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Presence of organohalide-respiring bacteria in and around a permeable reactive barrier at a trichloroethylene-contaminated Superfund site☆

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ABSTRACT

Trichloroethylene (TCE) is one of the most common groundwater contaminants in the United States; however clean-up efforts are a challenge due to its physical and chemical properties. TCE and several of its degradation products were detected in the groundwater of the Beaver Dam Road Landfill site (Beltsville, MD) at concentrations above accepted maximum contaminant levels. A permeable reactive barrier (i.e., biowall) was installed to remediate the groundwater. Microbial infiltration and colonization of the biowall with native site bacteria was expected to occur. An array of molecular biological tools was applied to survey the microbial community for presence of organohalide-respiring microorganisms at the site. Microorganisms belonging to methanogens, acetogens, sulfate-reducing bacteria, and chlorinated aliphatic hydrocarbon-metabolizing bacteria were identified, thus making way for the application of the microbial populations in the biowall bioaugmentation efforts. In concomitant laboratory studies, molecular approaches were used to monitor continuously-fed column reactors containing saturated biowall material spiked with a commercially-available, *Dehalococcoides*-containing culture (SDC-9), with or without zero-valent iron (ZVI) shavings. The column without ZVI had the highest abundance of *Dehalococcoides* spp. (2.7×10^6 cells g^{-1} material, S.D. $= 3.8 \times 10^5$ cells g^{-1} material), while the addition of ZVI did not affect the overall population. Although the addition of ZVI and biostimulation did change ratios of the *Dehalococcoides* strains, the results suggests that if ZVI would be applied as a biowall material amendment, biostimulation would not be required to maintain a *Dehalococcoides* population. These experimental results will be utilized in future remediation and/or biowall expansion plans to utilize the natural resources most effectively at the biowall site.

1. Introduction

Trichloroethylene (TCE) is one of the most common groundwater contaminants in the United States and was detected at 57% of the National Priorities List (NPL) sites in 2015 (ATSDR, 2015; ATSDR, 2017). TCE is of particular concern because of its classification as a human carcinogen and its detrimental effects to the nervous system (ATSDR, 2011). Moreover, vinyl chloride (VC), a degradation product of TCE, is 2.5 times more toxic than TCE and is also classified as a human carcinogen (ATSDR, 2006; US EPA, 2009). TCE remediation, however, is challenging due to the high volatility of TCE and propensity to form a dense non-aqueous phase liquid in an aquifer (Chiao et al., 1994; US EPA, 2016; Jacoby et al., 1998). TCE was detected in the groundwater of the Beaver Dam Road Landfill site (Beltsville, MD) at a concentration two levels of magnitude or more above the maximum contaminant level (MCL) of 5 ppb (US EPA, 2009). The site was included in the CERCLA (Superfund) Program, prompting lengthy site investigations and a feasibility study to determine the extent of the contamination.

Based on the findings from the feasibility study, a biowall was selected as the remedial action (BMT Entech Inc., 2009; Niño de Guzmán et al., 2018; US EPA, 2009). The composition of the biowall was determined based on laboratory experiments resulting in

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a mixture of 30% sand and 70% organic material mixture (Niño de Guzmán et al., 2018). The organic component was made up of mulch and compost (ratio 4:3). Although zero-valent iron (ZVI) shavings and glycerol were considered as potential amendments, they were not added at the time of installation in 2013.

Biowalls are a green technology installed to remove or reduce groundwater contamination, usually to resolve issues where the source cannot be directly managed and the source lifespan is unknown (Powell et al., 1998). Biowalls are active barriers and filters, where fill-materials comprised of organic matter and other materials trap and subsequently aid in degrading sorbed contaminants (Ozturk et al., 2012). As an open system, microbial infiltration and colonization into this porous structure was expected.

Microbial reductive dechlorination is an important conduit for TCE dechlorination. Activity measurements from on-site experiments have shown that the rates of dechlorination are dependent on the abundance of dechlorinating bacteria, soil properties, and the mass loading of reactive minerals (Dong et al., 2009). Therefore, it is advantageous to utilize this naturally occurring process and employ the microbial population of the site to improve the biowall activity by promoting simultaneous biotic and abiotic degradation inside the structure (Dong et al., 2009; Janssen et al., 2001).

ZVI is a strong reducing agent that has been used in studies for the treatment of chlorinated organic compounds without the help of additional materials (Chen et al., 2011; Farrell et al., 2000; Orth and Gillham, 1996). Although ZVI was not installed with the biowall at this time, it is still under consideration for use in the future. While ZVI is a powerful tool for remediation, studies have shown that ZVI also has the potential to inactivate or kill bacteria (Gu et al., 1999; Wilkin et al., 2003; Ingram et al., 2012; Lee et al., 2008; Diao and Yao, 2009; Chen et al., 2011). To avoid this, particle size, dose, and some form of biostimulation should be considered when employing ZVI.

Previous studies have utilized culture-dependent and culture-independent methods based on the 16S rRNA gene such as sequencing and quantitative PCR (qPCR) to characterize microbial populations in groundwater and other sediment systems (Davis et al., 2002; Semprini et al., 1997; Holliger et al., 1993; Da Silva et al., 2008; Fung et al., 2007). The discovery of *Dehalococcoides mccartyi* strain 195 (formerly *D. ethenogenes* strain 195) and its ability to reductively dechlorinate TCE to vinyl chloride (VC) and ethene was a benefit since TCE was previously considered as recalcitrant to biodegradation (Hendrickson et al., 2002; Maymó-Gatell et al., 1997; Seshadri et al., 2005; Löffler et al., 2013). Other organohalide-respiring bacteria and functional genes targeting chlorinated ethenes have since been identified (He et al., 2005; Cupples et al., 2004; Lee et al., 2006; Sung et al., 2006; Krajmalnik-Brown et al., 2004; Sharma and McCarty, 1996; Scholtz-Muramatsu et al., 1995; Gu et al., 2004; Chang et al., 2000). *D. mccartyi* strains 195 and FL2 contain the functional gene *tceA,* which encodes for a TCE reductive dehalogenase that transforms TCE to VC, while strains VS and GT utilize the gene *vcrA* for degradation of TCE to ethene; *D. mccartyi* strain BAV1 employs the gene *bvcA* to reduce VC to ethene (Johnson et al., 2005; He et al., 2003; Lee et al., 2006; Krajmalnik-Brown et al., 2004; Ritalahti et al., 2006). These genes can be used as biomarkers to determine the potential for microbial reductive dechlorination activity in a site (Rowe et al., 2012; Heavner et al., 2018; Dugat-Bony et al., 2012; Ritalahti et al., 2006).

Bioaugmentation of TCE-contaminated sites with *Dehalococcoides* spp. and other microbial consortia containing organohalide-respiring organisms has shown great success especially in conjunction with biostimulation efforts (McDonald et al., 2012; Scheutz et al., 2010; Santharam et al., 2011; Bradley and Chapelle, 1998). In some bioaugmentation strategies, microorganisms at the sites were isolated and reintroduced into the contaminated area to enhance the degradation process (Hood et al., 2008; Lendvay et al., 2003; Lee et al., 2012). The success of these efforts depended on the makeup of the targeted population, the inherent degradation capability of the population, and the cultivation or biostimulation efforts accompanying the bioaugmentation (Hood et al., 2008). Other studies successfully used a commercially available culture for degradation with or without biostimulation (Harkness et al., 1999; Ellis et al., 2000; Lee et al., 2010; Major et al., 2002).

The objectives of this study were to (1) conduct a survey of the soil microbial community at the site to identify microbial TCE degradation clusters, (2) evaluate the potential presence of *Dehalococcoides* species to support bioremediation efforts using native species, and (3) determine if *Dehalococcoides* spp. could survive ZVI amendment in a mock-up of the biowall. In this study, environmental samples were collected from areas showing known presence or absence of dechlorination activity for chlorinated solvents. Soil cores were collected from an uncontaminated control location and two areas upstream of the biowall with a high or moderate concentration of TCE. Fill-material samples from the biowall were collected in 2015, two years after its installation. The habitability of the biowall structure was investigated through a series of flow-through column experiments to determine if biostimulation was necessary to ensure the survival of the culture inside the biowall.

2. Materials and methods

2.1. Sample collection

Soil samples were collected in September 2013 from three locations upstream of the biowall which were undisturbed by the installation process (Fig. 1). The characteristics of these sites were: 1) Remedial Investigation Well 4 (MW4): no detected TCE contamination (method detection limit = 0.3 $\mu g\,L^{-1}$; BMT Designers and Planners, 2015); 2) approximately 30 m north of Biowall Well 4 (nBW4): low/moderate TCE contamination (<260 $ng\,L^{-1}$); 3) Remedial Investigation Well 6 (MW6): high TCE contamination (>260 $ng\,L^{-1}$). Saturated soil cores (0.9 m long, approximately 5 cm diameter) were collected using a Geoprobe soil borer (Geoprobe Systems, Salina, KS). The cores were encased in plastic sheaths, which were tightly wrapped with pallet plastic wrap and kept on ice until transport to the laboratory, where they were frozen until analysis. DNA was extracted from 5 cm sections from the middle of the saturated zone: 3.7–4 m below ground surface (bgs) at MW4, 3.0–3.4 m bgs at nBW4, and 4.0–4.3 m bgs at MW6.

Biowall samples were collected in May 2015 from three locations within the biowall: A) BW3 with low/moderate TCE contamination (260 $ng\,L^{-1}$); B) BW6 with high TCE contamination (>260 $ng\,L^{-1}$); C) BW8 with moderate contamination (130–260 $ng\,L^{-1}$) (Fig. 1). The cores were collected using a 1.8 × 15.2 cm (length x diameter) hand auger to penetrate the fill-material and collect the saturated samples. Each core was deposited onto a clean polyethylene bag, wrapped, and secured with duct tape. At BW3, the saturated zone extended from 0.9 to 1.8 m bgs. Due to the consistency of the core, only the top and bottom 0.3 m were separated and were referred to as BW3 shallow or BW3 deep. At BW6, the saturated zone extended from 1.2 to 1.8 m bgs, so the core was wrapped as two 0.3 m lengths and were referred to as BW6 shallow or deep. Lastly, at BW8 the saturated zone was encountered from 1.1 to 1.5 m bgs and was preserved as a single sample. These samples were kept on ice until transport to the laboratory, where they were frozen until analysis.