [7]. Chemical compounds (biostimulation) can also be added to contaminated samples to stimulate the biological degradation of the pollutants [8]. For example, Patil et al. reported 100 % PCE conversion to ethene in acetate-stimulated 24 week-microcosms relative to 15% in control samples [9]. For biostimulation, the compounds used are usually electron donors such as molasses, acetate, butyrate, propionate and lactate which release hydrogen during fermentation [10]. The important characteristic shared among all these compounds is their ability to generate hydrogen which acts as a strong reducing agent [11].

Anoxic conditions prevailing in aquifers are preferred by microbes performing reductive dechlorination (i.e., the substitution of chlorine by a hydrogen atom). Bacteria involved in reductive dechlorination fall into three phylogenetic clusters, among which the *Chloroflexi* group is of particular interest. This is because several of its member strains, *Dehalococcoides ethenogenes* (*Dhc*) 195, FL2, GT and BAV1 can completely dechlorinate chloroethenes to environmentally benign ethene and chlorine ions [12,13], although in nature this is carried out by microbial consortia [6,14,15]. In this process, hydrogen (H_2), primarily supplied by syntrophic organic fermenters is known to be a key electron donor for reductive dechlorination by *Dhc* [16–19].

The decision to carry out bioremediation is dependent on cost and the presence or absence of dechlorination potential of native microbial community in the contaminated environment. Using a laboratory based combined microbial and analytical approach can be helpful in designing effective *in-situ* bioremediation strategies [20]. Several laboratory studies have extensively assessed the role of *Dhc* mixed cultures for chloroethene removal by using molecular biological tools (MBTs). MBTs such as PCR based denaturing gradient gel electrophoresis (DGGE), quantitative real-time PCR (qPCR), allow the monitoring of specific groups or reductive dehalogenase enzymes [13,14,21,22]. Next generation sequencing tools (NGS) based on whole genome and amplicon can provide important information on microbial roles in contaminated environments [23].

Real world applications of these techniques to study microbial communities during commercial bioremediation are limited. For example, successful field based chloroethene degradation in aquifers by biostimulating and augmenting *Dhc* cultures have been reported [17,24,25]. However, some of the results were based on qPCR detection indicating only the presence or absence of the classical dechlorinator, *Dhc*. There are very few reports of NGS based dechlorinating microbial community studies [26,27], with limited reports of NGS based *in-situ* remediation study [28]. Fundamental knowledge of microbial community structure, dynamics and functionality is important for predicting contaminant degradation patterns and deciding which bioremediation strategy to implement [20]. Metagenomics, PCR-DGGE and qPCR can be effectively used to assess the overall remediation progress by studying microbial shifts and correlating dechlorination rates with specific or total community response during *in situ* trials. However, information from PCR-DGGE or qPCR can be biased, and focussed only at target groups. Case in contrast, metagenomic assays based on whole genome sequencing, provide important information on the different microbial groups present and their potential functions [29,30].

Therefore in this study, we describe the applications of MBTs for monitoring *in situ* bioremediation involving three treatments, biostimulation only (BS), biostimulation plus bioaugmentation (BS–BA) and monitored natural attenuation (MNA) for TCE remediation. Dechlorinating microbial community structures and dynamics before and after bioremediation were studied using a combination of metagenomics and quantitative PCRs. Generating a better understanding of changes to groundwater microbial communities during several different types of TCE remediation.

2. Methods

2.1. Site characterization

The study site shown in Fig. 1, has historically been used for light industrial activities including manufacturing, machining and warehousing of metal products, which has resulted in subsurface contamination with metals and halogenated organic compounds. Previous environmental assessment identified a TCE contamination source. Prior to the start of this study, this source was removed.

Soils and groundwater samples were characterised before and during treatment. Soil samples from the targeted zone consisted of orange brown, soft to firm, clay, minor gravel, sand. Soil porosity ranged between 40 and 45%; hydraulic conductivity estimated using slug test data was 0.02 m day^{-1},and groundwater velocity was approximately 0.005 m day^{-1}. For this study, twelve wells with various TCE levels ranging from 30 to 150 $\mu g\,L^{-1}$ and one well with comparatively lower TCE contamination (Control), 1.2 $\mu g\,L^{-1}$ were selected for use. Construction details of selected wells are listed in Table 1.

2.2. Groundwater collection

Groundwater samples were collected prior to the start of bioremediation treatments (PT) in May 2013 with sampling being carried out every month until November 2013. A total volume of 4 L of groundwater was collected from the screen interval between 5 and 8 mbTOC from all thirteen wells using a polypropylene bailer (Bunnings Warehouse, VIC, Australia). A flow-through cell (YSI, VIC, Australia) recorded pH, oxidation-reduction potential (ORP), specific conductance, temperature, dissolved oxygen (DO) and turbidity of groundwater. The flow-through cell was disconnected after the stabilization of geochemical parameters and replicate samples were collected without flow interruption. Sample containers consisted of sterile and N_2 – purged 4 L high density polyethylene Nalgene bottles with polypropylene screw caps (Thermo Scientific Australia, NSW) were filled to capacity. Upon collection, bottles were transported to the analytical laboratory. All samples were stored in the dark at 4 °C until further use.

2.3. Analytical procedures

Replicate groundwater samples from each contaminated well were analysed for chlorinated ethenes using a 5975C gas chromatography (GC) system equipped with a mass spectrometry (MS), flame ionizing detector (FID) and a Porabond Q column (0.32 mm by 25 m) (Agilent Tech, Australia). Chlorinated hydrocarbons were analysed in a 1 mL gas headspace. The GC settings were: injector temperature 200 °C; detector temperature 300 °C; oven temperature 3 min at 40 °C, followed by an increase of 10 °C min^{-1} to 70 °C, followed by an increase of 15 °C min^{-1} to 250 °C for 7 min; and carrier gas (He) with a flow rate of 2 mL min^{-1}. External standards at