Push-pull test evaluation of the in situ aerobic cometabolism of chlorinated ethenes by toluene-utilizing microorganisms

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Abstract Single-well, push-pull tests were conducted in a contaminated aquifer to evaluate the ability of toluene-oxidizing microorganisms to cometabolize chlorinated aliphatic hydrocarbons (CAHs), such as trichloroethene (TCE). Test solutions were injected into the aquifer using a standard monitoring well, and then were transported under natural-gradient conditions. Transport tests demonstrated similar transport characteristics of the conservative tracer and the reactive solutes. Biostimulation tests were then performed by injecting a test solution containing dissolved toluene substrate, hydrogen peroxide, bromide, and nitrate in order to increase the biomass of toluene-utilizing microorganisms. Decreases in toluene concentration and the production of o-cresol as an intermediate oxidation product indicated the simulation of toluene-utilizing microorganisms containing an ortho-monooxygenase enzyme. Transformation tests demonstrated that indigenous microorganisms had the capability to transform the surrogate compounds (e.g. isobutene) and both cis-dichloroethene (*cis*-DCE) and trans-dichloroethene (*trans*-DCE). Isobutene was transformed to isobutene oxide, indicating transformation by a toluene ortho-monooxygenase, and both *cis*-DCE and *trans*-DCE were transformed. In a final test, the utilization of toluene, and the transformation of isobutene, *cis*-DCE, and *trans*-DCE were all inhibited in the presence of 1-butyne, a known inhibitor of the toluene ortho-monooxygenase enzyme. The method assessed the activity of attached microorganisms under in situ conditions of bioremediation.

Keywords Bioremediation; cometabolism; push-pull test

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Introduction

Chlorinated aliphatic hydrocarbons (CAHs), such as trichloroethene (TCE) and cis-dichloroethene (*cis*-DCE), are among the most abundant groundwater contaminants at industrial and military sites. Results from laboratory and field studies indicate that indigenous microorganisms at many sites have the potential to degrade TCE aerobically and anaerobically (Hopkins *et al.*, 1993). A variety of substrates have been shown to stimulate aerobic cometabolism of CAHs under laboratory and field conditions (Semprini, 1997). Aromatic substrates (*e.g.* phenol and toluene) have been shown to stimulate aerobic cometabolism of TCE, cis-DCE, and vinyl chloride (VC) (Hopkins *et al.*, 1993; Hopkins and McCarty, 1995). For example McCarty *et al.* (1998) used a recirculating well system to inject toluene and stimulate the aerobic cometabolism of TCE in contaminated groundwater at Edwards AFB, CA. Toluene was injected at average concentrations as high at 15 mg/L, and was reduced to less than 1 μg/L in the treatment zone, and TCE concentrations were reduced by over 95%.

Push-pull test methods were developed as a means of evaluating the potential for in situ cometabolism. Toluene was tested as a substrate for stimulating in situ aerobic cometabolism of TCE and cis-DCE in contaminated groundwater at Fort Lewis, WA. In this field study a series of transport, biostimulation, and activity push-pull tests were performed. Toluene was used as the cometabolic and isobutene as a reactive tracer to detect and quantify the aerobic cometabolic activity of indigenous microorganisms.

Methods

Aerobic cometabolic transformations of toluene, cis-DCE, and TCE were investigated in situ using single-well push-pull tests. Tests were conducted in a shallow alluvial aquifer in the area of Fort Lewis known as the East Gate Disposal yard (EGDY). The EGDY was used as a disposal site for TCE between 1940 and 1970. The depth of groundwater at the site is approximately 3.1 m and groundwater velocities across EGDY range from 8 to 23 cm per day. LC191 and LC192 were multi-port monitoring wells selected for the push-pull tests. A series of tests were performed in Ports 1 and 2 (7.5 m and 10.5 m depths, respectively) in each well. The multi-port monitoring wells were of interest since they allow for the use of smaller injection volumes, which simplified test logistics. Groundwater samples from these wells had TCE and cis-DCE concentrations ranging from 100–500 μg/L and 50–400 μg/L, respectively. The aquifer was aerobic with dissolved oxygen concentrations of approximately 6 mg/L.

A series of push-pull tests were performed to evaluate transport characteristics, biostimulation, and transformation activity of the injected solutes. Test solutions were prepared with groundwater extracted from each port and well, amended with a suite of solutes (see below) and injected into the same location. Bromide was used as a non-reactive tracer in all tests. Additional solutes injected in specific tests included a growth substrate (toluene), dissolved oxygen (DO), non-toxic CAH surrogates (e.g. isobutene), and nitrate as a nutrient. The test solution (100 L) was prepared by adding bromide (125 mg/L), nitrate (50 mg/L) and hydrogen peroxide (106 mg/L) to the extracted groundwater. Groundwater (20 L) was purged with isobutene for 1 hour to achieve an aqueous concentration of 35 mg/L. Groundwater (5 L) was added to a collapsible Teflon bag and toluene was added to achieve a concentration of 250 mg/L. The different solutions were mixed together at different flow rates to achieve the desired concentrations in the injected test solutions. The aqueous concentrations of each solute injected into the aquifer were 10 mg/L for toluene, 5.6 mg/L for isobutene, 85 mg/L for hydrogen peroxide (40 mg/L DO), 100 mg/L for bromide, and 40 mg/L for nitrate (9 mg/L as N). Figure 1 shows the schematic of equipment used to inject test solutions into monitoring wells during field tests.

Transport tests evaluated the transport of the bromide tracer and the reactive organic solutes. Biostimulation tests evaluated the stimulation of indigenous microorganisms on toluene. Biostimulation tests were performed by injecting a test solution containing dissolved toluene, hydrogen peroxide, and nitrate in order to increase the biomass of toluene-utilizing microorganisms. These tests were performed under natural drift conditions by permitting the test solution to reside in the aquifer and be transported away from the well. Activity tests were used to estimate the rate of transformation of toluene, the CAHs of interest, and the reactive surrogate (isobutene). Activity tests were performed by injecting the prepared test solution, allowing the test solution to drift downgradient with the regional groundwater flow for 20 h (no pumping) and then extracting approximately 200 L at a flow rate of 1 L/min. Natural drift activity tests were performed similar to activity tests except that no extraction pumping was performed. Instead, samples were collected periodically from the injection location as the test solution drifted downgradient. Samples collected from both types of tests were analyzed for injected tracer and potentially reacting solutes, as well as reaction products formed in situ.

Results and discussion

A single transport test was conducted in each port and well prior to biostimulation. For these tests the injected solution was allowed to reside in the aquifer for about 20 h, and was then extracted at a rate of 1 L/min for 3.3 h. Extraction curves for toluene, isobutene, and nitrate were very similar to the breakthrough curve of the bromide tracer, indicating

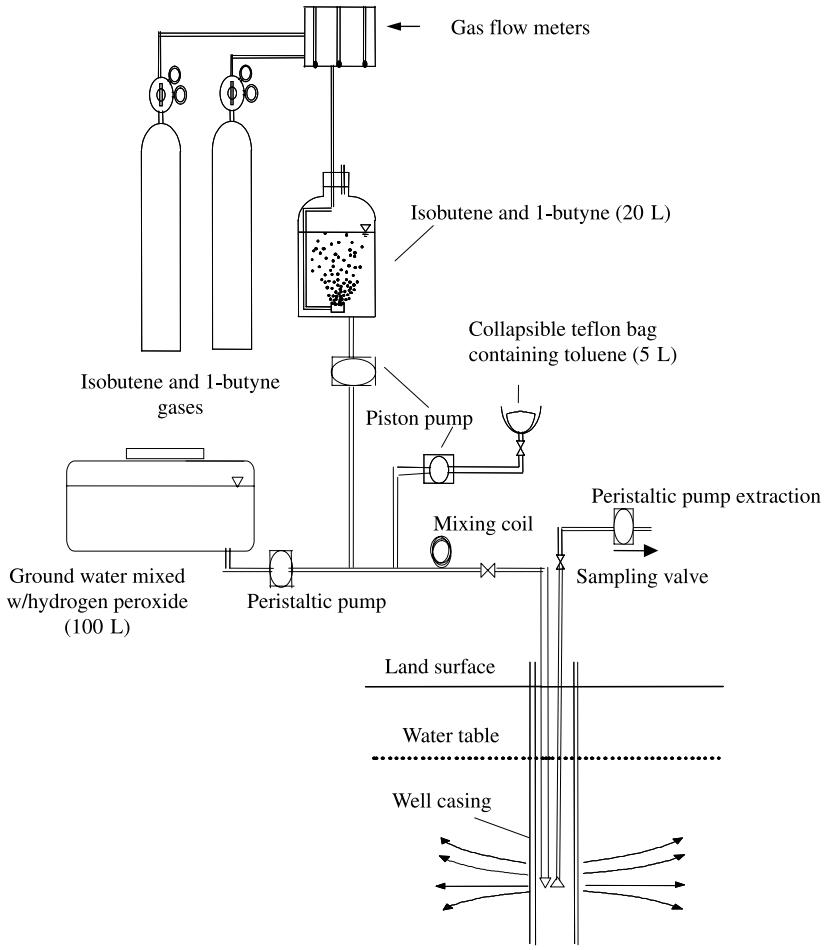

Figure 1 Field equipment used in field transport, activity, and natural drift tests

conservative transport of all injected solutes. Mass balances indicated nearly equal percent recoveries showing similar transport characteristics of the conservative tracer and the reactive solutes at both LC191 and LC192 wells. About 30–36% and 55–60% of injected organics and inorganic solute mass were recovered during the extraction phase of the transport test at LC191 and LC192, respectively (Table 1). The lower recovery at LC191 indicates relatively higher groundwater velocities compared to the LC192 well.

Activity tests evaluating toluene utilization were performed after five successive additions of toluene to biostimulate indigenous microorganisms. Activity tests showed decreases in injected toluene concentration and the production of o-cresol as an

Table 1 Quantities of injected and extracted solutes and percent recovery during transport tests

Test well location	Quantities	Isobutene (mmol)	Toluene (mmol)	NO_3^--N (mmol)	Br^- (mmol)
Transport LC191-P1	Injected mass	13.7	13.6	77.1	143.6
	Extracted mass	5.0	4.1	24.0	47.1
	Mass recovery (%)	36.5	30.1	31.1	32.8
Transport LC192-P2	Injected mass	12.4	13.3	79.4	148.7
	Extracted mass	7.1	8.2	44.0	98.3
	Mass recovery (%)	57.2	61.6	55.4	66.1

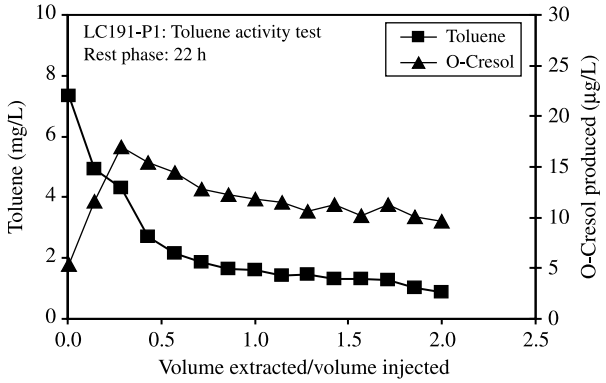

Figure 2 Toluene and o-cresol concentrations during toluene biostimulation tests at port P1, well LC191

intermediate oxidation product indicating the simulation of toluene-utilizing microorganisms containing an ortho-monooxygenase enzyme. Toluene utilization and o-cresol formation concentration versus volume injected/volume extracted at LC191-P1 are plotted in Figure 2. The o-cresol represents around 0.1 to 0.3% of the total toluene mass injected. Similar results were obtained at the other test locations.

Mass balances indicated a similar percent bromide recovery between transport and biostimulation activity tests conducted in LC191 and LC192 (Tables 1 and 2). Nitrate mass percent recovery in the biostimulation tests was 21.5% and 43% for LC191 and LC192, respectively (Table 2), which was less than 31.1% and 55.4% nitrate mass percent recovery observed in the transport tests (Table 1). Toluene mass percent recovery in the biostimulation tests was 26.7% and 38.6% for LC191 and LC192 (Table 2), which was less than the 30.1% and 61.6% toluene mass percent recovery observed in the transport tests (Table 2). The resulting decrease of toluene and nitrate mass recovery, along with the production of o-cresol, provided evidence that toluene-utilizers were successfully stimulated in the subsurface.

Activity test results showed a similar bromide mass percent recovery between transport and activity tests (Tables 1 and 3). A similar nitrate mass percent recovery between biostimulation and activity tests was observed (Tables 2 and 3), which was less than the nitrate mass percent recovery observed in the transport tests (Table 1). Toluene concentrations were reduced to almost below detection after 20 h. Toluene mass percent recovery in the activity tests was 2.6% and 5.2% for LC191 and LC192 (Table 3). No o-cresol was detected during the activity tests, likely because the injected toluene concentration was only 2 mg/L and toluene was rapidly transformed.

Table 2 Quantities of injected and extracted solutes and percent recovery during biostimulation activity tests

Test well location	Quantities	Toluene (mmol)	NO_3^--N (mmol)	Br^- (mmol)
LC191-P1	Injected mass	10.5	75.8	125.2
	Extracted mass	2.8	16.3	41.5
	Mass recovery (%)	26.7	21.5	33.1
LC192-P2	Injected mass	11.9	79.5	123.3
	Extracted mass	4.6	34.2	82.9
	Mass recovery (%)	38.6	43.0	67.2

Table 3 Quantities of injected and extracted solutes and percent recovery during activity tests

Test well location	Quantities	Isobutene (mmol)	Isobutene oxide (mmol)	Toluene (mmol)	NO_3^--N (mmol)	Br^- (mmol)
Activity LC191-P1	Injected mass	6.77	0.0	2.5	82.8	158.1
	Extracted mass	1.42	0.19	0.06	20.7	48.2
	Mass recovery (%)	21.0	* NA	2.6	25.0	30.5
Activity LC192-P2	Injected mass	6.77	0.0	3.47	80.2	162.0
	Extracted mass	2.55	0.33	0.18	34	100.4
	Mass recovery (%)	37.62	NA	5.19	42.4	62.0

* NA: Not Available

Isobutene mass recovery was reduced to 21% and 37.6% for LC191 and LC192, which was less than the 36.5% and 57.2% observed in the transport tests (Table 1). When isobutene was utilized, isobutene oxide was observed as an intermediate oxidation product. The ratio of the recovered mass of isobutene oxide produced to the isobutene mass injected was about 2.9 and 4.9% at LC191 and LC192 (Table 3). This small percentage could be due to rapid utilization of isobutene oxide by the toluene-utilizers and/or demineralization to CO_2 during the course of this experiment. Reduction in isobutene concentrations and the production of isobutene oxide as an intermediate oxidation product indicated the stimulation of toluene-utilizing microorganisms containing an ortho-monooxygenase enzyme. Similar results for isobutene oxidation by toluene-utilizing microorganisms were found in laboratory microcosm studies by Hicks (2002). In a final series of tests 1-butyne was added as an inhibitor of the toluene monooxygenase enzyme system. Similar recoveries of toluene, isobutene, cis-DCE, and trans-DCE as the bromide tracer indicated that their transformation was effectively inhibited by 1-butyne.

Conclusions

Single-well tests demonstrated the stimulation of indigenous toluene-utilizing microorganisms in a CAH contaminated aquifer. Activity tests demonstrated the stimulation of microorganisms that had CAH transformation potential. Isobutene was an effective reactive surrogate, with isobutene oxide easily detected as a cometabolic transformation product. Evidence that injected toluene stimulated organisms with the ortho-monooxygenase enzyme system was provided by the oxidation of injected isobutene to isobutene oxide and by the inhibition of toluene and isobutene oxidation in the presence of a coinjected 1-butyne inhibitor. Evidence was also obtained for the in situ transformation of injected cis-DCE and trans-DCE, but not TCE. The results demonstrated that push-pull tests can be used to evaluate the potential for in situ cometabolic metabolism of chlorinated ethenes.

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