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A Field Demonstration of Substrate Distribution for Accelerated Anaerobic Biodegradation at Dover AFB, Delaware

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ABSTRACT: In Spring 2006, Oak Ridge National Laboratory (ORNL) and URS Group, Inc. (URS) implemented accelerated anaerobic biodegradation (AAB) to remediate several large chlorinated solvent groundwater plumes at Dover Air Force Base (DAFB) in Delaware. Organic carbon substrate is injected along transects perpendicular to the flow of groundwater. Groundwater is extracted from alternating wells, amended with organic substrates (emulsified vegetable oil [EVO] and sodium lactate) and reinjected to create a push-pull effect. Distribution of vegetable oil (a longer lasting carbon source) across the plumes enhances the long-term performance of the AAB strategy. Therefore, understanding and demonstrating the distribution of vegetable oil during the injection process is important.

Total organic carbon (TOC) concentrations measured by HACH methods have been lower than calculated TOC concentrations in samples of the substrate injection solutions and in prepared standards, suggesting that the TOC analysis used does not measure a significant portion of the TOC contributed by the vegetable oil.

A field demonstration was conducted in October 2007 to assess the distribution of EVO during an injection event and evaluate alternative methods for detecting vegetable oil. The demonstration used Terra Systems' Slow Release Substrate (SRS®) EVO product. Several alternative methods for detecting vegetable oil were tested. These methods were in various stages of development during the demonstration and included: (1) Oil Red O dye for water samples, (2) fatty acid methyl ester (FAME) analysis specific to derivatives of vegetable oil in groundwater, (3) visual observation of vegetable oil droplets in groundwater samples under a microscope, (4) HACH method for volatile acids (VA), and (5) hexane extraction of vegetable oil from aquifer soil samples.

EVO can be visually observed in groundwater at concentrations as low as 50 to 100 mg/L. The Oil Red O dye with the use of a microscope can be used to quickly identify vegetable oil droplets in water samples. VA detects lactate and other components of EVO. FAME analyses can also be used to detect vegetable oil but is more time consuming and requires extraction, derivitization, and analysis on a gas chromatograph (GC). The first four methods listed above require further development. The hexane extraction method was useful for documenting oil distribution in soil samples; however, it was not considered for use as a real-time tool to be used during an injection event. The field demonstration evaluation showed that TOC and VA were detected in the groundwater but the emulsion was not observed either visually or with the Oil Red O dye test. The EVO was likely adsorbed to the soil. The hexane extraction method detected EVO in soil samples

from throughout the pilot area each as far as 22 ft (6.7 m) from the injection point and up to 5 ft (1.5 m) upgradient.

INTRODUCTION

Past maintenance activities resulted in chlorinated solvent releases that created large dissolved-phase plumes within the shallow water table aquifer at DAFB. In the spring of 2006, ORNL and URS implemented accelerated AAB to remediate several of the plumes (Bloom et al., 2007a and b). Organic carbon substrate is delivered to the subsurface using rows of permanent wells installed along transects perpendicular to the flow of groundwater. The wells are spaced approximately 50 ft (15.2 m) apart. Groundwater is injected and extracted from alternating wells to create a push-pull effect to distribute substrate laterally across the width of the plumes. Organic substrates (EVO and sodium lactate) are added to the groundwater before it is injected. TOC concentrations in groundwater are used to demonstrate the distribution of substrates, per standard industry practice (Konzuk et al., 2005).

To optimize the injection process and the distribution of substrates across the transects, ORNL, URS, ES&H, and Terra Systems, Inc. conducted a field demonstration in October 2007. The TOC analysis used to determine the distribution of EVO did not differentiate between the sources of injected organic carbon (namely sodium lactate, vegetable oil, and the emulsifiers used in the EVO). Additionally, it does not appear to measure a significant portion of the TOC contributed by the vegetable oil. Improved methods for detecting vegetable oil in soil and groundwater samples were needed to produce conclusive results for the demonstration.

Therefore, during the field demonstration, several methods currently under development to detect vegetable oil in soil and groundwater samples were evaluated. Ultimately, the methods may be used to provide near real-time assessment and confirmation of EVO distribution during an injection event.

A test EVO injection was carried out at the Area 6 plume injection/circulation transect (PICT) 9 at DAFB. Groundwater was extracted from two wells, amended with EVO, and re-injected into three other wells. The wells are screened between 20 and 40 ft (6.0 and 12.2 m) below ground surface (bgs). The test focused on injection well AB3133 and extraction well AB3134 (Figure 1). The average flow rate into the injection well was 2.9 gpm (11 L/min). Four pairs of monitoring wells are installed between the injection and extraction wells with screens between 23 and 26 ft (7.0 and 7.9 m) and deep screens between 35 and 38 ft (10.7 and 11.6 m) bgs. Approximately 1 month after EVO injection ended, soil samples were collected from five locations using a direct-push rig. The soil sampling locations (GP 1 through GP 5) are shown in Figure 1.

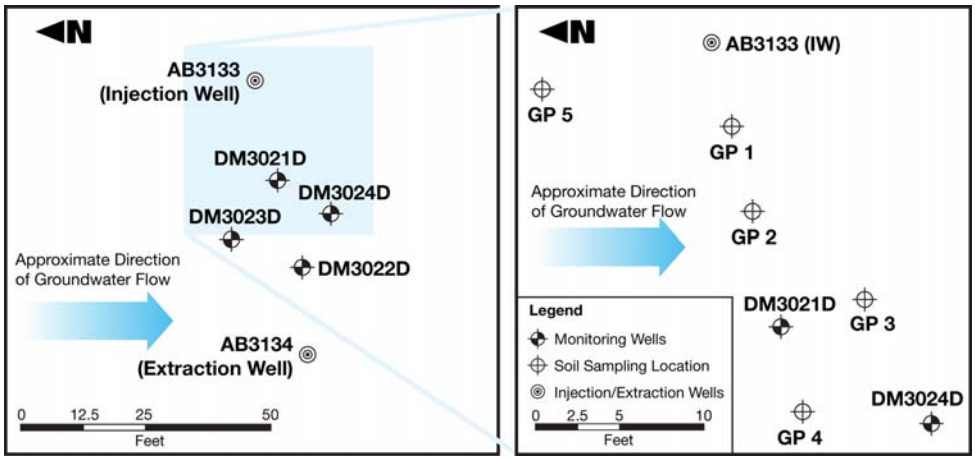

FIGURE 1. Site map.

VEGETABLE OIL DETECTION METHODS

Several methods have been used or are being developed to detect vegetable oil during injection events at DAFB.

TOC Analysis. Groundwater samples are analyzed for TOC using HACH low-to-high-range TOC test kits with a HACH DR5000 Spectrophotometer measured at 598 and 430 nanometers. TOC is determined by acidifying a groundwater sample, sparging to remove the inorganic carbon, and then placing an aliquot of the sample in a prepared TOC acid digestion vial containing persulfate, acid, and a pH indicator reagent tube. The sample is then heated and reacts with the persulfate and acid, producing carbon dioxide. The carbon dioxide diffuses into the pH indicator reagent tube and forms carbonic acid, which reacts with the indicator reagent and changes the color of the reagent tube to represent the amount of organic carbon present in the sample.

Volatile Acids (VA). VA are analyzed using a HACH method that converts fatty acids to acetic acid for analysis with a HACH spectrophotometer. The procedure involves esterification of the VA in the sample with ethylene glycol, sulfuric acid, and heat, followed by addition of hydroxyalamine hydrochloride, sodium hydroxide, and ferric chloride sulfuric acid. Results are reported as the acetic acid equivalents.

Oil Red O Dye. This method is currently being developed for the DAFB projects. Oil Red O dye is a hydrophobic fat stain that is insoluble in water. Field soil or groundwater samples are collected in glass sampling containers. Oil Red O dye and distilled water are added to the soil samples and Oil Red O dye only is added to groundwater samples. Both sample types are then shaken vigorously to bring the oil into contact with the dye. The presence of vegetable oil in a sample produces a color ranging from deep red to pale pink, depending on the concentration of vegetable oil in the sample.

Fatty Acid Methyl Esters (FAME). This method is also currently being developed for the DAFB projects. Soil or groundwater samples are collected in glass sampling containers. Groundwater samples are filtered using membrane filter of a specific micron size,

depending on the vegetable oil droplet size. The filter is removed and placed in a small sampling vial equipped with a screw-on lid. Methanolic 3N hydrochloric acid is added to the vial to break down the vegetable oil triglycerides to methyl esters. The vial is capped, placed in a digester, and heated at 70 degrees Celsius for 10 minutes. After digestion, the sample is cooled to room temperature. Hexane and water are added and the sample is shaken vigorously to allow the methyl esters to partition into the upper hexane layer. An aliquot of the hexane layer is collected and manually injected into a GC equipped with a flame ionization detector to detect the methyl esters. Soil samples are treated in the same manner, except that vegetable oil is first extracted from the soil with hexane. The upper layer of the extraction solution is then passed through the membrane filter, removing any solid particulates.

Visual Observation with Microscope. A fraction of the liquid from a groundwater sample or from a hexane extracted soil sample is placed on a microscope slide. If present, vegetable oil droplets are observed with a light microscope. Adding Oil Red O dye to the sample enhances the visibility of the oil droplets. Vegetable oil droplets appear glassy with a bright red color.

Hexane Extraction. The hexane-extractable oil is determined by transferring 10 g of the soil to a beaker, adding 5 g anhydrous sodium sulfate, and mixing to dry the soil. Then two 10 mL aliquots of hexane are added to the soil. The hexane is transferred to an aluminum foil weigh boat and the hexane allowed to evaporate in a fume hood. The resulting weight of extracted oil is then recorded.

RESULTS

The results of the various methods for detecting vegetable oil in samples collected during injection events at DAFB are discussed below. Due to the developmental status of the Oil Red O dye, the FAME analysis, and the visual observation with the microscope methods, limited data are available.

TOC Analysis. TABLE 1 compares measured TOC and VA concentrations of EVO and sodium lactate solutions to the calculated, or expected, TOC concentrations. Table 1 includes TOC results for four samples of injection solution collected during injection events at DAFB. The calculated TOC concentration represents the mass of carbon per liter of injection solution based on the dilution ratio of the injection solution. The measured TOC concentration for the injection solutions was 53 to 68 percent of the calculated TOC concentration. Standard solutions of vegetable oil, emulsifiers found in EVO, a combination of the two solutions, and sodium lactate were also prepared and analyzed for TOC and VA. The measured TOC concentration for the oil-only solution was 1 percent of the calculated TOC concentration due to the oil's limited solubility. In contrast, the emulsifier solution TOC result was greater than 100 percent of the calculated TOC concentration. For the mixed oil-emulsifier solution, the measured TOC was 55 percent of the calculated TOC, which is consistent with the dilution ratio for the solution. The two injection solutions with measured VA had an average of 938 mg/L of VA as acetic acid, which includes both the lactate and a portion of the fatty acids found in the vegetable oil.

TABLE 1. Measured and calculated TOC and VA concentrations in injection solutions and prepared standard solutions.

SAMPLE IDENTIFICATION	Calculated TOC (mg/L)	Measured TOC (mg/L)	Percent of Calculated TOC	VA as Acetic Acid (mg/L)
Injection Solution Sample 1	3239	2195	68	950
Injection Solution Sample 2	3239	1904	59	925
Injection Solution Sample 3	3239	1799	56	NS
Injection Solution Sample 4	3239	1731	53	NS
1 g/L SRS	507	389	77	11
1 g/L 60% Sodium Lactate	193	125	65	149
1 g/L Vegetable Oil Solution	767	5.4	1	NS
1 g/L Emulsifier Solution	617	786	130	NS
1 part 1 g/L Oil to 1 part 1 g/L Emulsifier Solution	692	378	55	NS

Notes:
Measured TOC = HACH method
Calculated TOC based on molecular weight of carbon and mass of organic carbon per volume of water
NS = not sampled

Table 2 presents the data for TOC and VA for the injection test at Area 6 PICT 9. The groundwater contained low concentrations of TOC (<1 mg/L) and VA (<6 mg/L) before EVO injection. TOC concentrations were first detected in both the shallow and deep screen of well DM3021 after 3 days of continuous injection. TOC and VA concentrations continued to increase in this well, located approximately 18 ft (5.5 m) from injection well AB3133 throughout most of the test. The VA concentrations were detected in both the shallow and deep screens of well DM3024 on day 5, with TOC detected first in the shallow screened interval and not until day 8 in the deep screened interval. Well DM3024 is approximately 25 ft from the injection well. Only low levels of VA were found in well DM3023, also approximately 25 ft from the injection well. There was no evidence of the emulsion in any of the wells based upon visual observations or tests with the Oil Red O dye. Based on the field measurements of specific conductivity, there was no evidence of the emulsion reaching well DM3022 or the extraction well AB3134, therefore samples for TOC and VA analysis were not collected from these wells.

Oil Red O Dye for Groundwater. FIGURE 2A shows the emulsion diluted 910 fold with and without the Oil Red O dye. The EVO solution is white and the dyed sample is red. A digital microscope picture of the dyed EVO is included as Figure 2B.

FAME. This procedure is under development and no results are available at this time.

Visual Observation with Microscope. Oil droplets have been easily observed in water and soil samples with a microscope under optic magnifications ranging from 400 to 1000 times. This method may be limited for oil droplets less than 1 micron, although addition of Oil Red O dye enhances the observation of smaller droplets (Figure 2b).

TABLE 2. TOC and VA in groundwater samples (mg/L).

Well	DM3021DS		DM3021DD		DM3023DS		DM3023DD		DM3024DS		DM3024DD	
Day	TOC	VA	TOC	VA	TOC	VA	TOC	VA	TOC	VA	TOC	VA
-4	0.6	ND	0.6	ND	ND	ND	ND	ND	ND	ND	ND	6
-2	NS	NS	NS	NS	ND	ND	ND	ND	NS	NS	NS	NS
-1	ND	5	ND	2	NS	NS	NS	NS	ND	6	ND	2
0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3	14.6	2	1.2	ND	NS	NS	NS	NS	NS	NS	NS	NS
4	29	41	17	32	NS	NS	NS	NS	NS	NS	NS	NS
5	52	75	29	49	ND	11	ND	12	ND	10	ND	14
6	70	133	54	113	ND	7	ND	10	1.6	16	ND	5
7	87	146	69	120	ND	2	ND	ND	8.2	9	ND	ND
8	100	194	86	125	ND	2	ND	9	23	36	23	26
9	101	179	64	101	NS	NS	NS	NS	34	56	NS	NS
10	109	156	78	116	ND	ND	ND	ND	40	63	128	216
11	108	176	94	155	NS	NS	NS	NS	54	112	92	218
13	123	347	124	306	NS	NS	NS	NS	85	261	74	213
17	115	222	149	229	ND	4	ND	5	103	178	31	52
19	142	236	172	254	NS	NS	NS	NS	109	193	NS	NS
21	181	359	210	345	NS	NS	NS	NS	122	221	NS	NS
24	218	340	203	304	NS	NS	NS	NS	133	207	NS	NS
31	105	214	NS	NS	NS	NS	NS	NS	143	269	NS	NS

Notes:
ND = not detected
NS = not sampled

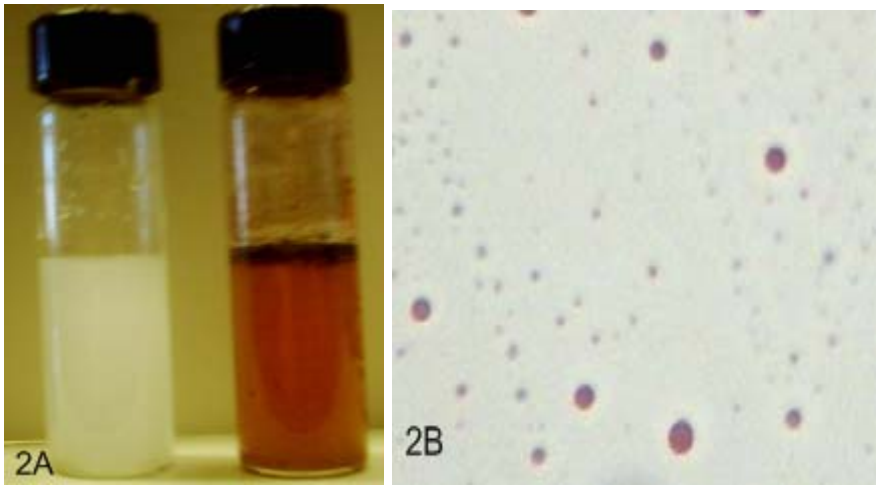

FIGURE 2. (a) Diluted EVO and diluted EVO with Oil Red O dye. (b) Digital photograph of EVO dyed with Oil Red O dye (1,000 times magnification).

Hexane Extraction. As part of the field demonstration, soil samples were collected at distances of 5, 10, 17, and 22 feet (1.5, 3.1, 5.2, and 6.7 m) from the injection well (FIGURE 1). Samples were collected at multiple vertical intervals at each location. Each sample was analyzed with Oil Red O dye as a field screening method and then analyzed using the hexane extraction method to determine whether vegetable oil was present in the soil sample. Results of both methods are presented in Table 3.

TABLE 3. Oil concentrations in soil by hexane extraction and results of Oil Red O dye screening.

Sample Number and Depth (ft)	Distance from Injection Well (ft)	Oil Concentration (mg/kg wet weight)	Oil Red O Dye Test
GP 1 20-24	5	28550	++
GP 1 28-32	5	1050	+
GP 1 36-40	5	940	-
GP 2 16-20	10	2630	+
GP 2 20-24	10	1860	+
GP 2 24-28	10	1180	+
GP 2 28-32	10	690	+
GP 2 32-36	10	3860	-
GP 2 36-40	10	1020	+
GP 3 20-24	17	1700	+
GP 3 28-32	17	950	+
GP 3 36-40	17	3430	+
GP 4 20-24	22	940	+
GP 4 28-32	22	2030	+
GP 4 36-40	22	970	NA
GP 5 20-24	5	30	+
GP 5 28-32	5	390	NA
GP 5 36-40	5	100	NA

Notes:
mg/kg = milligrams per kilogram
++ = strong positive dye response
+ = moderate positive dye response.
- = no dye response.
NA = not available, test not conducted.

The highest concentration of hexane-extractable oil (28,550 mg/kg) was detected in GP 1 at 20 to 24 ft (6.1 to 7.3 m) bgs. This soil sampling point was approximately 5 ft (1.5 m) away from the injection well and also had the strongest Oil Red O dye response. Lower concentrations of oil were found at the two deeper intervals for well GP 1, but the Oil Red O dye response for the deepest point was negative for vegetable oil. Hexane-extractable oil was found in all samples from GP 2 (located 10 ft or 3.0 m from the injection well), GP 3 (17 ft or 5.2 m from the injection well), GP 4 (22 ft or 6.7 m from the injection well), and GP 5 (about 5 ft or 1.5 m upgradient from the injection well). The Oil Red O signal dye responses generally corresponded with the hexane-extractable oil concentration.

OBSERVATIONS AND CONCLUSIONS

The following observations were made regarding the distribution of EVO.

- TOC results provided by the HACH method do not demonstrate clearly whether EVO has been distributed to a sampling location, since the sources of the TOC are not differentiated. The method does not appear to measure a significant portion of the TOC provided by the vegetable oil, due to interference between the method chemistry and the vegetable oil or some characteristic of the oil in water, such as limited solubility. Whether this is true for commercial laboratory TOC analysis has not been determined, but similar results are expected.
- The Oil Red O dye method is very promising as a field test for vegetable oil. Because the emulsifiers in the EVO can cause a similar color change as the oil, those using the dye method must be familiar with the differences in color

changes. Confusion is easily avoided by observing distinct oil droplets in a dyed sample under a microscope. Further work to determine an ideal sample collection technique is planned.

- Initial work with the FAME analysis is promising for the detection of vegetable oil in samples. One potential limitation, however, is that the method does not distinguish between FAMEs from oil and those from the surfactants. More work is required to develop the GC method and differentiate the two sources. Also, a column study may be conducted to determine whether there is a significant difference in vegetable oil and emulsifier transport during injection. If the two components transport through aquifer materials at similar rates, the need to distinguish FAMEs specific to the vegetable oil from FAMEs specific to the surfactants is less relevant.
- Visual observation of oil droplets with a microscope is another very promising method. Oil droplets can be conclusively identified, and the use of Oil Red O dye enhances the visibility of the droplets. This method can be used in the field.
- The hexane extraction method is a reliable confirmation for the distribution of vegetable oil after an injection. However, this method does not distinguish between vegetable oil and emulsifiers, so a low TOC result does not necessarily demonstrate the presence of vegetable oil. This method also requires the collection of soil samples, which makes it impractical as a real-time assessment tool. It may be more appropriate for smaller injection projects or pilot tests.

During the field demonstration, measured TOC concentrations, visual observations, and specific conductivity measurements of groundwater samples collected from the monitoring wells and extraction well did not provide evidence for distribution of the emulsion. The oil is believed to have adsorbed to the soil. The rate at which this adsorption occurs is not known. The use of Oil O Red dye for water samples and the microscope examination procedure were not developed at the time of this demonstration. Development of these methods is ongoing. The hexane extraction method showed the presence of oil in all of the soil samples up to 22 ft (6.7 m) from the injection well. The Oil Red O dye was a useful screening test for the oil.

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