Environ. Sci. Technol. **1998**, *32,* 1667−1675

Surfactant Foam/Bioaugmentation Technology for In Situ Treatment of TCE-DNAPLs

RANDI K. ROTHMEL,[†]
ROBERT W. PETERS,[‡]
EDWARD ST. MARTIN,[‡] AND
MARY F. DEFLAUN*,[†]

Envirogen, Inc., 4100 Quakerbridge Road, Lawrenceville, New Jersey 08648, and Argonne National Laboratory, 9700 Cass Avenue, Argonne, Illinois 60349

Chlorinated solvents such as trichloroethylene (TCE) are prevalent aquifer contaminants. Depending on the degree of contamination, their physical properties may cause them to occur as dense nonaqueous-phase liquids (DNAPLs) making them difficult to remediate by pump-and-treat methods. Successful in situ bioremediation requires mobilization and dispersion of DNAPLs in order to reduce sediment concentrations to levels nontoxic to degradative bacteria. A bench-scale study was conducted to evaluate a novel remediation technology that utilized surfactant foam for mobilizing and dispersing TCE-DNAPLs combined with a bioaugmentation technology to remediate TCE in situ. Results using the anionic surfactant Steol CS-330 showed that foam injected into TCE-DNAPL-contaminated sand columns enhanced mobilization of TCE-DNAPLs. Mobilization was maximized when the foam was injected in a pulsed operation. Injection of foam followed by artificial groundwater (AGW) and then by foam again resulted in flushing 75% of the initial TCE-DNAPL through an 8-in. column (884 cm^3 of sand). The residual TCE was dispersed within the column at concentration levels compatible with biodegradation (<500 μg/g). Adding the TCE-degrading bacterial strain ENV 435 simultaneously with the second pulse of foam resulted in 95−99% degradation of the residual TCE. This level of remediation was achieved with a total of 3 pore volumes (foam/AGW/foam + bacteria) and an aqueous column retention time of 1 h.

Introduction

Volatile organochlorine compounds (VOCs), such as trichloroethylene (TCE) and its sister compounds, vinyl chloride and dichloroethylene, are common contaminants of groundwater and sediments at many government and private sites. Although these compounds may be relatively soluble in water, other inherent chemical/physical properties make them particularly problematic as groundwater pollutants. One chemical/physical property of concern is the tendency for VOCs to form dense nonaqueous-phase liquids (DNAPLs), which are localized high concentrations of VOCs that form liquid deposits because the solvent's density is greater than that of water. Although often thought of as "puddles" or pools of free product, they may also include small, even microscopic, globules of material that may either rest atop consolidated materials or be entrained within such material. DNAPLs may also occur as ganglia that form as the DNAPL percolates toward the groundwater. It is estimated that it would take several decades to centuries for natural groundwater dissolution to deplete all the residual and pooled DNAPL (*1*). Pump-and-treat technologies have little effect on DNAPLs because it is difficult to generate water flows capable of moving, dislodging, or breaking up the materials (*2*). Alternative remediation treatments have focused on removing DNAPLs through the use of surfactant-enhanced aquifer remediation (SEAR) and vapor extraction. Surfactants can enhance the removal of DNAPLs by (a) solubilization mediated by the formation of micelles or (b) mobilization by reducing the interfacial tension (IFT) between the DNAPL and groundwater (*3*−*5*). However, a number of parameters limit the use of surfactant solutions in pump-and-treat systems such as channeling effects, aqueous-phase bypassing, and rate-limiting mass transfer (*6*). Air sparging, which can be more effective than pump-and-treat technologies for recovering DNAPLs, is limited in its radius of influence (regions directly above the injection area) and by channeling of the gas flow, especially if DNAPLs are pooled above clay lenses (*7*, *8*). Neither surfactant washing or air sparging addresses the residual concentration of contaminant that remains after the bulk of the DNAPL is removed.

Successful in situ bioremediation of aquifer sediments containing DNAPLs, therefore, requires both bulk extraction of the DNAPL and the dispersion of residual contaminant to a concentration level that is compatible with biological degradation. A bench-scale research project was conducted to evaluate a novel remediation technology that utilized experience from a number of previously unrelated areas for in situ treatment of TCE-DNAPLs. These research areas included the development of surfactant foam technology; the development of models and methods for foam-driven oil recovery; the development of physical/chemical methods for removal of pollutants from soils and sediments; the development of low-cost in situ bioremediation methods; and a bioaugmentation technology to remediate TCE in situ.

Rationale

There are several potential remediation advantages for using foam technology in conjunction with bioremediation for the treatment of DNAPLs. Foam technology research has demonstrated that (a) microbubble foams can deliver and retain oxygen and air in various sediments to enhance biodegradation (*9*−*12*); (b) foams tend to flow through porous media in a "plug-flow" manner, providing an effective areal sweep that allows for more uniform penetration with reduced channeling effects as compared to the flow of air or liquid (*13*−*15*); (c) foams provide an ideal transport vehicle for dispersing bacteria because of the strong sorption of bacteria to gas/water interfaces (*16*, *17*); and (d) foams provide a reduction in the IFT (*18*).

Specialized microorganisms have been developed over the past several years to address problems associated with in situ treatment of VOC-contaminated groundwater. Natural soil isolates have been optimized (without genetic engineering), for (a) constitutive degradative activity, e.g., maintainance of degradative expression in the absence of inducing chemicals (*19*); (b) reduced adhesion properties (*20*); and (c) growth to maximize their energy storage material for prolonged in situ activity with reduced oxygen demand (*21*). One such variant, ENV 435 (*20*), can degrade TCE for

* Corresponding author telephone: (609)936-9300; fax: (609)936-9221; e-mail: deflaun@envirogen.com.
[†] Envirogen, Inc.
[‡] Argonne National Laboratory.

S0013-936X(97)00980-2 CCC: $15.00
Published on Web 04/17/1998

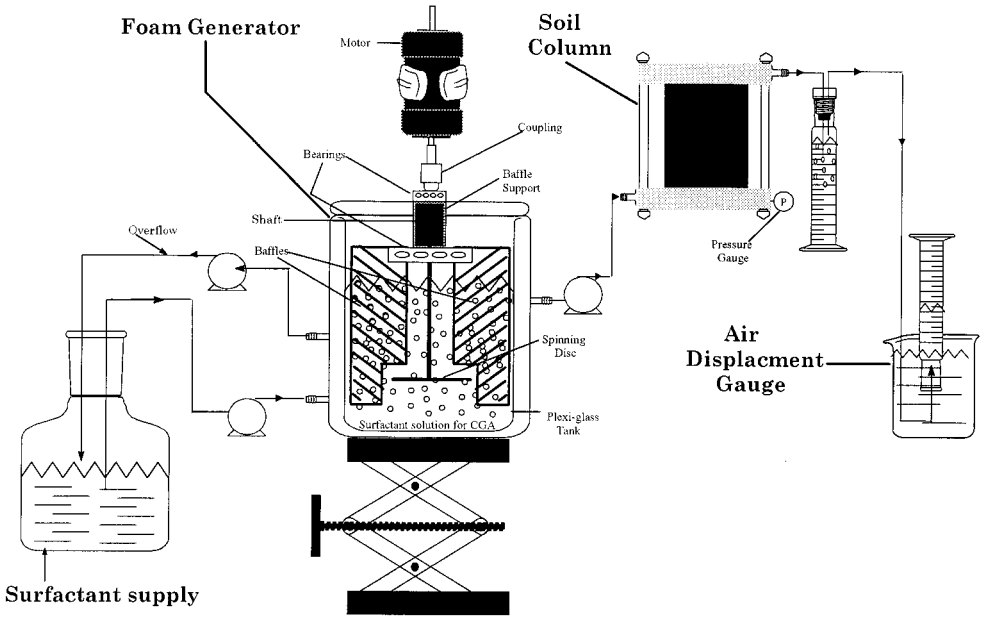

FIGURE 1. Schematic of the foam generator and column setup for treatment of TCE-DNAPL-contaminated sand.

at least 5 times longer than the wild-type *Burkholderia (Pseudomonas) cepacia* G4, 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-β-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 (e.g., DNAPLs).

Methods and Materials

Growth of Bacteria. The TCE-degrading organism ENV 435 was grown at 30 °C in basal salts medium (BSM). BSM contained (per liter of deionized water) the following: 4.25 g of $K_2HPO_4 \cdot 3H_2O$, 1.0 g of $NaH_2PO_4 \cdot H_2O$, 2.0 g of NH_4Cl, 0.123 g of nitrilotriacetic acid, trisodium salt monohydrate, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.012 g of $FeSO_4 \cdot 7H_2O$, 0.003 g of $MnSO_4 \cdot H_2O$, 0.003 g of $ZnSO_4 \cdot 7H_2O$, and 0.001 g of $CoCl_2 \cdot 6H_2O$. Cells were grown either in a 7-L fermentor or in 10-L batches using a 20-L glass carboy sparged with sterile air. DL-Lactic acid, sodium salt (20 mM), and phenol (1 mM) were used as carbon sources. To prevent contamination of the growth media, four antibiotics were added: nalidixic acid (15 μg/mL), ampicillin (75 μg/mL), streptomycin sulfate (30 μg/mL), and chloramphenicol (20 μg/mL). The cells were harvested in late stationary growth phase by centrifugation, washed, and resuspended in sterile saline prior to use.

Surfactant Emulsifying Ability. An initial assessment of the ability to emulsify TCE was carried out using anionic, nonionic, and cationic surfactants. TCE (100 μL) was added to 10 mL of surfactant solution (0.1%). The vials were vigorously mixed by vortex for 1 min, which resulted in the formation of foam bubbles and a TCE emulsion. The vials were allowed to sit for 1−2 h, and then visual observations were made as to the amount of foam still present and the persistence of the TCE emulsion. A stable emulsion resulted in the formation of a turbid solution, while unstable emulsions resulted in neat TCE pooling at the bottom of the vial and clearing of the aqueous solution. Twenty-six surfactants were screened. Thirteen were anionic surfactants and included 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). Ten nonionic surfactants were tested including 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). The three cationic surfactants tested included the following: Ammonyx KP (Stepan), Ammonyx Cetac-30 (Stepan), and dodecylpyridinium chloride (Aldrich).

Biocompatibility Screening. Biocompatibility screening was performed with 12 surfactants that adequately emulsified TCE (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). Ten milliliter of a freshly grown culture of ENV 435, suspended in artificial groundwater (AGW) at 4.9 $\times 10^8$ colony forming units (cfu)/mL culture, was distributed to sterile 50-mL conical culture tubes. The AGW consisted of 60 mg of $MgSO_4 \cdot 7H_2O$, 20 mg of KNO_3, 36 mg of $NaHCO_3$, 36 mg of $CaCl_2$, 35 mg of $Ca(NO_3)_2$, 25 mg of $CaSO_4 \cdot 2H_2O$, 0.2 μM $NaHPO_4$, and 0.35 mL of 1.0 N HCl per liter with a conductivity of 250 μS. The selected surfactants were then added to the culture tubes to give a final concentration of either 0.025% or 0.1% (v/v). The tubes were mixed, sealed, and incubated at 15 °C. Subsamples removed at days 0, 3, and 7 were plated on R2A agar (BBL; Becton Dickinson and Co.) to determine bacterial viability.

Generation of Foam. Foams were generated in all experiments with a spinning disk generator (Figure 1; Aphron Technologies, Blacksburg, VA). This device rapidly and efficiently generated microbubbles (colloidal gas aphrons, CGAs) from low-concentration surfactant solutions. Microbubbles were generated within seconds of reaching the critical disk rotation speed of nearly 7000 rpm. Once generated, the disk speed was reduced to between 3000 and 4000 rpm, enough to maintain good microbubble generation. The quality of the foams generated were typically between 65 and 75% (volume of air per 100 mL volume of foam). Foams were pumped from the generator to the experimental column apparatus using a peristaltic pump at a rate varying between 3.5 and 7 mL of liquid volume per minute.

Bacterial Delivery Studies. Bacterial delivery studies were conducted in glass columns packed with sand to simulate

a model aquifer system. Glass columns (Figure 1) measuring ~10 or 20 cm in length with an outer diameter of 8.2 cm (i.d. ~7.4 cm and the wall thickness was ~4 mm) were packed with a uniform washed silica quartz sand (used in the construction of sand filters). The sand had an average diameter of 0.45−0.5 mm and a composition of 99.4% SiO_2, 0.13% Al_2O_3, 0.03% CaO, 0.13% Fe_2O_3, 0.02% K_2O, 0.02% TiO_2, 0.01% Na_2O, and 0.01% Mg_2O and was supplied by a local sand and gravel company. This sand was used as a model for a porous aquifer sediment because the homogeneous matrix ensured that columns were as similar as possible and allowed for direct comparisons between column runs. Columns were filled halfway with AGW, and the sand was slowly poured into the column to avoid air pocket formation. Once the column was filled with sand, the top plate of the column was secured, and 1−2 pore volumes of AGW was pumped upflow through the column prior to adding bacteria. The 10-cm columns were packed with 650 g of dry sand, and the 20-cm columns were packed with 1300 g of dry sand. The pore volume in the packed columns (water saturated) was ~150 mL for the 10-cm column and ~300 mL for the 20-cm column. Pore volumes were determined by filling the columns with dry sand and measuring the volume of water required to saturate the column.

Surfactant solutions (~500 mg/L in AGW) employed in these experiments included Tergitol 15-S-12, Steol CS-300, and Biosoft D-40. An ENV 435 culture (washed with AGW to remove residual antibiotics and growth media) was added to the surfactant solutions to provide an optical density measured at 550 nm of 1.0 (~1×10^9 cfu/mL). Six pore volumes of the surfactant/microorganism solution were pumped through the sand column at a flow rate of 5 mL/min. Influent and effluent samples were taken for plate count analysis to determine the percent of viable cells that were transported through the sand column.

Surfactant foam experiments employed the same basic procedure as that described above for the surfactant solution experiments, except that foam was generated from the surfactant/ENV 435 solution. The surfactant/ENV 435 foams applied to the sand column had the same equivalent liquid flow rate (~5 mL/min) as that employed for the surfactant solutions.

TCE-DNAPL Mobilization Studies. TCE-DNAPL mobilization studies using Tergitol 15-S-12, Steol CS-300, and Biosoft D-40 were conducted in a fashion similar to the bacterial delivery studies except that a TCE-DNAPL was introduced near the bottom of the sand column. Sterile sand was poured slowly through a column halfway filled with AGW (trying to avoid air bubbles) to a depth of ~0.5 in. Between 1 and 2 mL of TCE was then delivered with a 5-mL glass pipet to the center of the column on top of the sand. Using a spatula, the TCE pool was carefully covered with a small amount of sand. The rest of the column was then filled by pouring sterile sand through the AGW. Once the column was filled with sand, the top plate of the column was secured, and 1−2 pore volumes of AGW was pumped upflow through the column before surfactant treatment. Approximately 4 pore volumes of surfactant foam or surfactant solution (0.1%) were then pumped upflow into the column at a flow rate of ~5 mL/min (aqueous volume). Foam was injected either on a continuous basis or was introduced in a pulsed operation (foam/AGW/foam/AGW). Following the run, the column was analyzed for TCE (see below).

TCE-DNAPL Remediation Studies. The foam/bioaugmentation treatment was performed to evaluate (a) the delivery of ENV 435 through the column, (b) the mobilization of the TCE-DNAPL, and (c) the degradation of the residual dispersed TCE. Twenty centimeter aquifer columns, constructed as described above, were washed with 450 mL of AGW and then were injected with Steol CS-330 foam (225 mL aqueous volume; 450 mL of air), followed by 200 mL of AGW followed by foam + ENV 435 (450 mL aqueous volume; 900 mL air volume). For comparison, control columns were run with an identical protocol except that either a Steol CS-330 solution or air-sparged AGW was used in place of the two foam injections. For the air-sparged columns, air was pumped into the influent of the column at 10 mL/min in order to deliver a total of 1350 mL of air during the first and second injections (450 and 900 mL, respectively). The total AGW delivered during the air-sparging operation was 675 mL. Abiotic foam control columns using a pulsed foam flushing without ENV 435 were also run. The Steol CS-330 surfactant foam or solution was injected at a 0.1% concentration at 5 mL/min liquid flow rate. The AGW was also pumped into the column at a flow rate of 5 mL/min. ENV 435 was delivered at ~1×10^8 cells/mL with the final surfactant foam pulse. The hydraulic retention time was 1 h for the 20-cm columns. Columns were analyzed for TCE and/or the presence of ENV 435 (see below). TCE mass balance experiments were also conducted under surfactant washing, surfactant foam, and air-sparging conditions. To quantitate the total TCE in the system, the entire sand column was extracted in methanol, and the effluent (surfactant plus air) was collected directly into air sampling (Tedlar) bags for TCE analysis. In addition, any liquid that drained from the column while removing the top plate from the sand column was collected for TCE analysis. The methods for TCE analysis are described below.

ENV 435 Activity Study. To estimate the duration of activity of ENV 435 applied to TCE-DNAPL-contaminated sediments, TCE degradation assays were performed with bacterial cells collected from the influent and effluent of a foam + ENV 435 treated TCE-contaminated sand column. Ten milliliter of the column influent or effluent was placed into 50-mL serum vials. Serum vials were crimp sealed with Teflon-lined septa, and then each vial was injected with 10 μL of a 40 mM TCE (trichloroethylene; stabilized, Baker Inc.) stock dissolved in methanol. Killed controls consisted of two vials with 100 μL of 50 mM mercuric chloride in addition to the TCE to monitor for any abiotic loss of TCE. All vials were placed on a rotary shaker at room temperature, and the headspace was monitored periodically for TCE using GC−PID. When significant TCE depletion was observed in the live samples, vials were reinjected with 10 μL of the 40 mM stock TCE solution. This procedure was repeated for up to two additional TCE injections.

ENV 435 Dosage Study. Twenty-centimeter aquifer columns contaminated with 1.5 mL of TCE were operated by injecting foam/AGW/foam. ENV 435 was added with the second foam injection at either a concentration of 1×10^8 cfu/mL or at 5×10^8 cfu/mL. Control columns were run without added ENV 435 to determine the TCE concentration in the absence of bioaugmentation.

TCE Analysis. Following a run, columns were sampled by taking a vertical core with a Teflon or stainless steel coring device. One or 2 cm vertical sections from the cores were extruded into vials that contained either acidified water or methanol. Methanol-extracted samples were analyzed directly for TCE, while vials containing acidified water were first crimped sealed and heated to 70 °C for 2−3 h before a headspace sample was analyzed for TCE. The analyses were performed using a VOCOL 60-μm capillary column and PID detector. For the TCE mass balance determinations, all fractions (aqueous drain, extracted sand column, and the effluent samples—air and liquid) were analyzed by purge-and-trap GC/MS using an FID detector.

Column Analysis—Colony Forming Units (cfu) Determination. Samples for bacterial enumeration were suspended in 10 mL of phosphate-buffered saline (PBS; 0.85% sodium chloride and 20 mM K_2HPO_4 at pH 7.0) and mixed

TABLE 1. Results of Biocompatability of ENV 435 with Surfactants

surfactants screened for biocompatability	concn (%)	pH	day 1 cell count (cfu/mL)	day 1 % survival[a]	day 3 cell count (cfu/mL)	day 3 % survival[a]	day 7 cell count (cfu/mL)	day 7 % survival[a]
control								
no surfactant	0	7.20	4.9E+8	100	2.6E+8	53	2.9E+8	59
anionic								
sodium docecyl sulfate	0.025	6.78	1.9E+8	39	8.0E+7	16	82.7E+7	6
	0.1	7.00	<1.0E+6	<0.2	3.0E+4	0.01	1.4E+3	0.0003
Polystep A-11 (isopropylamine dodecyl benzene sulfonate)	0.025	7.00	<1.0E+8	<0.2	1.0E+6	0.20	5.4E+5	0.11
	0.1	7.25	<1.0E+6	<0.2	9.0E+4	0.02	1.0E+4	0.002
Polystep A-16 (branched sodium dodecylbenzene sulfonate)	0.025	7.00	4.5E+8	92	3.2E+8	65	1.9E+8	38
	0.1	7.05	9.5E+8	19	1.9E+7	4	6.6E+6	1
Ninate 411 (amine dodecylbenzene sulfonate)	0.025	6.95	3.8E+8	77	1.9E+8	38	1.3E+8	27
	0.1	7.10	8.0E+6	1.6	9.0E+4	0.02	1.0E+5	0.02
Stepantan DT-60 (TEA-dodecyl benzene sulfonate)	0.025	7.05	3.1E+8	63	1.9E+8	38	1.5E+8	31
	0.1	7.10	<1.0E+6	<0.2	1.0E+6	0.20	1.0E+5	0.02
Biosoft N-300 (TEA-dodecyl benzene sulfonate, blend)	0.025	6.90	2.1E+8	42	2.8E+8	56	2.0E+8	40
	0.1	7.10	<1.0E+6	<0.2	1.0E+4	0.002	1.3E+3	0.0003
Biosoft D-40 (sodium dodecyl benzene sulfonate)	0.025	6.85	2.2E+8	55	nd[b]	nd	2.7E+8	45
	0.1	6.92	<1.0E+6	<0.2	<0.2	<0.2	1.0E+3	0.0002
Petronate L (petroleum sulfonate)	0.025	6.78	3.6E+8	72	2.1E+8	43	2.0E+8	45
	0.1	6.89	2.2E+8	44	1.8E+8	36	1.3E+8	26
Bioterge AS-40 (olefin sulfonate)	0.025	7.00	3.5E+8	71	2.0E+8	40	2.0E+8	31
	0.1	6.90	6.0E+7	12	3.7E+7	8	2.7E+7	0.02
Steol CS-330 (sodium laureth ether sulfate)	0.025	6.90	3.6E+8	73	2.5E+8	50	2.2E+8	44
	0.1	6.80	3.3E+8	66	9.5E+8	19	8.0E+7	16
nonionic								
Tergitol 15-S-12 (Alkyloxypolyethyleneoxyethanol)	0.025	6.95	3.4E+8	69	nd	nd	1.9E+8	79
	0.1	6.88	2.6E+8	53	53	53	3.9E+8	39
Triton X-705 (octoxynol-70)	0.025	6.85	4.3E+8	87	3.7E+8	74	3.6E+8	72
	0.1	6.83	3.8E+8	77	3.2E+8	64	3.5E+8	70

[a] % survival was calculated by comparing results to the day 1 no surfactant control since initial cell counts were less than day 1 analysis. [b] nd, not done.

TABLE 2. Surfactant Parameters

parameter	surfactant: Biosoft D-40	Steol CS-330	Tergitol 15-S-12	Triton X-705
manufacturer	Stepan	Stepan	Union Carbide	Union Carbide
chemical family	anionic	anionic	nonionic	nonionic
chemical formula	$C_{12}H_{25}C_6H_4SO_3Na$	$CH_3(CH_3)_{10}CH_2(OCH_2CH_2)_3OSO_3Na$	$C_{12-14}H_{25-29}O(CH_2CH_2O)_xH$	mixture
cmc (wt%)[a]	0.125	0.18	0.011	ND[b]
HLB[a]	ND	ND	14.7	18.7
biodegradable	yes	yes	yes	yes

[a] Information as supplied by manufacturer; cmc, critical micelle concentration; HLB, hydrophilic/lipophilic balance. [b] ND, not determined.

for 30 s on a vortex mixer at the highest setting before further dilution in PBS. Dilutions were plated on solid R2A medium agar plates containing antibiotics and incubated for 48 h at 30 °C. The results are expressed as the number of cfu per milliliter.

Results

Surfactant Screening. Twenty-six surfactants were screened for their ability to emulsify 0.1 mL of TCE added to 10 mL of water. Of the surfactants screened, 12 formed a stable emulsion in which the TCE-DNAPL was no longer visible. In addition, a foam layer that formed at the air−water interface remained stable for 1−2 h. The 12 surfactants that formed stable emulsions included 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.

Biocompatibility of these surfactants was evaluated using ENV 435. As shown in Table 1, greater than 40% of the ENV 435 inoculated survived when incubated with five out of the 12 surfactants at 0.025% concentration. At 0.1% surfactant concentration, ENV 435 survival was 15% or greater with Petronate L, Steol CS-330, Tergitol 15-S-12, and Triton X-705. Foam stability analysis (data not shown) showed that Biosoft D-40, Steol CS-330, and Tergitol 15-S-12 were the most stable foams, with the aqueous fraction draining in ~20 min from foams generated at a 0.1% concentration and 65% foam quality. Triton X-705 was not very stable and easily broke down, draining in half the time. Petronate L was not considered because of its low water solubility and formation of an oil/water suspension. The physical/chemical properties of the four selected surfactants are shown in Table 2. As shown, there is no direct correlation between cmc and foam stability.

Bacterial Delivery Studies. A series of experiments were performed in which ENV 435 was injected into sand columns with either a water flush, a surfactant flush, or a foam flush. Three different surfactants were used to perform these

TABLE 3. Delivery of TCE-Degrading Microorganisms Using Surfactant Solutions vs Surfactant Foams

		surfactant (0.05%)					
	control	Tergitol 15-S-12		Biosoft D-40		Steol CS-330	
parameter	water flush	solution	foam	solution	foam	solution	foam
	ENV 435 Delivered and Recovered						
colony-forming units/mL (cfu/mL)							
soil column influent	3.0E+7	2.6E+7	4.1E+7	1.5E+9	1.3E+9	3.1E+7	2.7E+7
soil column effluent	8.4E+6	1.6E+7	2.4E+7	9.3E+8	3.8E+8	1.9E+7	2.3E+7
% ENV 435 migration through column	28	62	59	62	29	61	85

TABLE 4. Use of Surfactants for TCE-DNAPL Migration/Dispersion in Sand Columns (4 In.) Containing a 2-mL TCE-DNAPL[a]

	μg/g (dry wt) TCE per core section (~1 cm each)							
	aqueous controls (5 mL/min)				foam at 18 mL/min foam vol (~5.0 mL/min liquid vol)			
core section	AGW (650 mL)	Tergitol (650 mL soln)	Biosoft D-40 (650 mL soln)	Steol CS-330 (650 mL soln)	Tergitol (650 mL foam)	Biosoft D-40 (650 mL foam)	Steol CS-330 (650 mL foam)	Steol CS-330 (350 mL foam/200 mL AGW/ 300 mL foam/250 mL AGW)
1 (bottom)	6 720	1 730	508	2 690	880	193	49	65
2	23 310	4 830	2 760	9 790	1 030	1 597	62	40
3	6 310	1 400	2 220	3 510	680	577	488	26
4	2 280	570	895	455	91	345	1 490	84
5	319	370	320	79	67	122	2 450	119
6	144	11	60	56	23	104	1 006	188
7	79	42	60	21	22	92	354	158
8	33	6.0	85	16	13	23	249	34
9 (top)	43	14	36	15	13	26	59	25

[a] All columns were first equilibrated with 450 mL of artificial groundwater (AGW) prior to injection of water, surfactant solution, or surfactant foam.

experiments: Tergitol 15-S-12, Steol CS-330, and Biosoft D-40. Results shown in Table 3 indicate that the mobilization of ENV 435 was enhanced when the bacteria were injected with surfactant solutions (61−62% bacterial recovery) as compared to the injection of ENV 435 suspended in AGW alone (29%). Transport of ENV 435 through the column was most efficient when Steol CS-330 surfactant foam was used as the medium to inject the ENV 435 cells. Injection of the ENV 435/Steol CS-330 foam mixture resulted in 85% of the bacterial cells being recovered in the column effluent. Use of Tergitol 15-S-12 foam did not improve the delivery of ENV 435, while Biosoft D-40 foam inhibited bacterial mobilization as compared to AGW. Increasing the concentration of Steol CS-330 from 0.05% to 0.1% resulted in nearly identical bacterial penetration and dispersion within the sand column (data not shown). This indicates that the lower concentration of surfactant provides adequate performance of the foam flush within the sand column and that the higher concentration does not result in an inhibitory behavior of the surfactant on the viability of the microorganisms.

TCE-DNAPL Mobilization Studies. TCE-DNAPL mobilization studies were performed using three surfactants: Tergitol 15-S-12, Biosoft D-40, and Steol CS-330 in 10 cm columns. Mobilization results, using 4.33 pore volumes (650 mL) of surfactant solution or surfactant foams (0.1% surfactant concentration), indicated that the Steol CS-330 surfactant foam was the most efficient in mobilizing the TCE-DNAPL (Table 4). Treatment with AGW did not result in adequate dispersion or mobilization of the TCE-DNAPL as shown by the AGW control column in which the TCE concentration is very high within the bottom four core sections (between 2280 and 23 310 μg/g of sand). Flushing the columns with the surfactant solutions did not alter the distribution of the TCE-DNAPL within the column, which was still located in the bottom three or four core sections, with core section 2 (location of injected DNAPL) still representing the highest TCE concentration. Treatment with the surfactant foams generated from Tergitol 15-S-12 or Biosoft D-40 also did not appear to mobilize or disperse the TCE-DNAPL as the highest observed TCE concentration was still observed in core section 2 (1030 μg/g for Tergitol 15-S-12; 1597 μg/g for Biosoft D-40). Only when the sand column was treated with Steol CS-330 surfactant foam was the TCE-DNAPL mobilized. Using Steol CS-330 foam, the highest TCE concentration (2450 μg/g) was observed in core section 5 (3 cm from the initial DNAPL location; Table 4). The superior stability of the Steol CS-330 foam was demonstrated by the fact that foam (60% quality) was collected in the effluent after delivery of ~2 pore volumes. 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 within the sand column.

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 of AGW, followed by 300 mL of foam, followed by 250 mL of AGW were injected. The TCE concentration in this sand column was reduced by nearly an order of magnitude, with the highest observed TCE concentration of 188 μg/g in core section 6 (Table 4). Under this pulsed foam injection regime, the bulk of the TCE appears to have been pushed through the column as compared to a single injection of foam. This result indicates that a pulsed injection of foam is significantly more effective in mobilizing and dispersing TCE-DNAPLs.

TCE-DNAPL Remediation Studies. *Effect of Foam Treatment on the Delivery of ENV 435.* Columns were operated under a pulsed operation of either surfactant/AGW/surfactant + ENV 435 or foam/AGW/foam + ENV 435. Each 20-cm column was treated with a total liquid flow-through volume of 875 mL (~3 pore volumes), consisting of 675 mL of surfactant and 200 mL of AGW. The surfactant foam used to treat the columns was composed of 675 and 1350 mL of air. Under these conditions, no intact foam formed in the effluent and the pressure in the columns during the foam injections ranged between 3 and 10 psi. There was no measured pressure gradient during the AGW, surfactant

TABLE 5. Survival of ENV 435 Inoculated with Steol CS-330 Solution or Foam Into TCE-DNAPL-Contaminated Columns[a]

	Steol solution + ENV 435		Steol foam + ENV 435	
	ENV 435		ENV 435	
column core sample (2 cm sections)	cfu/mL	% survival	cfu/mL	% survival
inoculum	1.3E+8	100	1.3E+8	100
1 (bottom)	<500	<0.0004	8.7E+7	67
2	<500	<0.0004	7.8E+7	60
3	<500	<0.0004	9.5E+7	73
4	<500	<0.0004	5.6E+7	43
5	<500	<0.0004	3.3E+7	25
6	<500	<0.0004	2.4E+7	18
7	<500	<0.0004	6.1E+6	5
8	<500	<0.0004	2.1E+7	16
9	<500	<0.0004	1.1E+7	9
10 (top)	<500	<0.0004	3.8E+5	<0.5

[a] Columns contained 1 mL of TCE which was introduced approximately 2 cm from the bottom of each column. Columns were equilibrated with 450 mL of AGW and then were injected with 225 mL of surfactant solution or surfactant foam followed by 200 mL of AGW and then 450 mL of solution or foam + bacterial cells.

solution, or air injections. Results, as shown in Table 5, indicate that the use of the surfactant foam resulted in improving both the dispersion and survival of the injected TCE-degrading bacteria as compared to the injection of the Steol-CS-330 surfactant solution. Up to 73% of the expected cfu were detected within a vertical section of the column core. In contrast, less than 0.001% of the expected ENV 435 cfu were detected in the control columns treated with surfactant solutions (Table 5). Only under conditions where foam was used to mobilize and disperse the TCE-DNAPL (to levels below bacterial toxicity) did the ENV 435 cells survive.

Effect of Foam/Bioaugmentation on the Remediation of TCE-DNAPLs. Columns were run under four treatment conditions as shown in Table 6. As described above, all columns were run with a pulsed operation and a liquid flow-through volume of 875 mL (comprised of either AGW or surfactant + AGW). For the air-sparged columns, a total of 1350 mL of air was delivered into the 675 mL of influent AGW, while in the foam-treated columns the foam itself consisted of 675 mL of surfactant plus 1350 mL of air. The control column treated with Steol-CS-330 surfactant solution (Table 6) resulted in only minimal dispersion of the introduced TCE-DNAPL (2.0 mL/2.92 g of TCE). The TCE concentration in the surfactant-flushed column was highest in the first core section (3430 μg/g of TCE) where the DNAPL was introduced. The column treated with air sparging also showed only minimal TCE mobilization, and the highest level of TCE was located near the influent (Table 6). Using a foam/AGW/foam flush, a significant amount of TCE was mobilized and the TCE concentration was fairly evenly distributed (200−470 μg/g) within the first seven core sections. The dispersed TCE was amenable to bacterial degradation as shown by the addition of ENV 435 with the second foam injection. The observed degradation of TCE was 98% or greater in the column treated with foam + ENV 435 as compared to the column treated with foam alone. The TCE concentration ranged between 0.6 and 8.4 μg/g with foam/biotreatment as compared to a range of 22−470 μg/g in the foam treated column.

TCE Mass Balance Determination. A separate set of columns was run under identical conditions to determine the mass balance of TCE. As shown in Table 7, 100% of the TCE was recovered in the surfactant-washed column, and the bulk of the TCE was detected in the aqueous drain from the column (2.24 g total; 77% of the introduced TCE). When columns were treated by air sparging, 82% of the total TCE was recovered, with 34% of total injected TCE recovered in the effluent (air + liquid fraction). In addition, a significant amount (18%) of the TCE was present in the liquid that drained from the column and was visually observed to be DNAPL. In contrast, treatment with foam injection resulted in nearly 70% of the injected TCE being mobilized through the sand column with a total recovery of 89% (sand column + effluent fractions). The column run under foam/biotreatment conditions also showed about 70% of the TCE-DNAPL being mobilized through the column. However, with bioaugmentation, only 42 mg (1.4%) of the total TCE-DNAPL remained in the sand column, which represents 93% degradation of the residual column TCE (assuming a residual TCE amount of ~645 mg as shown in the control column).

ENV 435 Activity Study. Nearly identical activity was observed for both influent and effluent cells for the first 24 h (Figure 2). At this point, the TCE in the headspace was nearly depleted. A second injection of TCE was completely degraded by the influent cells within 48 h, but was only 75% degraded by the effluent cells. After a third injection of neat TCE, the influent cells degraded >82% of the TCE within 72 h, but the effluent cells were active for only an additional 48 h and degraded only 40% of the headspace TCE. In both cases, however, ENV 435 showed TCE-degrading activity for 5 or more days without an added carbon source. Thus, ENV 435 applied in the sequential foam/AGW/foam + ENV 435 treatment of a TCE-DNAPL-contaminated column maintained much of its activity as compared to ENV 435 that were never exposed to the TCE-DNAPL in the sand column.

TABLE 6. Treatment of TCE-DNAPL-Containing Aquifer Columns with Surfactant Solution, Air Sparging, or Surfactant Foam[a]

	Steol solution (0.1%) treatment	air-sparged AGW	Steol foam (0.1%) treatment	Steol foam (0.1%) and ENV 435 1.0 × 10^8 cfu/mL)	
core samples	TCE (μg/g dry sand)	TCE (μg/g dry sand)	TCE (μg/g dry sand)	TCE (μg/g dry sand)	% degradation
1 (bottom)	3430	5182	203	4.2	97.9
2	223	518	379	7.5	98.0
3	133	264	470	8.4	98.2
4	101	144	313	1.8	99.4
5	42.0	76	288	1.3	99.6
6	19.0	4.9	404	1.5	99.6
7	13.0	2.2	202	0.8	99.6
8	8.3	1.6	76	1.5	98.0
9	6.9	1.0	49	2.1	95.7
10 (top)	4.4	0.6	22	0.8	96.6

[a] Columns contained 2 mL of TCE, which was introduced approximately 2 cm from the bottom of each column. Columns were equilibrated with 450 mL of AGW and then were injected with either 225 mL of surfactant solution, surfactant foam, or AGW sparged with air. This was followed by 200 mL of AGW and then a second 450-mL injection of surfactant solution, surfactant foam, or air-sparged AGW. ENV 435 was added to the second foam flush in the biotreated column.

TABLE 7. TCE Mass Balance Determination under Various Treatment Conditions[a] (Total TCE Recovered)

	Steol solution (0.1%) treatment	air-sparged AGW	Steol foam (0.1%) treatment	Steol foam/biotreatment
column samples				
total in sand column	516	840	645	60
column drain	2240	550	NA[b]	NA
total mg of TCE in column	2756	1390	645	42
effluent samples				
aqueous	341	821	1745	1755
vapor	NA	170	207	200
total mg of TCE in effluent	341	991	1952	1955
total TCE recovered	3097	2381	2597	1997
mass balance (% recovered)	106	82	89	68
% TCE flushed through column	12	34	67	67

[a] See Table 6 for column run conditions. [b] NA, not applicable.

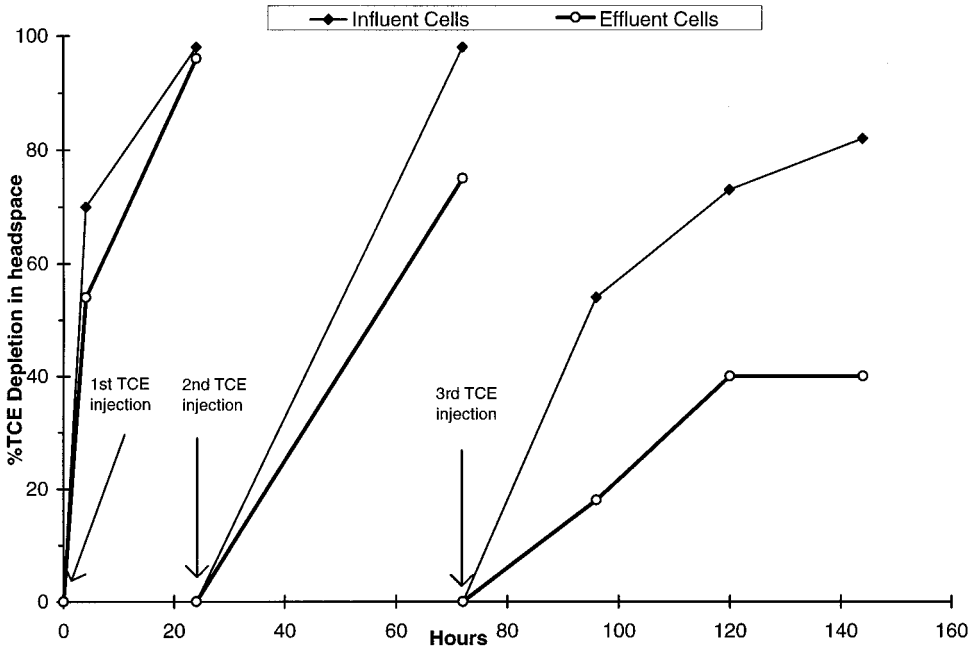

FIGURE 2. TCE-degrading activity of ENV 435 used in the foam/bioaugmentation treatment of a TCE-DNAPL-contaminated column. TCE bottle assays were conducted on column influent (♦) and effluent (○) samples to estimate the duration of activity of ENV 435 applied to TCE-DNAPL-contaminated sediments. Assays were conducted in the presence of 50 ppm TCE. Additional TCE was added (50 ppm) at 24 and 72 h as the degradation approached 100%.

ENV 435 Dosage Study. Columns were treated with an increased dose of ENV 435 to determine if TCE drinking water standards (<5 ppb) could be reached using this foam/biotreatment technology. A single high dose of ENV 435 (5×10^8 cfu/mL) resulted in a reduction of between 99.83 and 99.99% of TCE in 8 out of 10 core sections as compared to the abiotic foam control column (Table 8). The concentration in these eight core sections ranged from 7 to 28 ppb, which approaches the maximum level allowed for drinking water. In contrast, 700−9600 ppb of TCE was observed in the corresponding core sections when one-fifth as many cells (1×10^8 cfu/mL) were inoculated with the second foam injection. Only in core sections 1 and 2, representing the initial location of the TCE-DNAPL, was the TCE concentration similar for both the low and high ENV 435-dosed columns.

Discussion

The model aquifer system used to test the feasibility of a surfactant foam/biotreatment technology to remediate TCE-DNAPLs demonstrated that (a) TCE-DNAPLs could be effectively mobilized through a sand column using foam generated from Steol CS-330 surfactant; (b) bacterial delivery could be enhanced by Steol CS-330 foam; and (c) residual column TCE was reduced to a level that was compatible with biodegradation. Effective treatment was achieved in this model system with only 3 liquid pore volumes (total surfactant and AGW fraction) and a retention time of 1 h. Such a low treatment volume would be a significant improvement over current pump-and-treat methods that require many more pore volumes for TCE recovery.

The use of surfactant foams offers many advantages over surfactant solutions. Results from this study show that foams can be generated from low concentrations of surfactant solutions (0.1% and less), and their stability does not appear to be dependent upon inherent properties such as HLB or cmc. Two of the test surfactants, Tergitol 15-S-12 and Steol CS-330, that differed greatly in their cmc values (0.011% and 0.18%, respectively) formed equally stable foams at a 0.1% solutions (10 and 0.5 times their cmc, respectively). In contrast, the use of surfactant solutions for solubilization requires concentrations greater than the surfactants' cmc. Thus, high concentrations of surfactant solutions (1−10%) are often required to overcome dilution and binding effects in order to be effective (*4*, *22*, *23*). It has also been observed that high surfactant concentrations can result in plugging of

TABLE 8. Effect of Increased Bacterial Concentration on TCE Distribution in 8-in. Columns Containing 1.5 of mL of TCE Treated with Steol CS-330 (0.1%) Foam

	control surfactant solution no cells[a]	control surfactant solution + ENV 435 (1×10^8 cfu/mL)[a]	foam control no cells[b]	experimental columns[c]			
				foam/AGW/foam			
	surfactant soln/AGW/ surfactant soln	surfactant soln/AGW/ surfactant soln	foam/AGW/ foam	1×10^8 cfu/mL		5×10^8 cfu/mL	
column core sample (2-cm sections)	TCE (μg/g)	TCE (μg/g)	TCE (μg/g)	TCE (μg/g)	% degradation over mobilization	TCE (μg/g)	% degradation over mobilization
1	7210	4390	120	8.9	92.6	4.03	96.64
2	8160	830	401	2.8	99.3	6.70	98.33
3	5670	460	220	5.7	97.4	0.014	99.99
4	830	50	100	9.6	90.4	0.018	99.98
5	35	23	63	3.9	93.8	0.016	99.97
6	33	3	42	2.2	94.8	0.028	99.93
7	42	11	25	0.70	97.2	0.007	99.97
8	16	2.0	18	0.91	94.9	0.012	99.93
9	13	7.8	12	1.9	84.2	0.018	99.85
10 (top)	180	16	11	2.7	75.5	0.019	99.83

[a] Surfactant solution control column runs were operated as follows: 400 mL of surfactant solution then 200 mL of AGW and then either 600 mL of surfactant solution without cells or 600 mL of surfactant solution + 1×10^8 ENV 435 cells/mL. [b] Foam control column runs were operated as follows: 400 mL of surfactant foam then 200 mL of AGW and then 600 mL of surfactant foam without cells. [c] Experimental column runs were operated as follows: 400 mL of foam then 200 mL of AGW and then either 600 mL of foam + 1×10^8 ENV 435 cells/mL or 600 mL of foam + 5×10^8 ENV 435 cells/mL.

injection wells by the dispersion of fine materials or by the formation of viscous emulsions (*24*).

Surfactant foams, similar to Winsor Type III microemulsions, reduce the IFT between DNAPLs and the liquid phase (*18*). Under conditions of low IFT, capillary forces that trap DNAPLs in pores are reduced, and therefore a lower hydraulic gradient is required to mobilize DNAPLs. The difficulty in using Winsor Type III systems is that the formation of a Type III microemulsion follows a complex phase behavior and is very dependent upon the brine (salt) concentration (*3*, *6*, *25*). Therefore, maintenance of the transitional phase between a hydrophilic and hydrophobic system (phase in which microemulsions form) can be difficult when used in a pump-and-treat system. Foam formation, on the other hand, appears to be stable over a wide range of conditions, and preliminary results using the Steol CS-330 foam indicates that it remains stable with up to 1.0% $CaCl_2$ without precipitation of the surfactant (unpublished results). Furthermore, the high pressure associated with flowing foams (high flow resistance) improves the mobilization of DNAPLs (*26*, *27*). Results from this study showed that the upward hydraulic pressure gradient generated from the flow of foam (3−10 psi) was sufficient to overcome the downward migration of the DNAPL observed with the surfactant solution flushing. A TCE-DNAPL was observed in the aqueous drain from columns treated with surfactant solution but not with surfactant foam (Table 7). This implies that foams could overcome the danger of pushing DNAPLs deeper into fracture formations, which hampers DNAPL recovery (*28*, *29*). Without a sufficient upward hydraulic pressure, Chen (*30*) observed that the downward migration of tetrachloroethylene (PCE) microemulsions was faster than its recovery.

Other studies have shown that effluent concentrations of contaminants under surfactant flushing conditions can be significantly less than equilibrium values due to rate-limited mass transfer, aqueous bypassing, and aquifer heterogeneities (*7*, *4*). These effects may be reduced by the use of surfactant foams since they uniformly penetrate the contaminated media providing better contact with the DNAPL. Surfactant foams were 10 times more efficient than surfactant solutions in removing hydrophobic NAPL-like contaminants from porous media (even in the presence of clay lenses), and the residual NAPLs were more evenly dispersed following the foam flushes (*13*−*15*). In this study, TCE-DNAPLs were mobilized only when the sand column was treated with surfactant foam, leaving behind residual TCE that was evenly dispersed through more than half the sand column. The most effective treatment for mobilizing the TCE-DNAPL was a pulsed injection of foam and AGW (Table 4). The kinetics of this mobilization have not yet been investigated; however, it is hypothesized that the water pulse acts to temporarily increase the mobility of the foam and helps push the foam/TCE complex through the sand column. It will be necessary to determine the TCE flow through the column under foam and water injections in order to understand this phenomenon. It should also be noted that an injection rate of 5 mL/min (through the 20-cm columns) correlates to a flow rate of 4.8 m/day, which is a very reasonable velocity for pump-and-treat systems.

Results presented in this paper indicate that surfactant foams greatly enhance the mobilization of DNAPLs; however, a residual level of the contaminant will likely remain within environmental matrixes, even after exhaustive flushing, due to mass transfer limitations. This study has shown that this residual level can be effectively treated with degradative microorganisms. Even at fairly high levels of residual TCE (200−500 μg/g), ENV 435 was effective in degrading 95% or more of the residual TCE. It was observed that the residual TCE concentration could be reduced with an increased volume of foam flushes as shown by comparing results presented in Tables 3 and 4. The residual TCE concentration was significantly reduced when the foam throughput was twice as high (Table 3). Reducing the residual concentration of TCE before biological treatment is critical since the degradation of TCE itself is toxic to ENV 435 because of the intermediate formation of epoxides. The formation of these toxic intermediates accounts for the low survival of organisms observed within the sand columns (Table 5). However, the organisms that did survive and were collected in the effluent maintained a high competence for degrading TCE as shown by bottle assays. Although drinking water standards (<5 ppb) were not met under the treatment conditions used in this study, this technology has the potential to reach this cleanup standard as shown by the dramatically reduced TCE column concentration when treated with a high dose of ENV 435 (Table 8). Surfactant solutions have been shown to increase the bioavailability of hydrophobic compounds (*31*, *32*), however, the use of surfactant foams offers additional

advantages. This study showed that Steol CS-330 foam improved the transport of ENV 435 through the sand column (Table 3), and because of the low concentration needed for generating foams, it was biocompatible with ENV 435. In addition, it has been shown that foams enhance the oxygen mass transfer by delivering oxygen in a gaseous form, which is much more efficient than the use of oxygenated solutions (*9*−*11*).

To date, surfactant foams have been applied for enhancing the production of crude oil in tertiary oil recovery applications, but the use of foams for remediation of sites contaminated with nonaqueous-phase liquids (NAPLs) is in the early stages of development. Foams or colloidal gas aphrons (CGAs) have been applied in a pilot study at the Petro-Processors Superfund Site to treat petroleum and chemical plant wastes. CGAs were found to be superior to other flushing media, and three times more contaminant was displaced in a field demonstration (*33*).

Although this study was conducted under idealized conditions (clean, homogeneous sand), it does show the potential for the use of foams for mobilizing free-phase organics. Combining this technology with bioaugmentation to degrade the residual organics should lead to a comprehensive in situ remediation treatment for contaminated sites. Work is currently underway using heterogeneous soil and aquifer samples to determine the effect of organics, clays, and porosity on DNAPL mobilization by foams. In addition "weathered" soils are being used as source material to evaluate this technology with sorbed TCE. It will also be important to determine if foams are effective in fractured porous media, since DNAPL remediation is often difficult in such areas due to the complex fracture structure that impedes the flow of groundwater and the existence of the contaminant diffused within the matrix itself (*1*). Continued studies will involve further development and testing of the foam/biotreatment system as well as developing a predictive mathematical model for use in field remediation efforts.

Acknowledgments

This work was supported by a National Science Foundation Phase I SBIR Award 9561788.

Literature Cited

(1) Pankow, J. F.; Cherry, J. A. *Dense Chlorinated Solvents*; Waterloo Press: Portland, OR, 1996.
(2) MacKay, D. M.; Cherry, J. A. *Environ. Sci. Technol.* **1989**, *23*, 630−634.
(3) West, C. C.; Harwell, J. H. *Environ. Sci. Technol.* **1992**, *26* (12), 2324−2330.
(4) Pennell, K. D.; Abriola, L. A.; Weber, W. J. *Environ. Sci. Technol.* **1993**, *27* (12), 2332−2340.
(5) Shiau, B. J.; Sabatini, D. A.; Harwell, J. H. *Ground Water*, **1994**, *32* (4), 561−569.
(6) Abriola, L. M.; Dekker: T. J.; Pennell, K. D. *Environ. Sci. Technol.* **1993**, *27* (12), 2341−2351.
(7) McCray, J. E.; Falta, R. W. *Ground Water*, **1997**, *35* (1), 99−110.
(8) Fry, V. A.; Istok, J. D.; O'Reilly, K. T. *Ground Water*, **1996**, *34* (2), 200−210.
(9) Michelsen, D. L.; Wallis, D. A.; Sebba, F. *Environ. Prog.* **1984**, *3* (2), 103−107.
(10) Michelsen, D. L.; Wallis, D. A.; Sebba, F. *Proceedings of the Fifth National Conference on Management of Uncontrolled Hazardous Waste Sites*, Washington, DC; 1984; pp 398−403.
(11) Michelsen, D. L.; Wallis, D. A.; Lavinder, S. R. *Proceedings of the Sixth National Conference on Management of Uncontrolled Hazardous Waste Sites*, Washington, DC; 1985; pp 291−299.
(12) Jenkins, K. B.; Michelsen, D. L.; Novak, J. T. *Biotechnol. Prog.* **1993**, *9*, 394−400.
(13) Enzien, M. V.; Bouillard, J. X.; Michelsen, D. L.; Peters, R. W.; Frank, J. R.; Botto, R. E.; Cody, G. DOE Technical Report, TTP NO. CH-2-4-10-93. Argonne National Laboratory: Argonne, IL, 1994.
(14) Enzien, M. V.; Michelsen, D. L.; Wang, K. C.; Carney, A. F.; Peters, R. W.; Bouillard, J. X.; Frank, J. R. Presented at the 7th International Symposium on Gas, Oil, and Environmental Biotechnology, Colorado Springs, CO, December 1994.
(15) Peters, R. W.; Enzien, M. V.; Bouillard, J. X.; Frank, J. R.; Srivastava, V. J.; Kilbane, J.; Hayes, T. *Proceedings of the 33rd Hanford Symposium on Health and the Environment on In Situ Remediation: Scientific Basis for Current and Future Technologies*, Pasco, WA, November 1994; pp 1067−1087.
(16) Wan, J. M.; Wilson, J. L.; Kieft, T. L. *Appl. Environ. Microbiol.* **1994**, *60* (2), 509−516.
(17) *Genet. Eng. News*, June 1993, p 18.
(18) Jackson, A.; Roy, D.; Breitenbeck, G. *Water Res.*, **1994**, *28* (4), 943−949.
(19) Munakata-Marr, J.; McCarty, P. L.; Shields, M. S.; Reagin, M.; Francesconi, S. C. *Environ. Sci. Technol.* **1996**, *30*, 2045−2052.
(20) DeFlaun, M. F.; Oppenheimer, S. R.; Streger, S.; Condee, C. W.; Fletcher, M. Alterations in Adhesion, Transport, and Membrane Polymers in an Adhesion-Deficient Pseudomonad. In review.
(21) Oppenheimer, S. R.; Condee, C. W.; Fletcher, M.; DeFlaun, M. F. *Abstracts for the 96th American Society for Microbiology General Meeting*, New Orleans, LA, 1996; Q115.
(22) Oolman, T.; Godard, S. T.; Pope, G. A.; Jin, M.; Kirchner, K. *Ground Water Monit. Rem.* **1995**, 125−137.
(23) Fountain, J. C.; Starr, R. C.; Middleton, T.; Beikirch, M.; Taylor, C.; Hodge, D. *Ground Water* **1996**, *34* (5), 910−916.
(24) Nash, J. H. *Field Studies of In Situ Washington*; U.S. Environmental Protection Agency: Cincinnati, OH, 1987; EPA/600/2-87/110.
(25) Baran, J. R.; Pope, G. A.; Wade, W. H.; Weerasooriya, V.; Yapa, A. *Environ. Sci. Technol.* **1994**, *28* (7), 1361−1366.
(26) Kovscek, A. R.; Patzek, T. W.; Radke, C. *J. Soc. Pet. Eng.* **1993**, *26042*, 309−322.
(27) Kovscek, A. R.; Radke, C. J. *Foams: Fundamental Applications in the Petroleum Industry*; American Chemical Society: Washington, DC, 1993; Chapter 3.
(28) Fountain, J. C.; Waddell-Sheets, C.; Lagowski, A.; Taylor, C.; Frazier, D.; Byrne, M. *Surfactant-enhanced Subsurface Remediation: Emerging Technologies*; ACS Symposium Series 594; American Chemical Society: Washington, DC, 1995; pp 117−190.
(29) Chown, J. C.; Kueper, B. H.; McWhorter, D. B. *Ground Water* **1997**, *35* (3), 483−491.
(30) Chen, Y. Ph.D. Dissertation, University of Oklahoma, Norman, OK, 1995.
(31) Guha, S.; Jaffe, P. R. *Environ. Sci. Technol.* **1996**, *30* (4), 1382−1391.
(32) Brown, D. G.; Guha, S.; Jaffe, P. R. *Papers from Fourth International In Situ and On-Site Bioremediation Symposium: Volume 2*, New Orleans LA, May 1997; pp 581−586.
(33) Constant, W. D.; Chaphalkar, P. G.; Valsaraj, K. T.; Roy, D.; Robertson, E. Presented at the 1996 Spring National AIChE Meeting, New Orleans, LA, February 1996.

Received for review November 6, 1997. Revised manuscript received March 9, 1998. Accepted March 17, 1998.

ES970980W