Journal of Contaminant Hydrology 103 (2009) 157–167

Contents lists available at ScienceDirect

Journal of Contaminant Hydrology

journal homepage: www.elsevier.com/locate/jconhyd

Bioaugmentation with butane-utilizing microorganisms to promote *in situ* cometabolic treatment of 1,1,1-trichloroethane and 1,1-dichloroethene

Lewis Semprini [a,*], Mark E. Dolan [a], Gary D. Hopkins [b], Perry L. McCarty [b]

[a] *School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, OR 97331, USA*
[b] *Department of Civil and Environmental Engineering, Stanford University, Stanford, CA 94305, USA*

ARTICLE INFO

Article history:
Received 6 August 2008
Received in revised form 6 October 2008
Accepted 7 October 2008
Available online 17 October 2008

Keywords:
Bioaugmentation
Cometabolism
Butane
1,1,1-trichloroethane
1,1-dichloroethene
In situ

ABSTRACT

A field study was performed to evaluate the potential for *in-situ* aerobic cometabolism of 1,1,1-trichloroethane (1,1,1-TCA) through bioaugmentation with a butane enrichment culture containing predominantly two *Rhodococcus* sp. strains named 179BP and 183BP that could cometabolize 1,1,1-TCA and 1,1-dicholoroethene (1,1-DCE). Batch tests indicated that 1,1-DCE was more rapidly transformed than 1,1,1-TCA by both strains with 183BP being the most effective organism. This second in a series of bioaugmentation field studies was conducted in the saturated zone at the Moffett Field *In Situ* Test Facility in California. In the previous test, bioaugmentation with an enrichment culture containing the 183BP strain achieved short term *in situ* treatment of 1,1-DCE, 1,1,1-TCA, and 1,1-dichloroethane (1,1-DCA). However, transformation activity towards 1,1,1-TCA was lost over the course of the study. The goal of this second study was to determine if more effective and long-term treatment of 1,1,1-TCA could be achieved through bioaugmentation with a highly enriched culture containing 179BP and 183BP strains. Upon bioaugmentation and continuous addition of butane and dissolved oxygen and or hydrogen peroxide as sources of dissolved oxygen, about 70% removal of 1,1,1-TCA was initially achieved. 1,1-DCE that was present as a trace contaminant was also effectively removed (~80%). No removal of 1,1,1-TCA resulted in a control test leg that was not bioaugmented, although butane and oxygen consumption by the indigenous populations was similar to that in the bioaugmented test leg. However, with prolonged treatment, removal of 1,1,1-TCA in the bioaugmented leg decreased to about 50 to 60%. Hydrogen pexoxide (H_2O_2) injection increased dissolved oxygen concentration, thus permitting more butane addition into the test zone, but more effective 1,1,1-TCA treatment did not result. The results showed bioaugmentation with the enrichment cultures was effective in enhancing the cometabolic treatment of 1,1,1-TCA and low concentrations of 1,1-DCE over the entire period of the 50-day test. Compared to the first season of testing, cometabolic treatment of 1,1,1-TCA was not lost. The better performance achieved in the second season of testing may be attributed to less 1,1-DCE transformation product toxicity, more effective addition of butane, and bioaugmentation with the highly enriched dual culture.

1. Introduction

In situ aerobic cometabolism is a method for reducing groundwater contamination with chlorinated aliphatic hydrocarbons (CAHs) (Semprini et al., 1990; Hopkins and McCarty, 1995; McCarty et al., 1998). Detailed reviews of previous studies on cometabolic transformation of chlorinated solvents have been provided by Alvarez-Cohen and Speitel (2001) and Arp et al. (2001). Cometabolic transformation results from nonspecific enzymes fortuitously catalyzing these reactions. Because cometabolic transformation does not provide energy or carbon for organism growth, a primary substrate must be supplied to stimulate growth of the cometabolizing microorganisms. In oxidative cometabolism,

* Corresponding author. 102 Gleeson Hall, Oregon State University, Corvallis, OR 97331-2702, USA. Tel.: +1 541 737 6895; fax: +1 541 737 4600.
E-mail address: lewis.semprini@oregonstate.edu (L. Semprini).

0169-7722/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconhyd.2008.10.005

the enzymes use the primary growth substrate as an electron donor and oxygen as an electron acceptor, and often the growth substrate is the inducer for the cometabolizing enzymes, which are generally oxygenases.

1,1,1-TCA is a frequently observed groundwater contaminant (Squillace et al., 1999) with a maximum contaminant level (MCL) of 200 µg/L set by the U.S. EPA. 1,1,1-TCA can also be abiotically transformed in water to 1,1-dichloroethene (1,1-DCE) (Vogel and McCarty, 1987), which has a MCL of 7 µg/L. Thus there is interest in developing methods for the *in situ* treatment of both 1,1,1-TCA and 1,1-DCE. Microorganisms that grow on butane have the ability to cometabolize a broad range of CAHs (Hamamura et al., 1997; Kim et al., 1997; Hamamura et al., 1999; Kim et al., 2000), such as 1,1,1-TCA, 1,1-DCE, and 1,1-dichloroethane (1,1-DCA) (Kim et al., 2002b).

Semprini et al. (2007) recently reported the results from a field demonstration in which bioaugmentation with an enrichment culture of butane-utilizing organisms was conducted to promote the aerobic cometabolism of 1,1-DCE, 1,1,1-TCA, and 1,1-DCA. The bioaugmented test leg was shown to initially outperform the control test leg where only indigenous butane-utilizing organisms were stimulated. 1,1-DCE was most effectively cometabolized, followed by 1,1-DCA, and 1,1,1-TCA. However, with prolonged biostimulation through butane and oxygen addition, effective cometabolism was lost. By the end of that study, butane utilization was observed in both the bioaugmented and indigenous experimental legs, and 1,1-DCE was being cometabolized in both as well, but no 1,1,1-TCA transformation was observed in either leg. The results indicated that an indigenous population of butane utilizers that could effectively transform 1,1-DCE but not 1,1,1-TCA became dominant in both legs. Modeling analysis indicated that 1,1-DCE transformation toxicity was one possible reason why transformation potential was lost. Another possible reason was that insufficient butane may have been added.

Presented here are the results of the second season of field testing. The goal of this field testing was to determine whether long-term transformation of 1,1,1-TCA could be achieved in the bioaugmented test leg in the absence of high concentrations of 1,1-DCE, which was indicated to promote transformation product toxicity in the first test season. The second season also differed from the first in that a dual culture containing two *Rhodococcus* sp. strains (BP179 and BP183) was used for bioaugmentation, while an enrichment of mainly BP183 was added in the first season. Hydrogen peroxide was also added as an additional source of dissolved oxygen to permit the addition of more butane as a cometabolic substrate.

2. Materials and methods

2.1. Site description

A description of the field site, experimental tests legs, and protocols have been provided in detail by Semprini et al. (2007), and will only briefly be presented here.

Field studies were conducted at the Moffett Test facility, which has been used in past studies of *in situ* aerobic cometabolism (Semprini et al., 1990; Hopkins et al., 1993; Hopkins and McCarty, 1995). The test legs were located in a shallow confined alluvial aquifer composed of poorly sorted materials. Details of the site hydrogeology are provided by Roberts et al. (1990). Two experimental test legs were installed (Fig. 1). One leg served as the control test leg (west leg) where indigenous butane utilizers were stimulated, and the other test leg served as the bioaugmented test leg (east leg). Each test leg consisted of an injection well and an extraction well separated by about 7 m with monitoring wells in between. Tests were conducted using protocols described in previous studies (Roberts et al., 1990; Semprini et al., 1990; Semprini et al., 2007). Induced gradient conditions for each experimental test leg were created by injecting groundwater at 1.25 L/ min and extracting at approximately 8 L/min. The extracted groundwater was air-stripped to remove volatile components, and a portion was amended with the chemicals of interest and re-

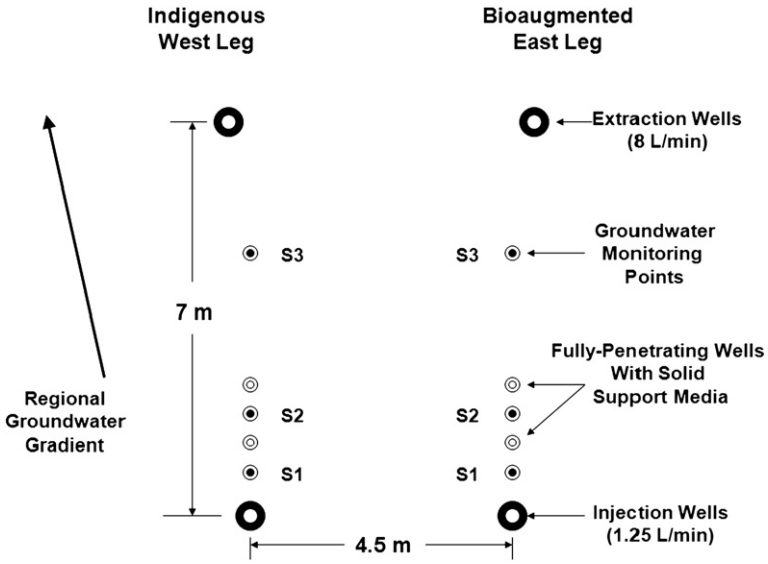

Fig. 1. Layout of the indigenous, or west, and the bioaugmented, or east, well legs at the Moffett Field test site. Groundwater monitoring points S1, S2, and S3 were placed approximately 1 m, 2 m, and 4 m from their respective injection wells. Wells designated FP were fully penetrating wells with support media to sample attached microbial mass.