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

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A B S T R A C T

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-dichloroethene (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 peroxide (H2O2) 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.

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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,
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-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-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, 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 test 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.](image-url)
Fig. 2. Scanning electron micrographs of the strain 183BP culture (left frame, 5 μm bar) and the strain 179BP culture (middle frame, 5 μm bar and right frame, 20 μm bar) show an even distribution of strain 183BP cells across the membrane and clumps of aggregated strain 179BP cells after growth in mineral media with a butane headspace.
injected. The concentrations of CAHs, dissolved butane and oxygen were measured on-site using an automated data acquisition system described by McCarty et al. (1998).

2.2. Microbial cultures

The butane-utilizing culture used in this study was developed from a parent culture enriched from aquifer sediments from the Hanford DOE site, Washington. The kinetics and inhibitory interactions of butane utilization and CAH cometabolic transformation by this culture were reported by Kim et al. (2002a,b). The butane enrichment culture used for bioaugmentation in the second season of testing consisted primarily of two strains of *Rhodococcus* sp., named strain 179BP and 183BP, which were differentiated by terminal restriction fragment length polymorphism (TRFLP) analysis. Bioaugmentation in the first season was performed with an enrichment culture containing strain 183 BP.

The two strains were isolated from the parent culture using serial plating. A frozen aliquot of the parent culture was grown in batch culture with a 4% butane headspace, harvested during log growth phase and serially plated onto agar containing only mineral salts media. The plates were incubated at 20 °C with a 3% butane headspace. Individual colonies were harvested and serially plated through four or more generations. This resulted in the isolation of organisms with TRFLP of 183BP (cultures B6 and B8) and 179BP (cultures B1 and B4).

Further enrichment was performed using standard streaking techniques in which the streak-plates were grown with a butane headspace, and the monocultures obtained were aliquoted into cryogenic tubes with 7% dimethyl sulfoxide (DMSO), and stored at −80 °C. The two different cultures obtained, strains 183BP and 179BP, were used in subsequent studies.

The two strains have different morphologies when grown in media (Fig. 2). Strain 179BP grew in clumps in media and colonized surfaces, while strain 183BP grew planktonically and did not stick to vessel walls, indicating that strain 183BP may transport better in the subsurface. When viewed using scanning electron microscopy, strain 183BP was evenly distributed and not aggregated, whereas strain 179BP grew almost exclusively in aggregates. 16S rRNA gene sequencing indicated strain 183BP was a close relative of *Rhodococcus* sp. USA-AN012 (nitrile-metabolizing actinomycetes), and strain 179BP was a 98% match (500BP fragment of 16S rRNA) to *Rhodococcus koreensis* and *Rhodococcus opacus*.

Batch kinetic tests were performed to evaluate the ability of the two highly enriched strains to cometabolize 1,1-DCE and 1,1,1-TCA using procedures described previously (Semprini et al., 2007). TRFLP analysis indicated that the cultures were highly enriched with either the 179BP or 183BP organism. The results indicated that the cultures enriched for the 183BP organism were able to oxidize butane at faster specific rates than the cultures enriched for the 179BP organism (data not shown). Resting cells tests were performed in which the cultures were grown first on butane, harvested, and then tested for their transformation abilities in the absence of growth substrate. Results are presented in Fig. 3, where the cumulative amounts of 1,1,1-TCA and 1,1-DCE transformed are shown following successive additions. Cultures B6 and B8 (183BP enrichments) transformed about 3 to 4 times more 1,1-DCE and 1,1,1-TCA than the B1 and B4 enrichments (179BP). 1,1-DCE was also much more rapidly transformed than 1,1,1-TCA (note different time scales on the axis), consistent with the results of detailed kinetic studies performed with the parent culture (Kim et al., 2002b).

In the first season of testing, the enrichment culture used contained predominantly the 183BP culture. Since transformation activity was eventually lost, we decided in the second season to use a more complex culture for bioaugmentation, one that contained both the 179BP and the 183BP enrichments. As shown in Fig. 2, the two strains have different morphologies that might prove to be a benefit in bioaugmentation. Strain 179BP grew in clumps and would likely attach better to aquifer solids and support a near well bioreactor, while strain 183BP, being more planktonic in nature, would likely be transported further from the injection well. The potential for both possibilities was of interest in developing an effective treatment zone. In addition, bioaugmentation
studies in microcosms containing aquifer solids and groundwater from the site showed most effective 1,1-DCE transformation when both strains were present (Semprini et al., 2005).

In order to produce a significant mass of strains 179BP and 183BP for field bioaugmentation, batch growth conditions were employed. Fortyeight 700 mL bottles containing 300 mL mineral media and 400 mL headspace with 4% butane in repeated butane injections were incubated on shaker tables oscillating at 200 rpm at 20 °C. As vacuum was created in the bottles due to butane and oxygen utilization, the additional oxygen needed was automatically drawn into the bottles. The batch cultures were harvested (producing approximately 3 g of culture dry weight for each strain), concentrated by centrifugation, and shipped to the field site in two refrigerated 500 mL centrifuge bottles.

3. Field tests

1,1,1-TCA was the main compound of interest in the study, since it was the contaminant that was least successfully transformed during the first season of testing (Semprini et al., 2007). 1,1-DCE was also monitored, since it was present as a background contaminant at low concentrations in the site groundwater (~4 μg/L), and was also present as a trace contaminant in the added 1,1,1-TCA, resulting in 1,1-DCE concentrations as high as 12 μg/L in the field tests.

Protocols for the field tests were similar to those used in the first season. The west test leg remained the control leg with indigenous microorganisms only. Otherwise, it was operated in a manner similar to that used in the bioaugmented east leg. As in the first season of testing, both experimental legs were operated under induced gradient conditions of injection and extraction. Table 1 presents the conditions of the tests during the second season of testing and the sequence of tests. The second season commenced about 7 months after the completion of the first season of testing. During the period between field seasons neither butane nor oxygen were added to the test legs. In the second season of testing 1,1,1-TCA was the main contaminant of interest and was continuously injected at concentrations ranging from 80 to 140 μg/L. Groundwater containing butane or DO and H2O2 was alternatively pulse-injected as described by Semprini et al. (2007), with a pulse cycle time for butane of about 45 min and oxygenated water containing H2O2 (10 to 45 mg/L) and DO (30 mg/L) for about 2 h. This resulted in pulse averaged butane concentrations ranging from 4 to 8 mg/L. The average pulse injected oxygen concentration, based on the DO present in the injected groundwater and the additional DO that would be produced upon the decomposition of the H2O2, varied from 8 to 22 mg/L.

The following sequence of tests were performed in both experimental legs: 1) bromide tracer tests and H2O2 addition under induced gradient conditions to study transport and hydrogen peroxide decomposition to DO; 2) addition of H2O2 and 1,1,1-TCA as a control phase prior to bioaugmentation and biostimulation to determine if 1,1,1-TCA was transformed as a result of H2O2 addition alone; 3) bioaugmentation with the culture (east leg) in the presence of 1,1,1-TCA and butane, and DO addition to both experimental legs to evaluate whether 1,1,1-TCA cometabolism was enhanced by bioaugmentation; 4) long-term biostimulation of both test legs with butane, DO and hydrogen peroxide injection to evaluate long term 1,1,1-TCA transformation; and 5) increased H2O2 and butane addition to both test legs to increase active biomass and to determine whether more effective 1,1,1-TCA cometabolism could be achieved in this manner.

4. Results

4.1. Tracer tests

Bromide tracer tests conducted on the east and west legs of the test site were used to evaluate the degree of concentration breakthrough of the injected groundwater at the monitoring locations. Essentially 100% bromide breakthrough was observed at the S1 east monitoring well, while 86%, and 93% were achieved at the S2 east, and S3 east monitoring wells, respectively (data not presented). The results were essentially the same as those achieved during the first season of testing. Under similar hydraulic conditions the west leg showed lower degrees of bromide breakthrough of 89%, 19%, and 89% at the S1, S2, and S3 west monitoring wells, respectively. Thus more complete breakthrough of injected groundwater was observed in the east leg compared with the west leg. Differences in tracer breakthrough extents were observed previously in other test site experimental legs and were considered to result from the aquifer being spatially heterogeneous over short distances (Roberts et al., 1990).

4.2. First 35 days of testing

Figs. 4–7 show DO, butane, 1,1,1-TCA, and 1,1-DCE concentrations, respectively during the first 35 days of testing at the three monitoring well locations along each experimental leg. The west (indigenous) leg and east (bioaugmented) leg

Table 1

<table>
<thead>
<tr>
<th>Test sequence</th>
<th>Duration (days)</th>
<th>Chemicals injected</th>
<th>Average concentration</th>
<th>Process studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 to 8</td>
<td>H2O2, Bromide</td>
<td>10 mg/L</td>
<td>Bromide tracer and H2O2 decomposition</td>
</tr>
<tr>
<td>2</td>
<td>9 to 23</td>
<td>1,1,1-TCA, Oxygen</td>
<td>10 mg/L, 140 mg/L</td>
<td>1,1,1-TCA transport with H2O2 addition and biotransformation prior to bioaugmentation and butane addition</td>
</tr>
<tr>
<td>3</td>
<td>23 to 30</td>
<td>1,1,1-TCA, Butane</td>
<td>10 μg/L, 100–115 μg/L</td>
<td>Bioaugmentation, biostimulation, and biotransformation of 1,1,1-TCA and 1,1-DCE</td>
</tr>
<tr>
<td>4</td>
<td>30 to 50</td>
<td>H2O2, 1,1,1-TCA</td>
<td>15 mg/L, 80–100 mg/L</td>
<td>Long term biostimulation and biotransformation with H2O2, butane, and 1,1,1-TCA addition</td>
</tr>
<tr>
<td>5</td>
<td>50 to 70</td>
<td>H2O2, 1,1,1-TCA</td>
<td>15 to 45 mg/L</td>
<td>Long term biostimulation and biotransformation with increased H2O2, butane, and 1,1,1-TCA addition</td>
</tr>
</tbody>
</table>

a Added until day 17. 

b Added day 17 to 23.
are presented together for comparison purposes. The tests were initiated with the introduction of hydrogen peroxide in order to evaluate its transformation to dissolved oxygen, and also as a means of potentially lowering the biomass present from the previous year’s bioaugmentation and biostimulation tests. The DO concentration at the monitoring wells of the east and west legs are shown in Fig. 4. Hydrogen peroxide (10 mg/L), in the absence of butane, was added during the first 15 days of the test. DO concentrations of approximately 5 mg/L were observed at the monitoring wells, which is in agreement with the stoichiometric amount that would be expected from the breakdown of H₂O₂ to ½ mol O₂ and 1 mol of H₂O. The results indicate that hydrogen peroxide decomposes to oxygen by the time of transport to the first monitoring well, S1. The experimental legs show nearly identical results.

A change from H₂O₂ to the addition of dissolved oxygen (DO) occurred around day 17 so that bioaugmentation would occur in the presence of DO, and not H₂O₂, which might harm the culture. A DO breakthrough to higher concentrations around day 20 was observed at both experimental legs. Consistent with the bromide tracer results, DO breakthrough to a lower concentration at the S2 east well resulted from incomplete breakthrough of the injected groundwater. DO also peaked at a lower concentration at the S2 west well, which is consistent with incomplete 1,1,1-TCA breakthrough (Fig. 6). The DO concentration decreased after day 23 as a result of butane addition and subsequent biostimulation of the test zone.

Bioaugmentation of the east test leg was started on day 23. Three grams (dry weight) of each of the two cultures, stains 172BP and 183BP, were diluted into 48 L of site groundwater at an approximate injection concentration of 1 × 10¹⁰ CFU/mL and injected over a period of several hours into the east leg injection well. The injection rate was maintained at 1.25 L/min, the same rate that was used throughout the field tests. Butane concentrations for both legs are shown in Fig. 5. Butane addition was started on day 22 just prior to bioaugmentation on day 23. Butane was observed to breakthrough to a maximum of about 4 mg/L in the west leg, and about 3 mg/L in the bioaugmented east leg. Butane concentration decreased to below its detection limit by day 25 in the bioaugmented east leg, and by day 26 in the indigenous west leg. Additionally, butane increased to over 3 mg/L in the S3 west leg, while the concentration never increased above 1 mg/L in the S3 east leg. The lesser maximum concentration and the more rapid consumption of butane in the east leg likely resulted from bioaugmentation there. It is interesting to note that butane was more rapidly consumed in the indigenous west leg than in the first season of field testing, where 40 to 50 days was required before oxygen and butane uptake were observed (Semprini et al., 2007). Thus, it appears that a much greater biomass of butane-utilizing organisms was present at the start of the second season of testing. A
significant number of butane utilizers apparently survived the seven-month period of starvation when neither butane nor oxygen was added. This is consistent with previous field studies at this site with methane-utilizing microorganisms (Semprini and McCarty, 1991). Also, the hydrogen peroxide addition at the beginning of the experiment does not appear to have disinfected the test legs.

The temporal response of 1,1,1-TCA at the monitoring wells is shown in Fig. 6. 1,1,1-TCA addition to the test zone was started around day 8 at 115 μg/L, in the presence of hydrogen peroxide. The presence of hydrogen peroxide did not cause any observable transformation of 1,1,1-TCA.

A sequential breakthrough of 1,1,1-TCA at the monitoring locations was observed in both test legs, consistent with the results from the first season of field testing. Prior to biostimulation, 1,1,1-TCA breakthroughs to injection concentrations indicates essentially no transformation taking place prior to bioaugmentation or butane addition. In the east leg maximum concentrations were observed after about 15 days of injection. The S1 east breakthrough concentrations are similar to the injection concentrations, and concentrations are maintained at this level until biostimulation on day 23. The 80% and 90% breakthrough of 1,1,1-TCA at the S2 and S3 east monitoring wells are consistent with bromide tracer test results, thus showing incomplete breakthrough of the injected groundwater. Also as a result of incomplete breakthrough of the injected groundwater, 1,1,1-TCA in the west leg never reached injection concentrations at any of the monitoring locations. The greatest fractional breakthrough was about 85% at the S3 monitoring well, while lower degrees of breakthrough of 80% and 32% were observed at the S1 and S2 monitoring wells, respectively. The results are similar to those achieved during the first season of testing. Bromide tracer tests conducted after these tests were completed showed incomplete breakthrough of the injected groundwater in the west test leg similar to that observed for 1,1,1-TCA (data not shown). The lack of complete breakthrough of DO observed at the S3 well on the west leg is also consistent with the results of the tracer tests.

The concentration of 1,1,1-TCA decreased in the east test leg in response to bioaugmentation and biostimulation on day 23. The subsequent concentration decreases coincided with decreases in oxygen and butane concentrations. The decrease was most rapid at the S1 well with results indicating most of the biotransformation occurred between the injection well and the S1 well. Also, butane was completely removed by the time flow reached the S1 well, indicating that most of the biological activity occurred within 1 m of the injection well. While the indigenous west leg in comparison did have similar uptake of butane and dissolved oxygen, 1,1,1-TCA concentrations did not decrease here. The concentration remained essentially constant at all the observation locations. Within our ability to measure and detect changes in 1,1,1-TCA concentration, the indigenous butane utilizers were not transforming 1,1,1-TCA.

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Fig. 7 presents concentrations of 1,1-DCE in the two test legs, which were present at a background concentration of about 4 µg/L. This compound was present at all monitoring locations even before 1,1,1-TCA was added to the test legs. It was also present throughout the study in the extracted groundwater used to make up the injected groundwater. When 1,1,1-TCA addition began around day 8, 1,1-DCE concentrations also increased in the injected fluid and at the monitoring locations because of its presence in the 1,1,1-TCA stock solution. With bioaugmentation and biostimulation of the east leg on day 23 the concentration of 1,1-DCE decreased significantly from the injection concentration. Consistent with the 1,1,1-TCA observations, the concentration decreased most between the injection and S1 east well, and only a little more thereafter. The 1,1-DCE concentration in the east leg was reduced by day 30 from 10 µg/L to about 2 µg/L. At the same time, 1,1-DCE concentration also decreased in the indigenous west leg from 10 mg/L to about 6 µg/L, indicating some transformation of 1,1-DCE was occurring there, again primarily within the first meter of transport through the test leg. These results showing 1,1-DCE transformation in both legs, but more so in the bioaugmented leg, are consistent with the results from the first season of testing.

During the first 40 days, DO was injected without hydrogen peroxide. At around day 32, the DO concentration decreased to below its detection limit (0.2 mg/L) in both experimental legs (Fig. 4). The reason for this is not completely known, but may have resulted from the increase in biomass in the experimental legs, resulting in more DO consumption through organism decay.

Around day 32, butane was also detected at monitoring wells in both experimental legs, as shown in Fig. 5. Concentrations were typically well below 1 mg/L, but there were several observations above 2 mg/L. The 1,1,1-TCA and 1,1-DCE concentrations (Figs. 6 and 7) also increased in the east leg during the 31 to 33 day period when DO concentrations were low and butane concentrations increased. The transformations of 1,1-TCA and 1,1-DCE correlate with the DO and butane concentration changes. This would be expected since their transformation requires oxygen. Also, the presence of butane has a strong inhibitory effect on the cometabolic transformations (Kim et al., 2002b). The low DO and high butane concentrations during this period did not result in an increase in 1,1,1-TCA in the west leg as biotransformation was not in effect there.

4.3. Days 35 to 70 of Testing

Figs. 8–11 present the temporal responses for days 35–70 of the test. During this period, changes in operating conditions were made to try to improve the amount of transformation achieved. In addition, performance of the system over a period of about 2 months of operation was assessed.

The DO concentration history in Fig. 8 indicates some of the operational changes that were made, and also provides some insight into conditions that affected the performance of
the system. During the period of 38 to 45 days, DO measurements were lost due to a malfunctioning DO probe. During this period, butane was still effectively removed in experimental legs (Fig. 9), and 1,1,1-TCA and 1,1-DCE transformations in the east leg returned to normal (Figs. 10 and 11), thus DO concentrations were probably adequate.

During days 40 to 70, hydrogen peroxide was added while DO addition was continued. Hydrogen peroxide addition was begun at 5 mg/L from day 40 to day 48, was increased to 15 mg/L over days 48 to 52, increased to 30 mg/L over days 52 to 60, and increased again to 45 mg/L at day 60. At the same time, the length of the butane pulse cycle was also increased to deliver more butane to the tests legs. The hydrogen peroxide additions resulted in sufficient DO in both experimental legs. A period of butane breakthrough was observed around 57 day in both experimental legs; however, DO was not completely depleted and it appears likely the high butane levels were caused by a malfunction in the butane delivery system, where the system became stuck on a butane pulse cycle, resulting in high butane concentrations.

Between days 30 and 50, the injection concentration of 1,1,1-TCA was gradually decreased from 120 μg/L to 80 μg/L, but the concentrations at the monitoring locations remained constant, or increased slightly, indicating a decreasing removal efficiency with time. Thus, the increases in hydrogen peroxide and butane addition did not result in a hoped for increase in 1,1,1-TCA transformation efficiency in the east leg. 1,1,1-TCA concentrations gradually decreased in the west leg, in response to the decrease in the injection concentration. The concentration of 1,1-DCE remained relatively constant in the east leg, where it was being more effectively transformed, while it decreased in the west leg in response to the decrease in the injection concentration.

From day 52 to 68, the injection concentration of 1,1,1-TCA was increased to 145 μg/L in the east leg and to about 160 μg/L in the west leg. The co-contaminant 1,1-DCE also increased in the injected groundwater. In response, the concentration of 1,1,1-TCA also increased at the monitoring locations in both the east and west experimental legs. However, the percentage removal remained relatively unchanged in the east leg, and the concentrations observed in the west leg are consistent with little or no transformation of 1,1,1-TCA there. Similarly the percentage removals of 1,1-DCE remained relatively unchanged in both experimental legs.

**Table 2** Percentage removals of 1,1,1-TCA and 1,1-DCE during different periods of the second season of field testing

<table>
<thead>
<tr>
<th></th>
<th>Bioaugmented 1,1,1-TCA % Removal</th>
<th>1,1-DCE % Removal</th>
<th>Indigenous 1,1,1-TCA % Removal</th>
<th>1,1,DCE % Removal</th>
<th>1,1,1-TCA % Removal</th>
<th>1,1-DCE % Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>East Leg</strong></td>
<td></td>
<td></td>
<td><strong>West Leg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27–35 days</td>
<td>70</td>
<td>83</td>
<td>27–35 days</td>
<td>0</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>35–50 days</td>
<td>52</td>
<td>66</td>
<td>35–50 days</td>
<td>0</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>58–68 days</td>
<td>56</td>
<td>60</td>
<td>58–68 days</td>
<td>0</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>
The percentage removals of 1,1,1-TCA and 1,1-DCE were estimated for the different periods of the test based on the average injection concentrations and the measured concentrations at the S3 monitoring wells (Table 2). The values were corrected for incomplete breakthrough of the injected groundwater, based on the bromide tracer tests on the east leg and the 1,1,1-TCA concentrations observed in the west leg prior to butane biostimulation, as well as the bromide tracer tests performed after the tests were completed. These estimates indicate that the best removals of 1,1,1-TCA and 1,1-DCE, 70% and 83%, respectively, were observed on the east leg from days 30 to 35 or about 7 to 12 days after the test leg was bioaugmented. The removal efficiency decreased over the course of the experiment to about 56 to 60%. The removal efficiency remained about the same regardless of 1,1,1-TCA concentration between 80 and 140 µg/L and 1,1-DCE concentration between 6 and 10 µg/L. In contrast, the west leg indigenous leg showed no measurable 1,1,1-TCA removal, and only about 40% 1,1-DCE removal. The addition of hydrogen peroxide together with more butane in the latter stages of the test did not result in more effective transformation of either 1,1,1-TCA or 1,1-DCE.

5. Discussion

Bioaugmentation with a butane-utilizing culture known to effectively cometabolize 1,1,1-TCA promoted its transformation in the field. Here, 1,1,1-TCA was transformed in the bioaugmented east leg but not in the west leg, which had no bioaugmentation. However, the consumption of dissolved oxygen and butane was comparable in both legs, thus indicating that the lack of 1,1,1-TCA transformation in the indigenous leg was not attributed to ineffective butane utilization. The transformation of 1,1,1-TCA and 1,1-DCE through bioaugmentation as well as biostimulation was maintained for the entire period of the 50-day test. This compares to the results from the first year of testing, where over a similar period of operation, 1,1,1-TCA transformation ability was lost (Semprini et al., 2007). One difference between the first and second seasons of testing is that the 1,1-DCE concentration was much lower in the second season, and thus less transformation toxicity or inhibition may have resulted. Another difference is that the bioaugmented inoculum differed. The first season bioaugmentation culture consisted of an enrichment that contained strain 183BP, which lacked the strain 179BP. Whether the tests benefited from the addition of the 179BP culture is not known. While this culture transformed 1,1,1-TCA more slowly (Fig. 2), it did form aggregated clumps and perhaps attached better to the aquifer solids. Also in the second season of testing, greater efforts were made to maintain dissolved oxygen and butane concentrations in the injected groundwater.

Consistent with the first season of testing, 1,1-DCE was transformed in both experimental legs, with more effective removal achieved in the bioaugmented leg. However, in the first season the removal percentage for 1,1-DCE was higher (Semprini et al., 2007) at 80 to 97% removal in the bioaugmented leg and 76 to 86% in the indigenous leg. The injection concentrations of 1,1-DCE were an order of magnitude higher in the first season of testing. Competitive inhibition may have contributed to the lower 1,1-DCE removals in the second season since the concentration of 1,1,1-TCA was higher and 1,1-DCE was lower.

Kim et al. (2002a,b) found that 1,1-DCE and 1,1,1-TCA competitively inhibit each other’s transformations, and butane inhibited the transformation of both substrates. The lower concentrations of 1,1-DCE may have resulted in greater inhibition by both 1,1,1-TCA and butane. It is also possible that the background contaminant in the field present at around 4 µg/L was not 1,1-DCE, but co-eluted with 1,1-DCE during the GC analysis performed at the field site. GC-MS analysis of groundwater at the Moffett site reported in previous studies (Roberts et al., 1990) showed, for example, 1,1,2-trichloro-1,2,2-trifluoroethane (CF3C1C2F3) as being a trace contaminant in the site groundwater at concentrations of less than 10 µg/L. This compound would not be cometabolically transformed, and thus if co-eluting with 1,1-DCE would cause estimates of 1,1-DCE removal to be too low. The amount of 1,1-DCE observed to be in the groundwater after treatment equals the concentration in the background groundwater prior to treatment (Figs. 7 and 11), which supports a co-eluting contaminant being present. Unfortunately, no attempt was made to determine the presence of a co-eluting contaminant at the time of the field test, which raises uncertainty in the 1,1-DCE results.

Transformation was maintained when hydrogen peroxide was added to the test legs as a source of dissolved oxygen. Thus hydrogen peroxide did not prove to be toxic to the butane utilizers at the concentration used. This is consistent with previous studies at the site with toluene and phenol-utilizing organisms (Hopkins and McCarty, 1995). However, the addition of more butane to the test legs, which higher DO concentrations permitted, did not result in more effective transformation of 1,1,1-TCA and 1,1-DCE. The reason for this is not known, but might be attributed to butane inhibition.

Detailed studies of why some butane utilizers can transform 1,1,1-TCA while others cannot have been documented in the literature. Differences, for example, were observed between the closely related 179BP and 183BP strains (Fig. 3). Also, the indigenous microorganisms stimulated in the west leg had limited or no ability to transform 1,1,1-TCA, but had some ability to transform 1,1-DCE. Jitnuyanont et al. (2001) found 1,1,1-TCA to be transformed in bioaugmented microcosms, but minimal transformation by indigenous microorganisms stimulated on butane. Hamamura et al. (1999) found diversity in the butane monooxygenases of Pseudomonas butanovora, Mycobacterium vaccae JOB5, and an environmental isolate CF8. This diversity may result in differences in 1,1,1-TCA transforming ability.

An interesting observation is that the 1,1,1-TCA removal achieved in the bioaugmented leg was similar to that achieved in a continuous-flow column study by Maremenda (2004). In that study a column packed with aquifer solids from the Moffett site was bioaugmented with an enrichment culture containing the 183BP strain. Other findings from the column study were that 1,1,1-TCA transformation could be maintained for several months in the absence of 1,1-DCE, but transformation was greatly inhibited upon the addition of 1,1-DCE. The best percentage removal of 1,1,1-TCA of around 80% that was achieved soon after bioaugmentation decreased to about 60% with time, similar to that observed in the field study.

Past studies of in-situ cometabolism at the Moffett Field In Situ Test Facility with microbes stimulated on methane, phenol, and toluene (Semprini et al., 1990; Hopkins et al.,
1,1,1-TCA could not be transformed. The results presented show promise in the cometabolic treatment of 1,1,1-TCA through bioaugmentation and use of butane as a cometabolic substrate.

The loss of activity over time may have been associated with the growth of indigenous butane-utilizing microorganisms that had limited ability to transform 1,1,1-TCA. It is encouraging that transformation activity in this second season was maintained for up to 50 days after bioaugmentation, indicating that members of the bioaugmented culture were not displaced by butane utilizers unable to transform 1,1,1-TCA, as appears to have happened during the first season of study. The results indicate that there is potential for bioaugmentation of aerobic cometabolic cultures to achieve more effective in situ treatment of CAH contaminated aquifers.

6. Conclusions

A successful controlled evaluation of the bioaugmentation of a butane-utilizing culture for the in situ aerobic cometabolic treatment of 1,1,1-TCA and 1,1-DCE was performed in a shallow confined alluvial aquifer. Tests were performed in two experimental legs; one leg served as the control test leg where indigenous butane utilizers were stimulated, and the other test leg served as the bioaugmented test leg. The tests legs were operated under similar induced gradient conditions of injection and extraction and similar injection concentrations of dissolved butane, DO, 1,1,1-TCA and 1,1-DCE. More effective in situ treatment of 1,1,1-TCA and 1,1-DCE was achieved in the bioaugmented leg compared to the control leg. Maximum removals of 1,1,1-TCA and 1,1-DCE in the bioaugmented leg were 70% and 83%, respectively. No measurable removal of 1,1,1-TCA was observed in the control leg, while a maximum removal of 1,1-DCE of 48% was achieved. With prolonged treatment 1,1,1-TCA and 1,1-DCE removals in the bioaugmented leg were 70% and 83%, respectively. The addition of more butane to the test legs did not result in enhanced 1,1,1-TCA and 1,1-DCE removals, possibly resulting from butane inhibition. The demonstration during the second season of testing was more successful than the first season with respect to 1,1,1-TCA treatment. Several factors likely contributed to the better performance, including the bioaugmentation with a mixed culture consisting of two highly enriched cultures, the presence of lower concentrations of 1,1-DCE resulting in less toxicity from its cometabolism and the more consistent delivery of butane and DO to the test zone.

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References


