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Thermally enhanced in situ bioremediation of groundwater contaminated with chlorinated solvents – A field test

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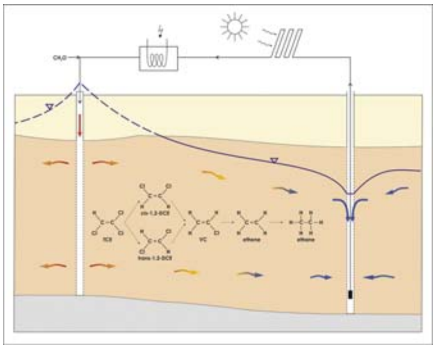
HIGHLIGHTS

- The technology was tested at a site contaminated by chlorinated ethenes.
- A groundwater circulation system was used for heating the aquifers.
- Hydrochemical and molecular genetic methods were used to analyse the processes.
- The application of heat and whey resulted in a fast removal of chlorinated ethenes.
- Temperature alone did not create favourable conditions for dechlorination.

GRAPHICAL ABSTRACT



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ABSTRACT

In situ bioremediation (ISB) using reductive dechlorination is a widely accepted but relatively slow approach compared to other technologies for the treatment of groundwater contaminated by chlorinated ethenes (CVOCs). Due to the known positive kinetic effect on microbial metabolism, thermal enhancement may be a viable means of accelerating ISB.

We tested thermally enhanced ISB in aquifers situated in sandy saprolite and underlying fractured granite. The system comprised pumping, heating and subsequent injection of contaminated groundwater aiming at an aquifer temperature of 20–30 °C. A fermentable substrate (whey) was injected in separate batches.

The test was monitored using hydrochemical and molecular tools (qPCR and NGS). The addition of the substrate and increase in temperature resulted in a rapid increase in the abundance of reductive dechlorinators (e.g., *Dehalococcoides mccartyi*, *Dehalobacter* sp. and functional genes *vcrA* and *bvcA*) and a strong increase in CVOC degradation. On day 34, the CVOC concentrations decreased by 87% to 96% in groundwater from the wells most affected by the heating and substrate. On day 103, the CVOC concentrations were below the LOQ resulting in degradation half-lives of 5 to 6 days. Neither an increase in biomarkers nor a distinct decrease in the CVOC concentrations was observed in a deep well affected by the heating but not by the substrate.

NGS analysis detected *Chloroflexi* dechlorinating genera (*Dehalogenimonas* and GIF9 and MSBL5 clades) and other genera capable of anaerobic metabolic degradation of CVOCs. Of these, bacteria of the genera *Acetobacterium*, *Desulfomonile*, *Geobacter*, *Sulfurospirillum*, *Methanosarcina* and *Methanobacterium* were stimulated by the

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substrate and heating. In contrast, groundwater from the deep well (affected by heating only) hosted representatives of aerobic metabolic and aerobic cometabolic CVOC degraders.

The test results document that heating of the treated aquifer significantly accelerated the treatment process but only in the case of an abundant substrate.

1. Introduction

Chlorinated ethenes are among the most abundant pollutants of groundwater and soil due to their frequent use in industrial applications. Since the mid-1990s, in situ biodegradation (ISB) has been a widely used remedial technology for the treatment of groundwater contaminated by these pollutants. Generally, it relies on sequential reductive dechlorination with each step in the process removing one chlorine atom and replacing it with a hydrogen atom (Bradley and Chapelle, 2010). Thus, tetrachloroethene (PCE) is converted stepwise to trichloroethene (TCE), dichloroethene (DCE), primarily to *cis*-1,2-DCE, then to vinyl chloride (VC) and finally to ethene (Stroo et al., 2014). In this process, chlorinated ethenes serve as the electron acceptor and molecular hydrogen and acetate, both released as by-products of organic substrate fermentation reactions, are used by dechlorinating bacteria as an electron donor and as a carbon source, respectively (Bradley and Chapelle, 2010).

The ability of reductive dechlorination of chlorinated ethenes is restricted to only a few bacterial genera. Microorganisms capable of sequential dechlorinating PCE down to *cis*-1,2-DCE include *Dehalobacter restrictus* (Holliger et al., 1998, 1993), *Desulfuromonas* sp. (Krumholz et al., 1996; Sung et al., 2003), *Geobacter lovleyi* (Sung et al., 2006), *Sulfurospirillum multivorans* (Luijten et al., 2003) and *Desulfitobacterium* sp. (Maillard et al., 2005). *Dehalococcoides mccartyi* is the only one known to be capable of gaining energy through dechlorination of DCE to VC and eventually to ethene (Löffler et al., 2013; Maymó-Gatell et al., 2001).

As in situ availability of electron donors is an important factor limiting the efficiency of microbial reductive dechlorination of chlorinated ethenes to ethene (e.g. Bouwer et al., 1994; Gibson and Sewell, 1992; McCarty, 1996), most practical applications of ISB lie in the delivery of a fermentable substrate (electron donor source). Various types of electron donor sources have been used including soluble, semi-soluble and solid substances (AFCEE, 2004). At sites where microorganisms that can perform full reductive dechlorination are absent or sparse, bioaugmentation i.e., the addition of exogenous cultures, may be utilized to enhance degradation (Ellis et al., 2000; ESTCP, 2010).

The role of temperature on reductive dechlorination was studied in laboratory experiments, which found that the growth of dechlorinating bacteria and the corresponding dechlorination activity depended highly on temperature (Zhuang and Pavlostathis, 1995; Friis et al., 2007; Ni et al., 2015). Friis et al. (2007) showed that degradation rates of lactate-amended TCE dechlorination increased approximately by a factor of 10 when the temperature was increased from 10 to 30 °C. Ni et al. (2015) tested a combination of underground storage energy systems (UTES) and ISB in batch experiments. They concluded that the overall removal rates of *cis*-1,2-DCE under UTES conditions (~25 °C) were from 8.5 to 13 times higher than under natural conditions (10 °C) depending on the configuration of the UTES. Reductive dechlorination of chlorinated ethenes at temperatures of 10, 20, 30 and 40 °C has previously tested in laboratory batch experiments (Najmanová et al., 2016). The highest degradation rates for chlorinated ethenes and the highest abundance of dechlorinating bacteria *Dehalococcoides* sp. were observed at 20 and 30 °C, whereas at 40 °C only incomplete dechlorination was observed. Therefore, increased temperatures can accelerate the bioremediation of sites contaminated by chlorinated ethenes and lessen the drawbacks of slow IRB observed at many sites.

To our knowledge, there are almost no articles published in the literature describing field testing of thermally enhanced IRB. Therefore, the present study was performed to assess the feasibility of this method at a real contaminated site with complex (hydro)geological settings. The remediation process was monitored using a combination of techniques ranging from physical and chemical analysis to molecular biology approaches. This enabled us to describe the effect of increased temperatures on a microbial consortium and to elucidate the treatment process in general.

2. Materials and methods

2.1. Test site

The pilot test was performed at a manufacturing site in the Czech Republic contaminated by chlorinated ethenes originating from historical degreasing activities. The hydrogeological system at the site comprises three aquifers. The upper phreatic aquifer is developed in Quaternary loams and has a saturated thickness of approximately 2 m. The middle aquifer is developed in a sandy eluvium of the granite massive. It is separated from the upper aquifer by a 1.0–1.5 m thick clay layer. The thickness of the confined middle aquifer is 1.5 to 2 m. Irregularly fractured granite holds the lower aquifer. The groundwater discharges into a local river located approximately 300 m from the site.

The groundwater of the upper and middle aquifers is of the Ca-Cl^- type and has elevated mineralization - total dissolved solids from 0.7 g/l (the middle aquifer) to 1.4 g/l (the shallow aquifer) and is slightly acidic (pH 6.4–6.8). The groundwater of the lower aquifer is of the Ca-SO_4^{2-} type and is characterized by low mineralization (total dissolved solids from 0.3 g/l to 0.5 g/l) and slightly acidic pH (6.2–6.5).

Historical use of TCE at the site as a degreasing agent has resulted in contamination of the subsurface at the manufacturing plant. Previous remediation of aquifers using pump and treat and in-situ reductive bioremediation, which terminated in 2012, reduced the amount of CVOCs. However, significant residual contamination remained. Whereas in the upper aquifer concentrations of chlorinated ethenes were below their respective limit of quantification (LOQ; 1.0 μg/l), in the middle and the lower aquifers the total concentration of CVOCs ranged from 249 to 1213 μg/l and from 5340 to 7004 μg/l, respectively.

As a consequence of the remedial activities, parent contaminants were significantly degraded to less chlorinated metabolites. The chlorine number (weighted average number of Cl atoms per molecule of ethene (Bewley et al., 2015)) in the groundwater ranged from 1.1 (the middle aquifer) to 1.8 (the lower aquifer) prior to the pilot test. Therefore, vinyl chloride (VC) and *cis*-1,2-dichloroethene (*cis*-1,2-DCE) were the dominant chlorinated contaminants.

2.2. Pilot application of the technology

The pilot test focused on the middle and lower aquifers. The treated zone was heated via groundwater circulation. Groundwater was abstracted from well HV-64 screened to both of the aquifers (average rate of 0.13 l/s), pre-heated in solar absorbers, then heated to the predefined temperature of 35 to 40 °C in an electrical heater and finally injected back via injection well IN-1, which was also screened to both of the aquifers. Heating and groundwater circulation lasted 3 months with