

BIOAUGMENTATION POTENTIAL AT A CARBON TETRACHLORIDE CONTAMINATED SITE

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ABSTRACT: A plume of carbon tetrachloride (CT) contaminated groundwater at the Y-12 site in Oak Ridge poses a difficult long-term remediation problem and several solutions including bioremediation are being considered. *Pseudomonas stutzeri* strain KC has the potential to degrade CT, without producing undesired degradation products (e.g., chloroform). However, specific environmental conditions, e.g., low iron - alkaline pH, appear necessary to achieve significant degradation with this strain. We designed a microcosm study to examine the feasibility of bioaugmentation with the KC strain at the Y-12 site. In this initial work, additions of nitrate, acetate, nitrilotriacetic acid [NTA], phosphate, and bacteria were tested, to determine if degradation was feasible. The microcosms contained site sediment (13 mL) and groundwater (17 mL) with an addition of 500 $\mu\text{g/L}$ CT. With the addition of 10^7 cells, the CT was degraded rapidly. Generally complete degradation was achieved within 48 hours and nitrate was exhausted to undetectable levels. No pH adjustments were necessary. However, in the presence of NTA, pH adjustment inhibited degradation. Apparently, neither the pH nor iron concentration in the CT plume posed a problem for CT degradation. Thus, the geochemistry of the site appears suitable for bioaugmentation with the KC strain.

INTRODUCTION

A plume of carbon tetrachloride (CT) contaminated water at the Y-12 site in Oak Ridge is present in fractured bedrock and apparently originates from a DNAPL source (ORNL, 1997). Thus, the plume poses a difficult long-term remediation problem. Pump-and-treat and bioremediation are being considered for site remediation.

Various laboratory studies have demonstrated the potential for *Pseudomonas stutzeri* KC to degrade CT, without producing undesired degradation products (e.g., chloroform). Several investigators have demonstrated CT degradation in field and laboratory studies (Criddle et al., 1990; Witt et al., 1995; Mayotte et al., 1996). Degradation apparently proceeds with the involvement of an extracellular low molecular weight molecule, pyridine-2,6-bis(thiocarboxylate) [PBTC] and the production of PBTC is critical to the process (Dybas et al., 1995a; Lee et al., 1999).

Specific environmental conditions, low iron, appear necessary for PBTC production and CT degradation (Tatara et al., 1993; 1995). In the presence of PBTC, CT transformation takes place in the proximity of actively growing or respiring cells (e.g., KC or indigenous aquifer flora). Thus, it is critical that iron is either at a level low enough to stimulate production of the extracellular PBTC. To achieve this at some sites, it has been necessary to adjust the pH in the CT plume to reduce iron availability (Dybas et al., 1995b, 1998).

We conducted a feasibility study with the goals of assessing the potential for bioremediation using this bacterial strain. The feasibility study focused on these questions. 1) Is in situ iron low enough to permit significant degradative activity? 2) Can potential amendments (e.g., pH alteration, chelator addition) control iron to the degree that significant degradation activity takes place?

MATERIALS AND METHODS

Field Site and Sampling. In late February 1999, 8 liters of groundwater from well GW606 and 8 liters of groundwater-sediment slurry from well GW605 were recovered (Fig. 1). Contaminants present in well GW606 were carbon tetrachloride (~ 150 µg/L), chloroform (~ 150 µg/L), tetrachloroethene (~ 8 µg/L), sulfate (~ 48 mg/L), and nitrate (N ~ 4.7 mg/L). The oxidation-reduction potential was ~ 150 mV and the dissolved oxygen ~ 0.2 mg/L (ORNL, 1997) indicating a near anaerobic environment. Concentrations are not available for the groundwater-sediment slurry from GW605 but the well was contaminated and there were some anaerobic degradation products of CT present. Groundwater pH was consistently slightly alkaline but addition of sediments caused a shift to lower pH values.

Groundwater sampling was performed in accordance with USEPA Region I “Low Stress (low flow) Purging and Sampling Procedure for the Collection of Ground Water Samples from Monitoring Wells.” A QED MicroPurge Flow Cell/Analyzer was used to analyze all intrinsic field parameters prior to sample collection. A dedicated submersible bladder pump, the associated pneumatic controller, and an oilless compressor were used to collect all the groundwater samples. Teflon-lined tubing was used for both the pneumatic and groundwater lines. The flow-through-cell was disconnected prior to groundwater sample collection. All groundwater samples were collected under a nitrogen blanket to minimize exposure to atmospheric air. In a field sampling bag flushed with 99% nitrogen, groundwater was collected in glass bottles, which had Teflon® caps. The bottles were completely filling the bottles to before capping, to maintain zero headspace.

Because fresh core material was not available for the study, we used sediments recovered from the bottom of well GW605 (Fig. 1) in order to examine degradation in the presence of aquifer solids. Sediment sampling began by agitating sediment

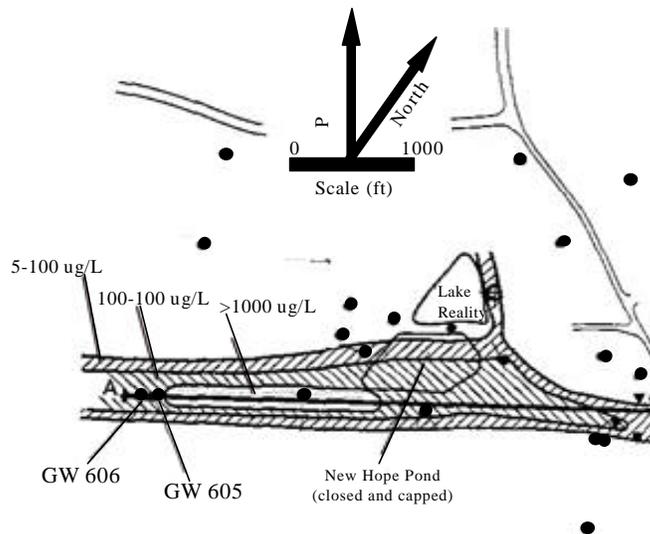


FIGURE 1. Map of Y-12 CT plume with sampling wells GW606 and GW605.

settled in the bottom of each well using a weighted tape (or equivalent). Sediment-laden groundwater was pumped from the bottom of the well to the ground surface using the same bladder pump system used for the groundwater sampling. With the bladder pump at a flow rate of approximately 1 L per minute, the sediment agitation continued throughout sampling of each well. Sediment-laden groundwater samples were collected in 10 L plastic bottles under a nitrogen blanket to minimize exposure to atmospheric air. The bottles were stored in an ice chest (4EC) for transportation under chain-of-custody procedures to our nearby laboratory.

Experimental Design. Triplicate microcosms were prepared in EPA vials with min-nert caps for repeated head-space sampling for each treatment. The microcosms contained 13 mL of the groundwater-sediment slurry (2.5 g dry weight sediment) and 17 mL of groundwater, plus a combination of the following CT (500 $\mu\text{g/L}$), acetate (0 or 500 mg/L), nitrate (0 or 50 mg/L), other nutrients (phosphate 10 mg/L), NTA (0 or 100 mg/L), bacteria (10^7 cells/mL), and pH adjustment. Supplement concentrations were set to yield a 100:10:1 C:N:P. ratio. The KC strain was grown overnight, in the phosphate-buffered mineral salts media (PBBM) described by Palumbo et al., (1995) modified by removal of iron and copper from the trace minerals, centrifuged, and the cell pellet was washed with anaerobic sodium chloride solution to remove residual medium. The washed cell pellet was resuspended in site groundwater. The cell concentration was determined microscopically and the stock suspension was diluted with site groundwater to the appropriate concentration for addition to the microcosms. The final cell concentration in the microcosms varied in the experiments from 10^{6-7} cells/mL. Incubations were at room temperature (about 22°C). A number of different controls were included in the experiments. Controls included vials, which contained no bacteria or bacteria killed with formaldehyde (negative controls) or bacteria in medium D (positive controls). Subsequent experiments focused on the effect of bacterial, acetate, nitrate and CT concentrations. We measured degradation at several time points, usually at 0, 1, 3 and 7 days using GC analysis for CT and degradation products. A secondary study was initiated using the stored (4°C) groundwater and groundwater-sediment slurry with differing concentrations of acetate, and nitrate. CT concentration was 500 $\mu\text{g/L}$. Acetate concentrations of 500 or 1000 mg/L were paired in all combinations with nitrate concentrations of 50 and 100 mg/L. Bacteria were added to a final concentration of 10^7 cells/mL. Time point measurements were 0, 1, 3 and 7 days. In this experiment, cells were grown on PBBM before inoculation. Due to problems in achieving degradation with cells grown on PBBM, as discussed below, medium D (Criddle et al., 1990), with 2100 mg/L acetate and 1400 mg/L nitrate, was used in all subsequent experiments.

RESULTS AND DISCUSSION

In this microcosm study significant CT degradation was detected in the presence of nitrate and acetate (Fig. 2A). Planned sampling at 7, and 14 days was

canceled due to the highly positive results from the first two time points in favor of additional studies on concentrations of nitrate and acetate. Results of this microcosm experiment indicated that acetate and nitrate stimulated the added bacteria to degrade 500 µg/L CT (Figure 2A and 2B). The rates of disappearance of CT were comparable to those seen in other microcosm studies using the KC strain (e.g., Tatara et al., 1993) in which the pH was adjusted to 8.2. The difference in our study was that groundwater pH was all ready in a favorable range and pH adjustment was not necessary.

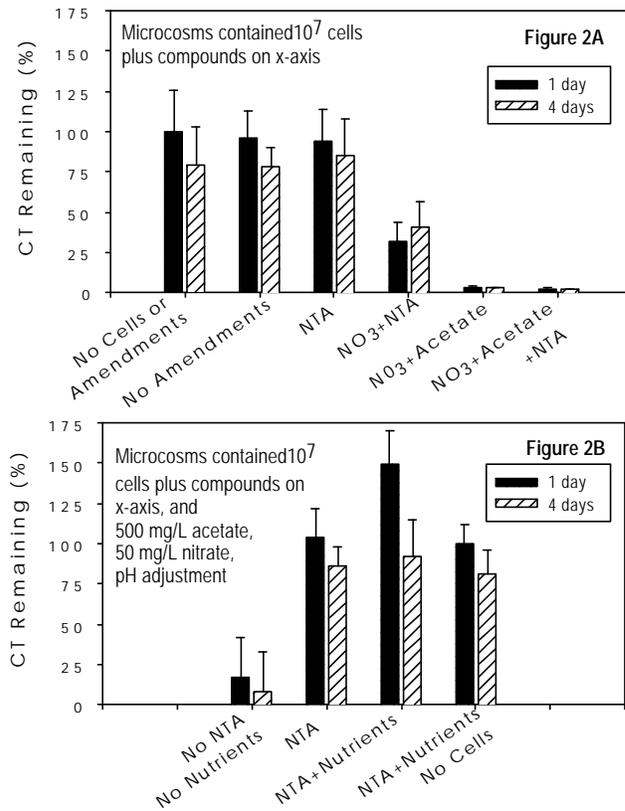


FIGURE 2. CT degradation in the first experiment.

Other adjustments, e.g., pH adjustment and addition of NTA did not have a positive effect. Adjusting the pH to 8.2 was not necessary (Fig 2B) and in the presence of NTA the pH manipulation appeared to inhibit degradation. This may have been an artifact of the manipulation required or due to geochemical interactions. The addition of NTA alone did not stimulate degradation (Fig. 2A). In the presence of nitrate, without pH adjustment or the addition of acetate, NTA did stimulate degradation (Fig. 2A). This may indicate the NTA was used as an electron donor.

Subsequent experiments (below) indicated that growth in medium D was necessary for reproducible degradation results. The bacteria for the first two experiments were grown in PBBM prior to inoculation rather than in medium D. The results of the second round of tests were negative due to the inability of cultures used to degrade CT (Table 1) even in the positive controls. Additional testing was performed to determine what growth conditions were required to ensure high degradation activity.

Up to this point we had been using PBBM to grow the cells for inoculation and upon switching to medium D reproducibility problems were eliminated and high rates of degradation were always achieved. With medium D the degradation was reproducible (e.g., Table 1). The additional investigations revealed our initial growth

medium apparently often inhibited the ability of the bacteria to degrade CT, perhaps due to small amounts of iron and copper in the medium (Tatara et al., 1993). The degree of inhibition observed with our original growth medium may depend on the trace amount of iron in the batch of materials used to prepare the medium. The subsequent microcosm studies, using medium D to grow the bacteria, all exhibited CT degradation using acetate (1000 mg/L), nitrate (50, 100, or 200 mg/L), and KC at 10^{6-8} cells/mL (e.g., Table 1).

TABLE 1. Example results from experiments with cells grown on PBBM and medium D.

Experiment	Carbon Tetrachloride Remaining (%)			
	Day 1		Last Day*	
	0 cells	+ cells	0 cells	+ cells
PBBM	108	107	113	113
medium D	111	79	102	33

* day 5 for medium D and day 7 for PBBM.

CONCLUSIONS

It appears that the site conditions are very suitable to bioaugmentation approaches. The pH and iron concentrations appear to pose no problems for achieving high degradation rates. Our findings also were in agreement with studies by Tatara et al., (1993) that indicated that media had a significant effect on the ability of the *Pseudomonas stutzeri* KC strain to degrade CT.

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