Bull. Environ. Contam. Toxicol. (2004) 72:87–93

DOI: 10.1007/s00128-003-0244-5

Environmental Contamination and Toxicology

Biobarrier System for Remediation of TCE-Contaminated Aquifers

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Received: 12 March 2003/Accepted: 8 September 2003

Groundwater at many existing and former industrial sites and disposal areas is contaminated by halogenated organic compounds that were released into the environment. At many trichloroethylene (TCE) spill sites, residual amounts of TCE persist in a pure liquid phase [commonly referred to as dense, non-aqueous-phase liquids (DNAPLs)] within pore spaces or fractures. The slow dissolution of residual TCE results in a contaminated plume of groundwater (EPA, 1998; Bedient et al., 1999). Because it is usually very costly and time-consuming to locate and remove the residual TCE, remediation must focus on preventing further migration of dissolved contamination. This plume control must be maintained for a long period of time. Therefore, more economic and less expensive approaches are desirable for groundwater remediation to provide for a long-term control of contaminated groundwater. One cost-effective approach for remediation of contaminated aquifers that is attracting increased attention is the installation of permeable reactive zones or barriers within aquifers. As contaminated groundwater moves under natural or induced hydraulic gradients through a permeable reactive zone, the contaminants are scavenged or degraded, and uncontaminated groundwater emerges from the downgradient side of the reactive zone (Burris, 1995; Kao and Borden, 1997; Gavaskar et al., 1998; Kao and Yang, 2000; Mcnab and Ruiz, 2000). Current evidence suggests that TCE can be degraded cometabolically by supplying an alternate primary substrate under aerobic conditions (Schollhorn et al., 1997; EPA, 1998). Several aerobic microorganisms or microbial communities have the ability to synthesize oxygenase enzyme systems that catalyze the initial step in oxidation of their respective primary or growth substrates and have the potential for initiating the oxidation of TCE and other chlorinated aliphatic hydrocarbons (Hirl and Irvine, 1997; Komatsu et al., 1997; Gossett and Zinder, 1997; Magnuson et al., 1998; Zenker et al., 2000). Biological sludge cake and cane molasses are wastes from domestic wastewater treatment and sugar industry, respectively. The sludge cakes used in this study were thickened biological sludges from the second clarifier of a domestic wastewater treatment plant. Cane molasses, which is brown to black thick liquid, was collected from a sugar cane factory (Taiwan Sugar Company, Kaohsiung, Taiwan). Both sludge cake and cane molasses have the following characteristics that make them good candidates for this type of application: (1) they are rich in carbon, an essential energy source for biodegradation; (2) they have the potential to exhibit sufficient carbon bioavailability for cometabolsim to occur; (3) they are relatively inexpensive.

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The above discussion suggests that a passive sludge cake or cane molasses biobarrier system is a practical method to biodegrade TCE to non-toxic end products. Sludge cake or cane molasses can be placed in remediation wells or trenches, which are installed perpendicular to the groundwater flow direction. The remediation wells or trenches act as diffusion sources for primary substrates (Kao and Yang, 2000). This remediation technology has the potential to biotransform TCE to nontoxic end products. Specific objectives of this bench-scale study were to: (1) determine the bioavailability of alternative primary substrates (cane molasses and sludge cakes) by microbial consortia; and (2) evaluate the feasibility of biodegrading TCE by different microbial consortia using substrates released from cane molasses and sludge cakes. Based on the experimental results, the favorable bioremediation conditions can be determined and in situ biobarrier system can be designed to bioremediate TCE contaminated groundwater passively and cost-effectively.

MATERIALS AND METHODS

A bioavailability test was conducted in this study. The bioavailability test was a modified biochemical oxygen demand (BOD) experiment. This modified BOD test was used to examine whether cane molasses and sludge cakes contain decomposable organic matters using microbial consortia under aerobic conditions. In this test, six two-liter serum bottles (batch reactors) (labeled as A, B, C, D, E, and F) were used to perform the aerobic bioavailability experiment. Reactors were purged with air and contained primary substrates (Reactors A and B: 2.5 g of sludge cake; Reactors C and D: 0.5 g of cane molasses; Reactors E and F: no primary substrate addition), 980 mL of mineral medium solution, and 20 ml of aerobic activated sludge collected from an industrial wastewater [mainly straight-chain chlorinated compounds (e.g., dichloroethylenes, vinyl chloride, tetrachloroethylene, dichloroethane) treatment plant. Reactors had the following features: (1) continuous monitoring of pH, and (2) a three-way outlet attached on the top of the reactor for water sample collection. Since activated sludge contained some unknown organics, Reactors E and F were prepared without cane molasses or sludge cake additions. This was used to differentiate the organic utilization between activated sludge and cane molasses/sludge cake. Reactors B, D, and F were used as control sets, which were autoclaved and contained 250 mg/L $HgCl_2$ and 500 mg/L NaN_3. Cane molasses and sludge cakes were collected from a sugar cane factory and a domestic wastewater treatment plant, respectively. The components of mineral medium were described in Kao and Yang (2000). The pH of this buffer solution was 7.5, and the solution was autoclaved before use. Duplicate water samples (2-mL each) were collected from all reactors each day for chemical oxygen demand (COD) measurements during the 25-day operation period. COD analysis was performed in accordance with the dichromate reflux method described in Standard Methods (APHA, 1995).

Aerobic microcosm experiments were conducted to examine the feasibility of TCE biodegradation using cane molasses and sludge cake as the primary substrates under aerobic cometabolic conditions. The inocula used in this microcosm study included contaminated aquifer sediments from the mid-plume area (dissolved oxygen level was approximately 1.2 mg/L) of a TCE-spill site and aerobic activated sludge used in the bioavailability test.

Table 1. Components of microcosms.

Inocula	Reactor Number	Components	Conditions
Aquifer Sediments	A1	Sludge cake 0.1 g + 10 g sediments + mineral medium	Live
Aquifer Sediments	A2	Sludge cake 0.1 g + 10 g sediments + mineral medium + $HgCl_2$ + NaN_3	Kill Control
Aquifer Sediments	A3	Cane molasses 0.1 g + 10 g sediments + mineral medium	Live
Aquifer Sediments	A4	Cane molasses 0.1 g + 10 g sediments + mineral medium + $HgCl_2$ + NaN_3	Kill Control
Activated Sludge	S1	Sludge cake 0.1 g + 1 mL sludge + mineral medium	Live
Activated Sludge	S2	Sludge cake 0.1 g + 1 mL sludge + mineral medium + $HgCl_2$ + NaN_3	Kill Control
Activated Sludge	S3	Cane molasses 0.1 g + 1 mL sludge + mineral medium	Live
Activated Sludge	S4	Cane molasses 0.1 g + 1 mL sludge + mineral medium + $HgCl_2$ + NaN_3	Kill Control

Each microcosm was constructed with 35 mL nutrient medium (described in the above section, 10 g sediments or 1 mL activated sludge of inocula, 5 mL TCE solution, and 0.1 g of cane molasses or sludge cake in a 70-mL bottle sealed with Teflon-lined rubber septa. Table 1 lists the components of each microcosm. The aquifer sediments were purged with nitrogen gas before use. The initial TCE concentrations in microcosm bottles were approximately 7.5 - 8 μM (after equilibration). The pH of the microcosm solution was 7.5. Control bottles contained 250 mg/L $HgCl_2$ and 500 mg/L NaN_3, and inocula used for the control groups were autoclaved before use. TCE analyses were performed in accordance with U.S. EPA Method 601, using a Tekmer Purge-and-Trap Model LSC 2000 with a Varian Model 3800 Gas Chromatograph (GC). Duplicate microcosms were sacrificed at each time point and analyzed for TCE concentrations.

RESULTS AND DISCUSSION

The variations in COD concentrations versus time for each aerobic reactor are presented in Figure 1. In the kill control group with sludge cake as the primary substrate (Reactor B), COD was 66 mg/L on day 1 and went up to 163 mg/L on day 7. Similar COD measurement (182 mg/L) was observed on day 25. In the kill control group with cane molasses as the primary substrate (Reactor D), COD was 87 mg/L on day 1 and went up to 502 mg/L on day 5, then slowly increased to 552 mg/L on day 25. This indicates that the soluble organic materials released from the cane molasses and sludge cake caused the increase in COD measurements in both reactors. No significant difference was observed between COD measurements in Reactors E (live control) and F (kill control). Consistent COD measurements were observed in both reactors (approximately 7 to 8 mg/L). Compared to the kill control reactors (B and D), significant decreases in COD measurements were observed in Reactors A and C with sludge cake and cane molasses as

the substrates, respectively. This suggests that the released organics (COD) from substrates were biodegraded by the inoculated activated sludge. COD measurements were averaged for the period from day 7, when stabilization was reached, to the end of the experiment on day 25. The averaged COD measurements for Reactors A, B, C, D, E, and F were 135, 167, 386, 490, 7, and 8 mg/L, respectively. After subtracting the contribution from the activated sludge (8 - 7 = 1 mg/L), approximately 31 mg/L of COD (167 - 135 - 1 = 31 mg/L) in Reactor B and 103 mg/L of COD (490 - 386 - 1 = 103 mg/L) in Reactor C were biodegraded after a 25-day incubation. This indicates that approximately 12.4 mg of biodegradable COD and 206 mg of biodegradable COD can be released from 1 g of sludge cake and 1 g of cane molasses, respectively.

Aerobic microcosms (Groups A1 to A4) using aquifer sediments as inocula and sludge cake and cane molasses as primary substrates were incubated for 60 days. The average concentration of the duplicate analysis of TCE versus time is presented in Figure 2. In Microcosm A1 with sludge cake as the primary substrate, almost complete TCE degradation was observed after 23 days of incubation with a measurable lag period (approximately 4 days). In Group A3 with cane molasses as the primary substrate, complete TCE degradation was also observed after 45 days of incubation with a measurable lag period (8 days). Results show that both substrates can be used to enhance the aerobic cometabolism of TCE using aquifer sediments as the inocula. In the control microcosms (Microcosms A2 and A4), no significant TCE decrease was observed throughout this experiment (Figure 2). Microcosms with aerobic activated sludge as the inocula and sludge cake (Microcosms S1 and S2) and cane molasses (Microcosms S3 and S4) as the primary substrates were incubated for 60 days. Figure 3 presents the analytical results for this microcosm study. Complete TCE degradation was observed in Microcosms S1 and S3 after 12 and 25 days of incubation with 1 and 4-day lag periods, respectively. Compared to aquifer sediments, activated sludge could use both substrates as the carbon sources more efficiently and enhance TCE cometabolism more effectively. No TCE decrease was observed in the control microcosms (S2 and S4) (Figure 3).

TCE degradation ratios were calculated to compare the activity of the microbial consortia. The selected incubation period for the ratio calculation did not include the lag time, and therefore, days 4, 8, 1, and 4 were used as the starting degradation time points for Microcosms A1, A3, S1, and S3, respectively. The calculated TCE degradation ratios for A1, A3, S1, and S3 were 0.04, 0.02, 0.59, and 0.33 μM/day/g or mL of inocula, respectively. This indicates that the microbial population in activated sludge contained more active microorganisms, which can synthesize oxygenase enzyme for subsequent TCE cometabolism during their degradation of sludge cake and cane molasses. Table 2 presents the calculated degradation ratios for the microcosm study. Microcosms with activated sludges as the inocula had more efficient TCE degradation. Longer lag periods for TCE removal were observed in microcosms with aquifer sediments as the inocula. Furthermore, the calculated TCE degradation ratios were lower using sediments as the inocula. These findings suggest that the aquifer sediments collected from the TCE-contaminated site had little beneficial effects on TCE removal compared to the activated sludge with high and versatile microbial populations from the industrial wastewater treatment plant.

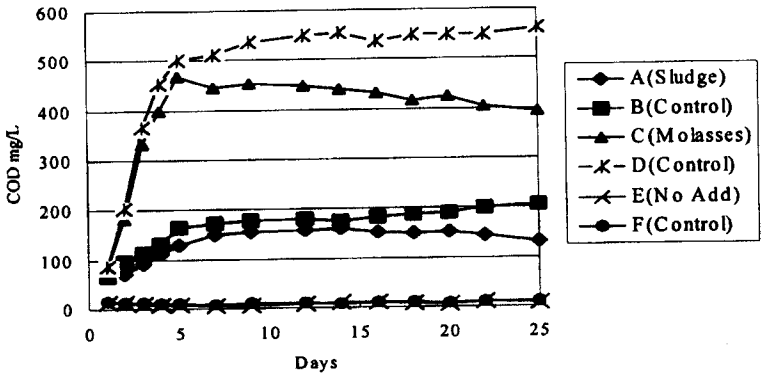

Figure 1. Variation in COD versus time in the bioavailability study.

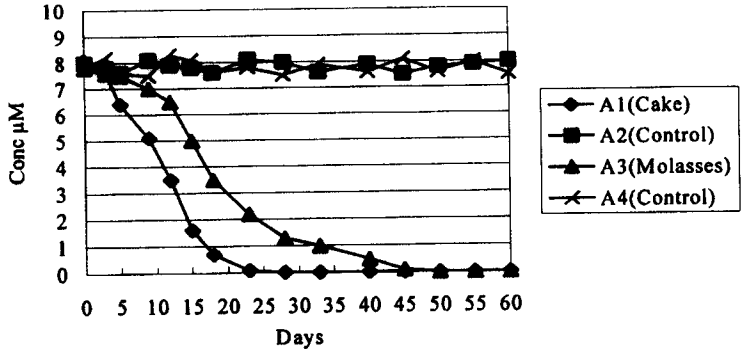

Figure 2. Microcosm experiments with aquifer sediments as the inocula.

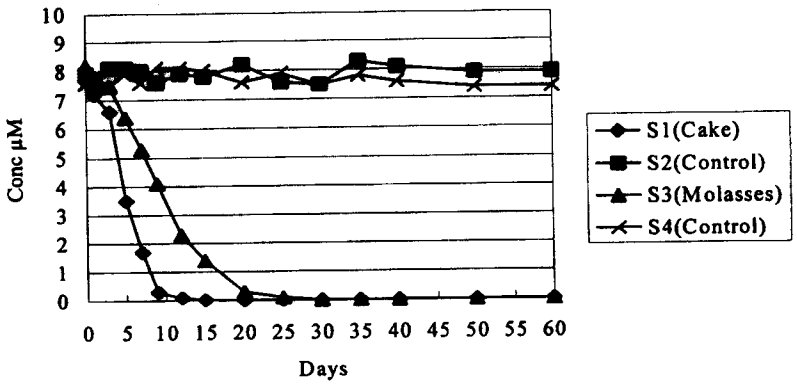

Figure 3. Microcosm experiments with sludge as the inocula.

Table 2. Calculated TCE degradation ratios.

Microcosm	Inocula	Description	Ratio (μM/day/g or mL of inocula)*
A1	Sediment	TCE removal from day 4 to 23	0.04
A3	Sediment	TCE removal from day 8 to 45	0.02
S1	Sludge	TCE removal from day 1 to 12	0.59
S3	Sludge	TCE removal from day 4 to 25	0.33

*(Initial concentration – final concentration)/reaction period/g or ml of inocula

Results from this study show that biodegradable materials were released from both sludge cake and cane molasses and utilized by microorganisms. Degradation of the released organic materials also caused the decrease in COD measurements. Based on the results from biodegradability tests, 1 g of sludge cake and 1 g of cane molasses could release approximately 12.4 and 206 mg of biodegradable COD, respectively. The released bioavailable organics could cometabolize significant amount of TCE and remediate the contaminated groundwater. Moreover, organic materials can be released from sludge cake and cane molasses continuously. Thus, sludge cake and cane molasses could be applied for a longer period of remediation time in the field. Results from this study also show that cane molasses contained more COD than sludge cakes (Figure 1). However, sludge cakes caused higher TCE removal ratio (Table 2). The following two potential hypotheses for the inconsistent results can be derived: (1) sludge cakes could release more biodegradable compounds which can be used by the inoculated microbial consortia as the primary or growth substrates, and (2) the inoculated microbial consortia could synthesize more oxygenase enzyme systems for TCE cometabolism during the biodegradation of sludge cakes.

From an engineering point of view, bacteria enrichment or organisms isolation may not be needed to achieve an effective TCE removal ratio for the practical remedial application. For a TCE spill site lacking of active microbial consortia, activated sludge can be applied to accelerate the TCE removal rates. This makes the biobarrier system containing sludge inocula more feasible and applicable. These findings make the sludge cake and cane molasses biobarrier containing sludge inocula more feasible and applicable at the TCE-contaminated site. Results show that the proposed biobarrier has the potential to become an environmentally and economically acceptable technology for the bioremediation of chlorinated-solvent contaminated groundwater. Results of this study will aid in designing a system for field application. This system could be applied for other hazardous waste contaminated sites to biodegrade other recalcitrant contaminants.

Acknowledgments. This project was funded in part by National Science Council in Taiwan. Additional thanks to Prof. C. K. Liu of National Sun Yat-Sen University for his support and assistance throughout this project.

REFERENCES

APHA, American Public Health Association (1995) Standard Methods for the Examination of Water and Waste Water, Part 500, 19th ed., APHA-AWWA-WEF, Washington DC, U.S.A.

Bedient PB, Rifai HS, Newell CJ (1999) Ground water contamination – transport and remediation, Chap. 11. PTR Prentice-Hall, Inc., New Jersey.

Burris DR (1995) Sorption of trichloroethylene and tetrachloroethylene in a batch Reactive metallic iron-water system. Environ Sci Tech 29: 2850-2855.

EPA, US Environmental Protection Agency (1998) Technical Protocal for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water. EPA/600/R-98/128, Washington DC, U.S.A.

Gavaskar AR, Gupta N, Sass BM, Janosy RJ, O'Sullivan D (1998) Permeable barriers for groundwater remediation, Chap. 1. Battelle Press, Columbus, U.S.A.

Gossett JM, Zinder SH (1997) Microbiological aspects relevant to natural attenuation of chlorinated ethenes. In Proc. of the Symposium on Natural Attenuation of Chlorinated Organics in Ground Water, Washington, DC, p. 12.

Kao CM, Borden RC (1997) Enhanced biodegradation of BTEX in a nutrient briquet-peat barrier system. J of Environ. Engr 123:18-24.

Kao CM, Yang L (2000) Enhanced bioremediation of trichloroethene contaminated groundwater by a biobarrier system. Wat Sci Tech 42: 429-434.

Komatsu T, Shinymo J, Momonoi K (1997) Reductive transformation of tetrachloroethylene to ethylene and ethane by an anaerobic filter. Wat Sci Tech 36: 125-132.

Hirl PJ, Irvine RL (1997) Reductive dechlorination of perichloroethylene anaerobic sequencing batch biofilm reactors (AnSBBR). Wat Sci Tech 35:49-56.

Magnuson JK, Stern RV, Gossett JM, Zinder SH, Burris DR (1998) Reductive dechlorination of tetrachloroethene to ethene by a two-component enzyme pathway. Appl Environ Microbiol 64:1270-1275.

McNab WW and Ruiz R (2000) In-situ destruction of chlorinated hydrocarbons in groundwater using catalytic reductive dehalogenation in a reactive well: testing and operational experiences. Environ Sci Tech 34:149-153.

Schollhorn A, Savary C, Stucki G, Hanselmann DW (1997) Comparison of different substrates for the fast reductive dechlorination of trichloroethene under groundwater conditions. Wat Res 31:1275-1282.

Zenker MJ, Borden RC, Barlaz MA, Lieberman MT, Lee MD, 2000. Insoluble substrates for reductive dehalogenation in permeable reactive barriers. In: Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds. Edited by Wickramanayake GB, Gavaskar AR, Alleman BC, and Magar VS, Battelle Press, Columbus, Ohio, p.47.