Treatment of Trichloroethylene-Contaminated Soils Using Foams Coupled with Bioaugmentation: Soil Column Studies

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Abstract

The feasibility of using surfactant foams to disperse trichloroethylene (TCE)-dense nonaqueous phase liquids (DNAPLs) in a model aquifer system was demonstrated. The foams also served as an effective delivery system for Envirogen's TCE-degrading bacterial strain (ENV 435). Over 20 surfactants were screened and Steol CS-330 (Stepan, Inc.) was found to meet five tested criteria: (1) it emulsified TCE; (2) it formed a highly stable foam; (3) it mobilized TCE-DNAPLs in porous media; (4) it was biocompatible with ENV 435; and 5) it evenly dispersed ENV 435 within a sand column when bacteria and foam were injected together. Foam injection studies, using sand columns as a model aquifer system, showed that mobilization of a TCE-DNAPL could be maximized when the Steol CS-330 foam was injected into the sand column in a pulsed operation. Injection of foam followed by artificial groundwater (AGW), then by foam again resulted in 90-95% mobilization of TCE through an eight-inch column containing 884 cm^3 of sand. The TCE remaining within the sand column after this pulsed operation was reduced to a level compatible with biodegradation (between 5 and 500-μg TCE per gram of sand). When ENV 435 [$1x10^8$ colony forming units (CFU)/mL] was added with the second pulse of foam, as much as 99% degradation was observed in some sand samples.

Introduction

Successful *in-situ* remediation of aquifer sediments containing DNAPLs requires mobilization and dispersion of the DNAPL in order to reduce local concentrations to a level that can be easily biodegraded. This project evaluated a novel remediation technology which combined work in a number of previously unrelated areas for *in-situ* treatment of TCE-DNAPLs. The research included the development of "designer foams" and methods for foam-driven removal of pollutants from soils and sediments, and the development of low cost *in-situ* bioremediation methods that utilize specialized degradative microorganisms.

The advantage to using foams for enhanced *in-situ* bioaugmentation is the ability of foams to extract and disperse NAPL-like contaminants from porous media. Work at ANL has shown that foams are 10-times more efficient than surfactant solutions at similar concentrations in mobilizing hydrophobic contaminants through porous media [Enzien et al., 1994a]. Visual observation also indicated that residual NAPLs were more evenly dispersed after foam flushes than with surfactant solutions. Foams tend to scour trapped NAPLs and leave residual sediment concentrations which are more amenable to biodegradation than flushing solutions. The biocompatibility of residual foam contaminated soil has been tested using nonionic surfactants and PAH contaminated soil. The results indicated no significant decrease in viable heterotrophic and naphthalene-degrading microorganisms after foam flushes.

Foam technology provides several potential remediation advantages for *in-situ* treatment of DNAPLs as follows. (1) Research and some pilot-plant activities have demonstrated that microbubble foams can deliver and retain oxygen and air in various sediment matrices and contaminated harbor sediments [Michelsen et al., 1984a, 1984b, 1985]. (2) Microbubbles can enhance the intrinsic biodegradation of dissolved and dispersed organics by adding oxygen to the environment [Michelsen et al., 1984a,b]. Injection of foams can also result in the formation of microbubble-treatment barriers (zones that retain oxygen) to enhance biodegradation [Jenkins et al., 1993]. (3) Work at Argonne National Laboratory (ANL) has shown that unlike aqueous-based fluids, foams tend to flow through porous media in a "plug-flow" manner providing effective areal sweeps [Enzien et al., 1994a; 1994b; Peters et al., 1994]. This allows for more uniform penetration and contact of the mobile phase with the porous media. (4) The strong sorption of bacteria with gas/water interfaces [Wan et al., 1994] should make foams an ideal transport vehicle for dispersing bacteria in a porous media. (5) Foams provide a reduction in surface tension at sediment particle surfaces by surfactant action [Jackson et al., 1994]. This reduction in surface tension should also reduce bacterial adhesion to sediment particles and may speed desorption of contaminants.

Specialized microorganisms for *in-situ* treatment of VOC-contaminated groundwater have been developed over the past several years at Envirogen, Inc. Starting with natural soil isolates that are very efficient at degrading TCE and other chlorinated solvents, organisms have been developed (without genetic engineering), that: (1) maintain expression of their

degradative genes in the absence of inducing chemicals (constitutive); (2) have reduced adhesion properties (adhesion deficient); and (3) can be grown to maximize their energy storage material (energy enriched) for prolonged *in-situ* degradative activity. These organisms have been tested for their ability to remove TCE and related VOCs from contaminated aquifer material, to penetrate aquifer sediments, and to efficiently utilize energy reserves to prolong degradative activity and minimize oxygen demand. The development of these specialized microorganisms has addressed a number of problems associated with using bioaugmentation for the remediation of VOCs in aquifers. One such organism, ENV 435 is constitutive for TCE-degrading activity, can migrate through sand at the same rate as a conservative chloride tracer, and can contain as much as 60% of its dry weight in the energy storage polymer, poly-beta-hydroxybutyric acid (PHB). However, these specialized TCE degrading bacteria, although tolerant of very high concentrations of contaminant, do not survive when in contact with free product TCE.

Delivery of microorganisms within a foam offers many advantages over currently available technologies for *in-situ* bioaugmentation. These advantages include: (1) enhanced transport of bacteria through sediment by dispersion with the foam; (2) delivery and retention of oxygen and nutrients to extend microbial activity; and (3) reduced toxicity as the DNAPL is mobilized and dispersed by the scouring effect of the foam resulting in higher bacterial survivability. The goal of this study was to demonstrate the efficacy of combining these two complementary technologies; surfactant foams and bioaugmentation, to remediate VOC-contaminated aquifers. Results from this work suggest that foam-facilitated transport of specialized microorganisms into subsurface aquifer sediments will result in the remediation of DNAPLs through mobilization and dispersion of the DNAPL and delivery of viable organisms for biodegradation.

Experimental Methods

The initial assessment of the emulsifying activity of anionic, nonionic, and cationic surfactants was carried out as follows. TCE (100 µL) was added to 5 mL of surfactant solution (0.1%). The vials were vigorously mixed for one minute (vortexed) and allowed to sit for 1-2 hours. Visual observations were made as to the degree of TCE emulsification and the presence of foam in the vials. Twenty-six surfactants were screened. Anionic surfactants tested were Polystep A-11 (Stepan), Polystep A-16 (Stepan), Ninate 411 (Stepan), Biosoft N-300 (Stepan), Biosoft D-40 (Stepan), Pyronate 40 (Witco), Petronate L (Witco), Stepantan DT-60 (Stepan), Bioterge AS-40 (Stepan), Steol CS-330 (Stepan), Gemtek (Gemtek), sodium dodecyl sulfate (Sigma) and diocytylsulfosuccinate (Sigma). Nonionic surfactants tested were Brij 35 (ICI Spec. Chem), Triton X-100 (Union Carbide), Triton X-705 (Union Carbide), Tergitol 15-S-12 (Union Carbide), Tween 80 (ICI Spec. Chem), Igepal CO-520 (Rhone-Poulenc), Igepal CO-720 (Rhone-Poulenc), Igepal CO-880 (Rhone-Poulenc), Igepal CO-990 (Rhone-Poulenc), and Microstep H-301 (Stepan). Cationic surfactants tested included Ammonyx KP (Stepan), Ammonyx Cetac-30 (Stepan), and dodecylpyridinium chloride (Aldrich). Those surfactants showing good emulsification were screened for biocompatibility.

Biocompatibility with 12 selected surfactants [Polystep A-11, Polystep A-16, Ninate 411, Biosoft N-300, Biosoft D-40, Petronate L, Bioterge AS-40, Stepantan DT-60, Steol CS-330, sodium dodecyl sulfate, Triton X-705, and Tergitol 15-S-12] was tested using the TCE-degrading ENV 435. Following the growth of ENV 435, cells were concentrated, washed with buffered saline and suspended in an artificial groundwater media (AGW). Ten mL of cells were incubated with selected surfactant solutions at either 0.025% or 0.1% (v/v) concentration. Subsamples were removed at day 0, 3, and 7 to determine bacterial viability using standard plate counts on R2A agar containing nalidixic acid, streptomycin sulfate, and chloramphenicol.

Glass columns (~10 cm [4 in.] long x ~7.4 cm [3 in.] diameter) were packed with dry sterile sand. A fine-mesh screen was placed on top of the sand column and was covered with glass beads to retain the sand in the column. After calibrating the flowmeter to achieve the desired flow rate, deionized water was applied to the sand column. The time required to fully saturate the column was noted, enabling the void volume of the column to be estimated. Surfactant solutions were prepared to provide a concentration of ~500 mg/L. Surfactants employed in these experiments included: Tergitol 15-S-12, Steol CS-300, and Biosoft D-40. ENV 435 was added to the surfactant solutions to provide an optical density measured at 550-nm of ~1.0 (approximately 1 x 10^9 CFU/mL). Six (6) pore volumes of the surfactant/microorganism solution were applied to the sand column at a flow rate between 5 to 7 mL/min. Afterwards, the sand column was drained, and a soil boring was collected approximately along the center of the column. This coring was then sliced into 1-cm lengths for CFU determinations. The residual sand was also monitored for its residual moisture content.

Surfactant foam experiments employed the same basic procedure, except that a foam was generated from the surfactant/microorganism solution using a foam generator apparatus. The foam was made to have a nominal 65%

quality. The surfactant/microorganism foams applied to the sand column had the same equivalent liquid flow rate (~5-7 mL/min) as that employed with the surfactant solutions.

Samples of the bacterial solutions at the influent and effluent of the sand columns were diluted in phosphate buffered saline (PBS) and plated out on solid R2A medium agar plates containing antibiotics. The sliced sand core samples (1-2 grams each) were resuspended in 10 mL of PBS in screw cap glass tubes and mixed for 30 sec. on a vortex Genie mixer at the highest setting for 30 seconds before dilution in PBS and plating on R2A medium. The number of Colony Forming Units (CFU) was determined after 48 hours incubation at 30°C. The results are expressed as the number of CFU per mL of solution or per gram of sand. The amount of sand in each sample was determined by using preweighed sample tubes.

Results and Discussion

Three operational configurations were tested in conjunction with operation of sediment columns: (1) injection of 400 mL surfactant solution followed by 200 mL AGW, then a second injection of 400 mL surfactant solution and finally injection of 600 mL bacteria suspended in AGW; (2) injection of 400 mL surfactant foam followed by 200 mL AGW, followed by a second injection of 400 mL surfactant foam, and then injection of 600 mL bacteria in AGW; and (3) injection of 300 mL surfactant foam followed by 200 mL AGW and then injection of 600 mL foam with bacteria. Control columns were run in parallel under the same operational mode but without the bacteria.

TCE-DNAPL mobilization studies were performed with Tergitol 15-S-12, Biosoft D-40, and Steol CS-330. Dispersion results, using 650 mL of surfactant solution or 650 mL surfactant foams (0.1% surfactant concentration), indicated that the Steol CS-330 surfactant foam was the most efficient in mobilizing the TCE-DNAPL (Table 1). Treating with artificial ground water did not result in dispersing or mobilizing the TCE-DNAPL, as shown by the AGW column in which the TCE concentration is very high within the bottom four core sections (between 2,280 and 23,310 µg/g of sand). The highest concentration was measured in core section #2. Flushing the columns with the surfactant solutions did not alter the distribution of the TCE-DNAPL (TCE still located in bottom 3 or 4 core sections; core #2 still representing the highest TCE concentration), although the TCE concentrations themselves are somewhat lower (between 508 and 9790 µg/g TCE) compared to the AGW flushed column. The reduced TCE concentration may indicate that there was some dispersion of the TCE within a vertical core section or may reflect the variation in the actually coring of the columns and location of the actual TCE-DNAPL within the column. However, there is no indication that TCE was mobilized through the column using surfactant solution flushes since higher concentrations of TCE were not observed in the middle or top of the column. Treatment with Tergitol 15-S-12 surfactant foam or Biosoft D-40 surfactant foam also shows a lower TCE concentration within the DNAPL region of the columns, but again there is no apparent sweeping of the TCE through the column as the highest TCE concentration is still observed in core section #2 (1,030 µg/g for Tergitol 15-S-12; 1,597 µg/g for Biosoft D-40). Only when the sand column was treated with Steol CS-330 surfactant foam was the TCE-DNAPL mobilized. Table 1 shows that the highest TCE concentration using Steol CS-330 foam (2,450 µg/g) was observed in core section #5 (3-cm up from the initial DNAPL location). It should also be noted that foam (60% quality) was collected in the effluent only with the Steol CS-330 treatment. Foam was apparent in the effluent after pumping ~2 pore volumes of foam into the column. No foam was observed in the effluent using Biosoft D-40 or Tergitol 15-S-12 indicating that these two surfactant foams were not as stable in the sand column.

The effect of the foam pumping velocity on mobilizing and dispersing the TCE-DNAPL was tested using Steol CS-330. Results shown in Table 2, indicate that DNAPL mobilization is enhanced at the higher pumping rate of 5.0 mL/min liquid volume compared to 3.5 mL/min liquid volume. Results indicate that the TCE is moving as a pulse of TCE through the column when the foam is pumped at the higher velocity. While control columns show that the TCE-DNAPL is concentrated in the first 3 to 4 core sections, injection of foam at 5 mL/min resulted in TCE being concentrated in cores sections #4 through #6. At the lower pumping velocity, TCE is still concentrated over the first 4 core sections, although the highest TCE concentration was observed in core section #4 (2,420 µg/g) compared to the control columns where the highest TCE concentration was in core section #2.

In addition to altering the pumping velocity, the effect of pulsing the Steol CS-330 foam into the column was also examined. For this column, 350 mL of foam, followed by 200 mL AGW followed by 300 mL foam followed by 250 mL of AGW, was injected into the column. As shown in Table 2, the TCE concentration in this sand column was reduced by an order of magnitude, with the highest observed TCE concentration of 188 µg/g in core section #6. Under this pulsed foam injection regime, the bulk of the TCE (~90%) was mobilized through the column as compared to a single injection of foam. A total of 650 mL of foam was injected into the columns with both the single and pulsed

injections. This result indicates that a pulsed injection of foam is significantly more effective in mobilizing and dispersing TCE-DNAPLs.

Table 1. Result Using Surfactants on TCE-DNAPL Migration/Dispersion in Sand Columns (4-in) Containing TCE-DNAPL.

Core Section	µg/g (dry wt.) TCE per core section (~1 cm each)						
	Aqueous Controls (5 ml/min)				Foam at 18 ml/min foam volume (~5.0 ml/min liquid volume)		
	AGW (650 ml)	Tergitol Surfactant Soln. (650 ml)	Biosoft D-40 Surfactant Soln. (650 ml)	Steol CS-330 Surfactant Soln. (650 ml)	Tergitol Surfactant Foam (650 ml)	Biosoft D-40 Surfactant Foam (650 ml)	Steol CS-330 Surfactant Foam (650 ml)
1 (bottom)	6,720	1,730	508	2,690	880	193	49
2	23,310	4,830	2,760	9,790	1,030	1,597	62
3	6,310	1,400	2,220	3,510	680	577	488
4	2,280	570	895	455	91	345	1,490
5	319	370	320	79	67	122	2,450
6	144	11	60	56	23	104	1,006
7	79	42	60	21	22	92	354
8	33	6.0	85	16	13	23	249
9 (top)	43	14	36	15	13	26	59

All columns were first equilibrated with 450 ml of artificial ground water (AGW).

Table 2. TCE Concentration vs. Depth in Sand Columns (8-in) Using Steol CS-330 (0.1%) Surfactant

Column Core Sample (2 cm sections)	TCE Concentration µg/g sand (ppm)					
	Controls			Experimentals		
	Surfactant Soln. (400ml surf/200ml AGW/400ml Surf/600ml AGW) 1.5 ml TCE	Surfactant Foam (300 ml foam/200ml AGW/600ml foam) 1.0 ml TCE	Surfactant Foam (400ml foam/200ml AGW/400ml foam/600ml AGW) 1.5 ml TCE	Surfactant Soln. (400ml surf/200ml AGW/400ml surf/600ml cells) 1.5 ml TCE	Surfactant Foam (300ml foam/ 200ml AGW/600ml foam+cells) 1.0 ml TCE	Surfactant Foam (400ml foam/200ml AGW/400ml foam/600ml cells) 1.5 ml TCE
1 (bottom)	7210	470	24	4390	4.1	6.3
2	8160	550	9.7	830	3.6	3.2
3	5670	22	6.0	460	2.2	0.7
4	830	20	5.9	50	1.0	1.8
5	35	14	6.6	23	0.8	1.9
6	33	12	10	3	0.6	2.5
7	42	5.5	7.4	11	0.6	1.6
8	16	7.8	6.6	2.0	0.7	1.3
9	13	19	3.1	7.8	1.0	1.4
10 (top)	180	6.0	4.1	16	1.2	1.3

Pulsing foam-water-foam significantly enhanced TCE mobilization and dispersion. Therefore, two foam injection protocols using a pulsing mode were tested for the ability to evenly distribute ENV 435 within TCE-DNAPL contaminated sand columns. The two protocols were as follows: (1) 300 mL of foam followed by 200 mL AGW followed by 600 mL foam [Run 3A]; and (2) 400 mL of foam followed by 200 mL AGW followed by 400 mL foam followed by 600 mL AGW [Run 3B]. For comparison, columns using a surfactant solution (Run 3C) were also run in a pulsing mode as follows: 400 mL surfactant solution followed by 200 mL AGW, then 400 mL surfactant solution and finally 600 mL AGW. The columns (7.5 cm [i.d.] x 20 cm [h], loaded with either 1.0 or 1.5 mL of TCE), were first flushed with approximately 450 mL of artificial groundwater (AGW). The surfactant foam or solution, Steol CS-330, was injected at a 0.1% concentration at 5 ml/min liquid flow rate. The AGW was also pumped into the column at 5 mL/min. Six hundred mL of ENV 435 ($1x10^8$ cells/mL) were delivered either with the final surfactant foam application or the final artificial ground water injection. Other foam injection scenarios were not tested as they called for the initial injection of bacterial cells into the column, which would have resulted in cell death as shown by the results of the

surfactant solution injection treatment Column 3C-2 (Table 3). In this column, the TCE DNAPL was not sufficiently dispersed prior to the injection of ENV 435 and resulted in >99% cell death.

Table 3 shows that the use of surfactant foams under both run modes resulted in dramatically improving the dispersion and survival of injected TCE-degrading bacteria compared to injection of the surfactant solution. Under the foam/AGW/foam operation (Column 3A-2) the average cell survival in core sections #1 through #9 was 27%, while Column 3B-2 (foam/AGW/foam/AGW) showed an average survival of 32% throughout the column. This compares to < 0.001% cell survival in Column 3C-2, treated with surfactant solution and ENV 435. In addition, the influent and effluent analysis of Column 3B-2 shows 100% bacterial survival after passage through TCE-contaminated columns flushed with foam prior to adding bacteria (Table 6). Only under conditions where foam was used to mobilize and disperse the TCE-DNAPL (to nontoxic levels) could the ENV 435 cells survive.

Table 3. Bacterial Counts (CFU) vs. Depth in TCE-DNAPL Sand Columns (8-in) Using Steol CS-330 (0.1%) Surfactant.

Sample	Colony Forming Units - [CFU/ml, aqueous or CFU/g, sand column]					
	Run 3A (Column 3A-2) Surfactant Foam (300ml foam/ 200ml AGW/600ml foam+cells)		Run 3B (Column 3B-2) Surfactant Foam (400ml foam/200ml AGW/ 400ml foam/600ml cells)		Run 3C (Column 3C-2) Surfactant Solution (400ml soln/200ml AGW/ 400ml soln/600ml cells)	
	Cell counts[1]	% Survival	Cell counts	% Survival	Cell counts	% Survival
Aqueous conc.						
influent cells	1.8E+8	100	1.3E+8	100	1.3E+8	100
effluent cells	<1.0E+5	<0.05[2]	1.4E+8	100	<500	<0.004
predicted in column[3]	5.4E+7	100	3.9E+7	100	3.9E+7	100
Core Section (2 cm segments)						
1 (bottom)	2.0E+7	51	2.0E+7	51	<500	<0.001
2	1.8E+7	46	6.1E+6	16	<500	<0.001
3	2.2E+7	56	4.4E+6	11	<500	<0.001
4	1.3E+7	33	4.0E+7	100	<500	<0.001
5	7.7E+6	20	1.0E+7	26	<500	<0.001
6	6.1E+6	16	1.2E+7	31	<500	<0.001
7	1.4E+6	3.6	1.1E+7	28	<500	<0.001
8	4.9E+6	12.6	1.1E+7	28	<500	<0.001
9	2.6E+6	6.7	8.0E+6	21	<500	<0.001
10 (top)	8.7E+4	0.22	4.7E+6	12	<500	<0.001

[1]Cell counts are expressed as CFU/ml for aqueous samples and CFU/g (dry wt) for core samples

[2]The apparent low bacterial survivability in this sample is due to the fact that the cells are moving with the foam front which just reached the end of the column as indicated by the cells counts in core #10.

[3]Predicted bacterial concentration is based on the initial aqueous bacterial concentration and a sand moisture retention of 0.3 ml water/g dry sand.

Comparison of the two foam run scenarios, indicates that ENV 435 was more dispersed and had greater viability when delivered in the AGW following the second foam injection, rather than with the 2nd foam injection. As shown, in Table 3, the cell survival in the top four core sections(#7-10) of Column 3B-2 was between 12 and 28%, while there was only between 0.22% and 12.6% cell survival when ENV 435 was injected with the 2nd foam application (Column 3A-2). However, the TCE concentration in control columns operated as foam/AGW/foam is higher than columns operated as foam/AGW/foam/AGW. Higher TCE concentrations, therefore, might account for the lower bacterial viability. In addition, the strong sorption of bacteria to gas/water interfaces [Wan et al.,1994] suggests that ENV 435 might travel with the foam as it moves through the column. Since the foam was just nearing the top of the Column 3A-2, a lower bacterial count would be expected at the top compared to Column 3B-2 which was flushed with 2 pore volumes (600 mL) of AGW + cells. Cells injected with AGW would not sorb to the foam and would travel with the AGW (assuming little adhesion of the cells to the sand).

Summary and Conclusions

Results from this study demonstrated the feasibility of using a surfactant foam/biological treatment for treating TCE-DNAPLs. A pulsed surfactant foam application itself was capable of scouring TCE-DNAPLs from model sand columns leaving behind a nontoxic level of TCE. When the foam was combined with a TCE-degrading strain, ENV 435, the residual TCE in the column could be degraded to greater than 99% of the initial concentrations. The most

effective treatment regime tested in this study [i.e., maximum TCE mobilization, dispersion, and degradation; and maximum bacterial dispersion, viability, and activity] was as follows: (1) TCE-DNAPL columns were initially flushed with Steol CS-330 surfactant foam (approximately 1.3 pore volumes based on the liquid volume of surfactant foam); (2) approximately 2/3 pore volume of AGW was then pumped into the column; and (3) finally two pore volumes of foam + ENV 435 ($5x10^8$ CFU/mL) was pumped into the column. The initial injection of foam followed by AGW (no TCE-degrading organisms) allowed for sufficient mobilization and dispersion of the TCE-DNAPL to below toxic levels and the second injection of foam in the presence of TCE-degrading bacteria resulted in greater than 99% percent degradation of the remaining TCE.

This research demonstrated the feasibility of using the combination of the foam technology and specially-developed TCE-degrading organisms to remediate sediments contaminated with DNAPLs of TCE. This technology has distinct advantages over competitive processes because of several unique developments in biocatalyst design and application methods. Because of the effectiveness of this *in-situ* technology, it should be applicable to remediation of many VOC-contaminated sites located nationwide. Specialized microorganisms have been developed at Envirogen for *in-situ* remediation of VOCs; these organisms are constitutive, adhesion deficient, and produce energy storage material to prolong their degradative activity *in-situ*. The organisms have been tested for their ability to remove TCE and related VOCs from contaminated aquifer material, to penetrate aquifer sediments, and to efficiently utilize energy reserves to prolong degradative activity, and minimize oxygen burdens. Argonne National Laboratory (ANL) has shown that foams are 10-times more efficient than surfactant solutions in removing (scouring) hydrophobic contaminants from porous media and that residual NAPLs are more evenly dispersed after foam flushes. Foams also enhance the oxygen mass transfer by using microbubbles versus oxygenated solutions. Oxygen delivery in gas is much more efficient than dissolved oxygen delivery. Delivery of microorganisms with a foam thus offers many advantages over currently available technologies for *in-situ* remediation.

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