

Enhanced Anaerobic Bioremediation of Chlorinated Solvents Utilizing Vegetable Oil Emulsions

William A. Newman

Ronald C. Pelle

The use of vegetable oil as an electron donor to enhance the reductive dechlorination of chlorinated solvents as an in situ remediation technology is gaining significant traction. Vegetable oil is a cost-effective slow-release electron donor with greater hydrogen-release efficiency than other electron-donor products. However, neat vegetable oil can inhibit distribution in aquifers due to the oil droplets blocking the flow of groundwater through the smaller pore spaces in the aquifer materials. This issue has been partially overcome by applying the vegetable oil as an oil-water emulsion, which typically is created in the field. However, the field preparation results in a mixture of droplet sizes, including larger droplets that can make the emulsions unstable and reduce the soil permeability by blocking soil-pore throats with oil. RNAS, Inc., has developed a kinetically stable soybean oil emulsion ("Newman Zone") consisting of submicron droplets with less droplet-size variation than field-prepared emulsions. This product is composed of a blend of fast-release (sodium lactate) and slow-release (soybean oil) electron donors. The emulsion is produced in a stable factory environment in which it is pasteurized and packaged in sterile packaging. This material can be utilized as an electron donor without further treatments or amendments in the field. This article discusses factors associated with selecting electron donors and the development of vegetable oil-based products. A case study of an application of Newman Zone at a former adhesives manufacturing facility is then presented. The case study demonstrates the effect of Newman Zone in reducing chlorinated solvent concentrations in groundwater by both rapidly stimulating initial microbial activity and supporting long-term reductive dechlorination with a slow-release electron donor. © 2006 Wiley Periodicals, Inc.

INTRODUCTION

Natural attenuation of chlorinated solvents by reductive dechlorination often occurs at sites where an electron donor (food source or substrate for microbes) is present in groundwater contaminated with chlorinated solvents. At sites where such a donor is not present, addition of electron-donor compounds has proven effective in stimulating reductive dechlorination of chlorinated solvents (US EPA, 2000). A variety of organic matter has successfully been used to enhance reductive dechlorination, including sugars, alcohols, wood mulch, chitin, dairy whey, vegetable oil, and sodium lactate (Air Force Center for Environmental Excellence [AFCEE], 2004). Anaerobic bioremediation of chlorinated solvents using electron-donor addition can be implemented through direct injection into source-area soils, with groundwater recirculation systems, or by installing bands of electron-donor injection points across a dissolved-phase plume to create a reactive treatment barrier.

Although almost any organic material can be used to stimulate reductive dechlorination, several factors determine whether the substrate can be applied in a cost-effective manner. These factors include electron-donor cost, hydrogen-release efficiency, longevity of electron-donor activity, ease of application, and electron-donor distribution in the subsurface. Both the electron donor's intrinsic properties and site-specific conditions, including hydrogeology, soil or bedrock matrix, and geochemistry, need to be carefully considered to achieve effective treatment.

Selection of a Cost-Effective Electron Donor

The primary measure of electron-donor activity is the mass equivalent of molecular hydrogen that is released from the anaerobic biodegradation of the electron donor.

The cost of electron-donor material is a significant factor in the design of enhanced anaerobic bioremediation systems for reductive dechlorination. The primary measure of electron-donor activity is the mass equivalent of molecular hydrogen that is released from the anaerobic biodegradation of the electron donor. A low cost of material per pound may not be the most cost-effective electron-donor choice if it has poor hydrogen-release efficiency. As an example, sodium lactate costs about \$1.00/pound of pure compound, while a factory-processed, oil-in-water vegetable oil emulsion (Newman Zone) contains 46 percent vegetable oil by weight and costs about \$1.25/pound. In terms of the lowest cost per pound of ingredient, the sodium lactate is less than half the cost of the emulsion. However, vegetable oil is a more efficient source of hydrogen with 0.115 pounds of hydrogen produced per pound of oil as compared to only 0.036 pounds of hydrogen per pound of sodium lactate (AFCEE, 2004). Using the above costs and hydrogen-release efficiency estimates, the vegetable oil emulsion costs approximately \$24/pound of hydrogen, while the sodium lactate costs \$28/pound.

The estimate of 0.115 pounds of hydrogen per pound of vegetable oil is based on the primary fermentation of Linoleic Acid to acetic acid and molecular hydrogen (16 moles per mole) without acetic acid utilization. If it is assumed that the acetic acid is utilized for hydrogen production as well, then 50 moles of hydrogen are produced per mole of Linoleic Acid. This increases the estimated hydrogen production to 0.36 pounds of hydrogen per pound of vegetable oil. With this more generous number, the cost comparison widens further, with the emulsion providing hydrogen at \$7.68/pound as compared to \$28/pound for the hydrogen produced by sodium lactate.

Costs associated with effectively delivering electron donors into the subsurface need to be considered, as well as the electron donor's price per pound of hydrogen produced. Inexpensive soluble electron-donor materials, such as ethanol, sodium lactate, or sugars, are rapidly consumed by microbes and normally persist for only 7 to 60 days (AFCEE, 2004), requiring multiple injections per year to sustain reductive dechlorination activity. Although initial injections of soluble donors are easily completed, multiple direct-well injections or injection wells associated with recirculation systems often encounter problems with biofouling of well screens and reduction in soil permeability. Reduced injection rates and the subsequent need to clean fouled injection wells can add to the project costs.

Slow-release electron donors have the potential to greatly reduce the frequency of electron-donor injection and reduce project operation and maintenance costs. A variety of slow-release electron donors have been effectively used to provide hydrogen-release activity for a year or longer from a single injection, including polylactic acids, chitin, vegetable oil, and cellulose. Most slow-release electron donors are not water-dispersible and must use small-volume injections of granular solid material or viscous liquids into a

large number of closely spaced injection locations to distribute the electron donor effectively. Direct injection of small volumes of slow-release compounds, such as pure vegetable oil, polylactic acid formulations, or chitin, partially rely on groundwater flow to distribute the slow-release soluble electron donors between the injection locations. At sites with limited advective groundwater flow, this can result in poor distribution of electron donors between injection points.

Vegetable Oil as a Slow-Release Electron Donor

Recent work with vegetable oil emulsions suggests that small-droplet, oil-in-water emulsions can be used to produce a water-dispersible, easily injected slow-release electron donor. The first injections of vegetable oil used neat oil, but this hydrophobic liquid is more viscous than water and has a strong tendency to block soil-pore throats. Blocking soil-pore throats greatly diminishes the permeability of the aquifer. This reduced permeability will inhibit groundwater flow through the treated aquifer materials and, in barrier designs, result in contaminated groundwater flow around the barrier rather than through it. In the case of source-area treatment, blocking soil pores results in poor distribution of the electron donor because of the diminished surface contact between the oil and water and the lack of any distribution from advective groundwater flow through the treatment area. Furthermore, reducing the permeability by blocking soil-pore throats can inhibit the ability to inject the desired volume of electron donor. Field demonstrations have compared injections of pure vegetable oil to the delivery of fine emulsions of vegetable oil and determined that emulsions more effectively distribute electron donors (Borden et al., 2001).

Early work with vegetable oil emulsions used field processing to reduce soil-pore throat clogging and to increase the distribution of the vegetable oil in the subsurface (Borden et al., 2001; Boulicault et al., 2000; Skladany et al., 2001; Zenker et al., 2000). Using high shear emulsion processing equipment in field applications is difficult due to the high cost and high-energy requirements of the processing equipment. In addition, field production of emulsions often results in a wide range of droplet sizes, with some droplets exceeding 10 microns in diameter. Although the oil-in-water emulsions produced in the field were a great improvement over injection of pure vegetable oil, the larger oil droplets have the ability to block soil-pore throats and reduce soil permeability. Column studies by Soo et al. (1996) suggest that oil droplets need to be one-third of the size of soil-pore throats to avoid being trapped by soil-pore throats by straining. To ensure efficient injection without aquifer fouling, vegetable oil emulsions should contain oil droplets that are at least three times smaller than the median soil-pore throats.

Even relatively fine emulsions that contain droplets over one micron may not be kinetically stable. Droplets larger than one micron can coalesce into even larger droplets and cause phase separation by "creaming." One example is raw milk, which contains fat droplets ranging from 1.0 to 10.0 microns. When allowed to stand for several hours, the large droplets coalesce into larger droplets, until creaming occurs. However, by homogenizing the milk to reduce the fat droplets to an average size of about 0.5 microns, the milk fat stays suspended for weeks. By using similar factory-processing methods, small-droplet vegetable oil emulsions can be produced for use as electron-donor products.

Utilizing an optimal blend of stabilizing agents and advanced factory processing methods, RNAS, Inc., has produced a kinetically stable soybean oil emulsion consisting entirely

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of submicron droplets (U.S. Patent #6,806,078). Factory processing produces an oil-in-water emulsion with oil droplets ranging from 0.15 to 0.6 microns, with a median oil-droplet size of 0.3 microns (particle size was determined by a laser diffraction method and reported as a volume distribution). The product is marketed under the trademark "Newman Zone" and contains a blend of fast- and slow-release electron donors. The emulsion formulations contain 4 percent sodium lactate by weight to provide a rapidly utilized electron donor and 46 percent vegetable oil by weight to act as a slow-release electron donor. The emulsion is pasteurized and packaged in sterile packaging such that it has a shelf life of one year or more when refrigerated and can be stored onsite without refrigeration for months.

After the emulsion is diluted to less than 5 percent oil, the injection fluid behaves essentially the same as water; vegetable oil emulsion can be successfully injected into any formation that accepts significant volumes of water.

The first full-scale production run of the product was completed in February 2002. Since then, over one million pounds of the emulsion product has been sold, with dozens of projects completed in the United States, as well as international projects in Australia, Canada, Denmark, and South Africa. Dilute oil emulsions ranging from 0.25 to 5.0 percent oil have been injected into a variety of soils, ranging from sands to low-permeability silts and clay soils. After the emulsion is diluted to less than 5 percent oil, the injection fluid behaves essentially the same as water; vegetable oil emulsion can be successfully injected into any formation that accepts significant volumes of water. The first full-scale field application is described in the following case study, with 52,000 pounds of emulsion injected in March 2002.

CASE STUDY—REMEDICATION OF A FORMER ADHESIVES MANUFACTURING FACILITY

Site History

A former adhesives manufacturing facility located within a primarily industrial area of Tampa, Florida, was active between 1972 and 1985. Trichloroethene (TCE) and 1,1,1-trichloroethane (1,1,1-TCA) used in the manufacturing process were delivered to the site by rail car and off-loaded into aboveground storage tanks (ASTs). The storage tanks were housed in a concrete secondary containment structure located along the south side of the manufacturing/warehouse building. In 1985, the storage tanks, associated piping, and rail spur were removed from the facility after cessation of adhesives manufacturing.

A Phase II environmental site assessment performed in January 1992 indicated the presence of volatile organic compounds (VOCs) in groundwater at concentrations greater than Florida groundwater protection standards. Subsequent monitoring at the site confirmed that TCE, 1,1,1-TCA, and their associated daughter products, 1,1-dichloroethene (1,1-DCE), *cis*-1,2-dichloroethene (cDCE), and vinyl chloride (VC), were present in groundwater. No specific release or catastrophic failure was recorded during the operation of the adhesives manufacturing facility, and it is believed that the groundwater and soil contamination resulted from a number of small spills that occurred during transfer of the solvents from rail cars to the storage tanks.

A pump-and-treat system was operated from August 1996 until March 1999 as an interim remedial action. The system was effective in meeting the following site-specific treatment goals: (1) to provide hydraulic control of the plume and (2) to begin removal of contaminant mass from the surficial aquifer. By 1999, the dissolved plume had been reduced to the immediate vicinity of the former AST containment area, and more than 90 kg of VOC contaminant mass had been removed.

Site Geology and Contaminant Distribution

The site lies at an elevation of approximately 1.5 to 3.0 meters above sea level, approximately 1 kilometer north of McKay Bay. The site geology consists of one meter of fill (mixed sand, gravel, and slag material), with underlying native sediments consisting of fine sand and silty sand to a depth of 4 to 5 meters below ground surface. The uppermost fine-sand unit overlies a slightly coarser medium-sand unit from 5 to 7 meters below ground surface. A thin layer of clayey sand, 1 meter thick or less, is present under the sand unit across part of the site. A continuous layer of blue clay approximately 1 meter thick underlies the surficial sand and acts as a confining unit for the underlying intermediate aquifer. The intermediate aquifer below the blue clay consists of interbedded layers of clay, partially lithified sandy limestone, and marl. The intermediate aquifer is characterized by artesian groundwater flow, with the potentiometric surface rising as much as 1.5 meters above ground surface.

The blue clay appears to have prevented contaminants from migrating from the surficial aquifer to the intermediate aquifer. Four monitoring wells (MW-17D through MW-20D) were completed within the intermediate aquifer (screened intervals range from 9.75 to 12 meters below ground surface). The wells were double-cased and show no signs of contamination from the surficial aquifer.

Chlorinated solvent constituents are concentrated in the deeper portion of the surficial aquifer beneath the tank pad, with the highest contaminant concentrations detected in sandy clay immediately above the blue-clay confining unit (Exhibit 1). The groundwater data indicate that the plume is limited to the surficial aquifer, primarily in the area around the AST pad (source area). The soil data indicate that the contaminants have not penetrated a significant distance into the blue clay, and no contaminants have been detected in the intermediate aquifer below the blue clay.

Design and Implementation

Monitoring after the pump-and-treat system was terminated indicated that reductive dechlorination was naturally active at the site. Remediation technologies that might harm the natural attenuation activity, such as air sparging or chemical oxidation, were rejected. An enhanced reductive dechlorination bioremediation approach that would complement the natural site activity was selected for the contaminant source area. During operation of the pump-and-treat system, extraction wells were limited to flow rates ranging from three to five liters per minute. A simple lactate recirculation system was considered but rejected because of concerns about well fouling and the relatively low anticipated extraction and injection flow rates. The site is currently used by a steel supply company, and aboveground structures would interfere with site operations.

Vegetable oil was selected as a long-term electron donor, and a small amount of sodium lactate was added to the amendment to stimulate a rapid microbial response. The lactate is consumed within a month or less, and the vegetable oil slowly ferments to produce molecular hydrogen and volatile fatty acids (VFAs), such as acetic acid. The emulsified oil amendment selected for the site is marketed by RNAS, Inc., under the name "Newman Zone," and consists of 50 percent soybean oil by volume, 4 percent sodium lactate by weight, and the remainder of the solution composed of food-grade stabilizing agents. A total of 26,000 liters of emulsified oil amendment was delivered to

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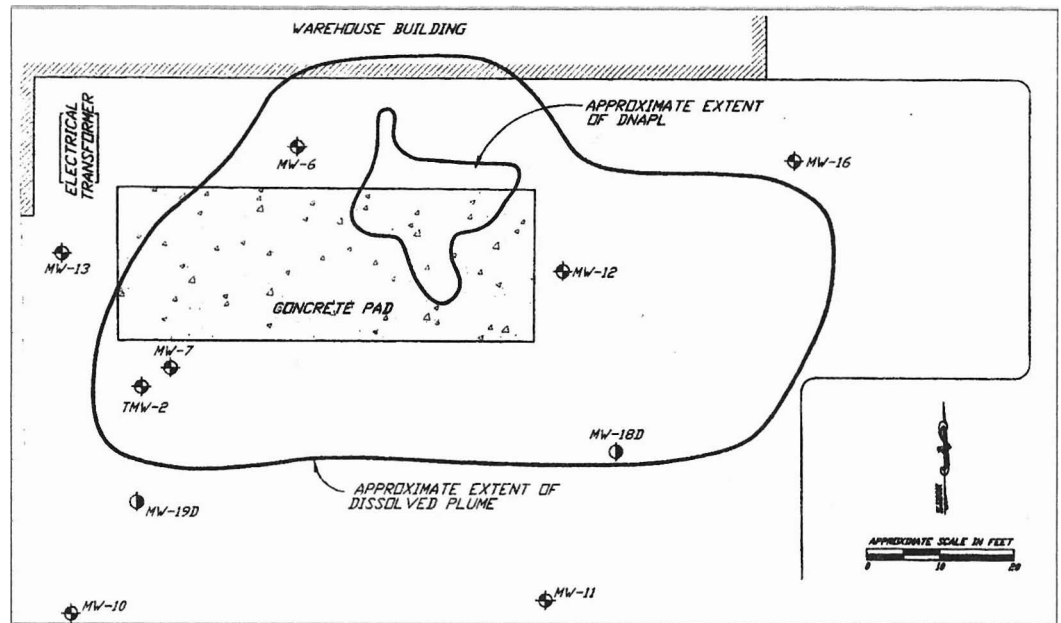


Exhibit 1. Map of the source area showing the approximate aerial extent of dissolved plume and DNAPL in the surficial aquifer

the site in 1,000-liter disposable totes. Injection began with the southern- and eastern-most line of wells, installed as a downgradient oil barrier (Exhibit 2). The emulsion was then injected into the contaminant source area through the remaining wells.

A proportional feed system was used to continuously inject 4.9 percent emulsified oil amendment by volume into multiple well points with precise control of the oil content (patent #6,806,078). Although the fine sand allowed injection flow rates of an average of only approximately four liters per minute, the injection system allowed for simultaneous injection into ten wells at a precise vegetable oil concentration. Each point received approximately 2,700 liters of injection fluid, with a total of 265,000 liters of fluid delivered over a two-week period. Other emulsified oil practitioners have described injections of concentrated emulsified oil followed by injections of water intended to disperse the emulsion (Borden et al., 2001; Zenker et al., 2000). If the single-point, multistep injection process described by other practitioners had been used, the emulsion injection would have required several months to complete, because the water dispersion process is typically conducted on a well-by-well basis.

Results

The presence of the oil-in-water emulsion was observed (milky-white appearance) immediately after injection in all of the monitoring wells within the treatment zone. After one month, some emulsion (milky-white appearance) was still visible, but none was observed after two months. At no time was separate-phase vegetable oil observed in any wells.

Prior to the amendment injection, the source-area geochemistry indicated an anaerobic environment. After amendment injection, the groundwater geochemistry indicated a strongly anaerobic environment with increasing concentrations of dissolved iron, manganese, sulfide, methane, and ethene. Decreasing values for oxidation-reduction poten-

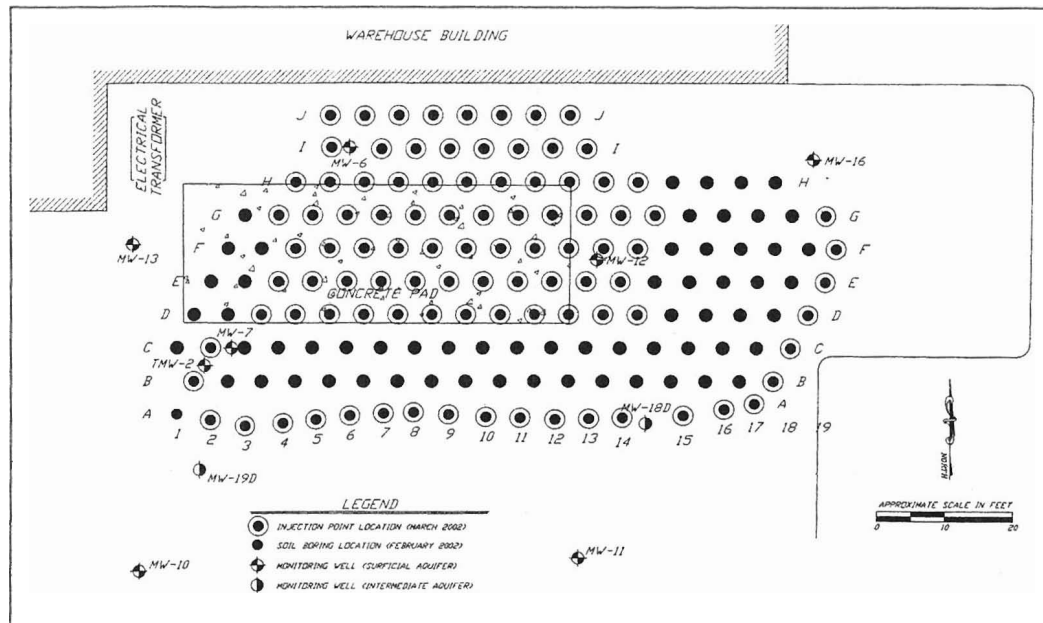


Exhibit 2. Site plan soil boring locations and ERD injection point locations (open circles) relative to the aerial extent of contamination in the surficial aquifer

tial (ORP), dissolved oxygen (DO), nitrate, and sulfate were observed within the treatment area following the injection. Background total organic carbon (TOC) concentrations were measured prior to the emulsion injection and were consistently below 2 mg/L in all of the monitoring wells within the treatment area, although these data are not presented in this article.

Chlorinated solvent concentrations for the primary contaminants TCE and 1,1,1-TCA, as well as their selected daughter products, are presented for the treatment area wells TMW-2, MW-6, MW-7, and MW-12 (Exhibit 3). High initial concentrations of TCE and 1,1,1-TCA made detection of the chloroethane and dichloroethane degradation products in MW-12 difficult, and data for chloroethane and dichloroethane are not included in Exhibit 3. Three years after the emulsion injection, the TCE and 1,1,1-TCA concentrations had been reduced to levels that allowed for reasonable detection limits, and both monochloroethane and dichloroethane daughter products have been observed since then in MW-12. Data for VFAs and TOC are presented as indicators of continued vegetable oil fermentation. Ethene is the final dechlorination end product of the chlorinated ethenes. TCE, *cis*-1,2 DCE, VC, and ethene data are presented in Exhibit 3.

Discussion

Injection flow rates of the emulsified oil (4.9 percent by volume) remained constant, with no observed increase in injection pressures. Flow rates ranged from 0.3 to 3.0 gallons per minute (gpm), depending on the permeability of soils adjacent to the injection point. Emulsified oil was observed in monitoring wells adjacent to a given injection point at times and distances that represented the expected volumetric displacement of the injection fluid (milky-white appearance). Follow-up drilling is needed to confirm the residual soybean oil distribution in the treatment area, but field observations suggest

Exhibit 3. Groundwater data from treatment-area monitoring wells

TMW-2	Elapsed Time	1,1,1- TCA	TCE	1,1- DCE	cDCE	VC	Ethene	VFAs	TOC
Date	From Inj. Days	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L
12/21/2001	Preinjection	< 5	< 5	23	240	260	NM	NM	NM
3/29/2002	2	< 25	83	77	1,600	950	< 50	NM	500
6/14/2002	79	< 25	< 25	26	1,700	640	NM	NM	NM
9/18/2002	175	< 1	< 1	< 1	6	22	4.6	NM	2
3/14/2003	352	< 5	< 5	38	300	98	22	94	79
3/9/2004	713	< 0.10	21	0.99	95	13	6.9	256	110
9/30/2004	888	< 0.46	5.7	< 0.45	40	12	5.5	82.4	130
3/23/2005	1,062	< 0.46	0.950	< 0.45	13	4	1.5	118	110
3/3/2006	1,407	< 0.46	< 0.46	1	3	2	0.56	69	NM

MW-6	Elapsed Time	1,1,1- TCA	TCE	1,1- DCE	cDCE	VC	Ethene	VFAs	TOC
Date	From Inj. Days	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L
12/21/2001	Preinjection	< 2,500	64,000	3,400	4,100	< 2,500	NM	NM	NM
3/29/2002	2	< 1,000	60,000	2,700	8,200	< 1,000	NM	NM	1,200
4/30/2002	34	430	13,000	1,700	17,000	6,200	NM	NM	NM
5/24/2002	58	300	2,900	950	7,600	4,800	NM	NM	NM
6/14/2002	79	< 250	4,000	1,000	11,000	8,900	NM	NM	NM
7/23/2002	118	< 250	2,000	490	7,800	6,800	NM	NM	NM
8/22/2002	148	< 250	< 250	< 250	3,400	7,300	NM	NM	NM
9/18/2002	175	< 250	< 360	< 250	8,200	6,800	460	332	130
3/14/2003	352	83	< 50	61	960	2,500	620	142	74
9/30/2003	552	< 50	< 50	< 50	260	1,400	980	102	51
3/9/2004	713	< 0.50	< 0.50	< 0.65	66	340	310	133	24
3/24/2005	1,063	< 0.46	0.830	< 0.45	15	38	26	58	20
3/3/2006	1,437	< 0.46	< 1.4	< 2.3	72	440	120	11	NM

(continued)

the oil emulsion was delivered to the surficial aquifer as easily as water and without a reduction in soil permeability.

Visual observations and TOC measurements taken two days after the injection suggest that the emulsified oil moved freely during the injection but then rapidly adsorbed onto soil surfaces. Very little suspended oil could be observed in source-area wells after one month (milky-white appearance), and no milky suspension was observed in any of the wells after two months. The injection fluid had a TOC content of over 38,000 mg/L, but two days after the injection, the highest groundwater TOC concentration was measured in MW-6 at only 1,200 mg/L. This suggests that the majority of the vegetable oil adsorbed onto soil surfaces rather than remaining suspended in the soil-pore water within days of injection. With a groundwater flow velocity of 20 feet per year, it was unlikely that the emulsion or even the sodium lactate

Exhibit 3. Continued

MW-7 Date	Elapsed Time From Inj. Days	1,1,1- TCA µg/L	TCE µg/L	1,1- DCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	VFAs mg/L	TOC mg/L
12/21/2001	Preinjection	< 25	200	58	920	670	NM	NM	NM
3/29/2002	2	< 25	410	120	1,900	540	NM	NM	25
4/30/2002	34	< 10	< 10	< 10	470	650	NM	NM	NM
5/24/2002	58	< 2.5	< 2.5	< 2.5	73	160	NM	NM	NM
6/14/2002	79	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5	NM	NM	NM
7/23/2002	118	< 1	< 1	< 1	6	63	NM	NM	NM
8/22/2002	148	< 1	< 1	< 1	4	36	NM	NM	NM
9/18/2002	175	< 100	< 100	< 100	2,800	460	75	610	320
3/14/2003	352	< 1	< 1	< 1	11	15	1.3	ND	1
9/30/2003	552	< 1	< 1	< 1	7	14	0.6	ND	1
3/9/2004	713	< 0.10	< 0.10	< 0.13	5	8	0.44	ND	< 0.53
3/24/2005	1063	< 0.46	< 0.28	< 0.45	0.92	2	0.12	ND	2
3/3/2006	1437	< 0.46	0.460	1	3.2	2	0.33	ND	NM

MW-12 Date	Elapsed Time From Inj. Days	1,1,1- TCA µg/L	TCE µg/L	1,1- DCE µg/L	cDCE µg/L	VC µg/L	Ethene µg/L	VFAs mg/L	TOC mg/L
12/21/2001	Preinjection	16,000	29,000	4,100	2,000	< 500	NM	NM	NM
3/29/2002	2	150,000	250,000	45,000	< 2,500	< 2,500	NM	NM	230
4/30/2002	34	130,000	230,000	30,000	8,200	< 2,500	NM	NM	NM
5/24/2002	58	90,000	100,000	15,000	15,000	< 5,000	NM	NM	NM
6/14/2002	79	82,000	140,000	8,800	10,000	< 5,000	NM	NM	NM
7/23/2002	118	34,000	35,000	11,000	16,000	3,400	NM	NM	NM
8/22/2002	148	18,000	18,000	7,400	14,000	7,000	NM	NM	NM
9/18/2002	175	20,000	27,000	5,900	17,000	4,100	430	221	130
3/14/2003	352	4,300	4,800	1,600	3,900	3,500	1,400	149	75
9/30/2003	552	19,000	13,000	3,200	17,000	5,300	590	79	31
3/9/2004	713	16,000	12,000	1,600	14,000	11,000	370	129	20
3/24/2005	1,063	870	1,100	130	1,200	2,600	960	176	61
3/3/2006	1,437	8200	9,000	1,400	3,800	8,500	240	31	NM

electron donors would be washed out of the injection area within a few months of the injection.

The adsorbed vegetable oil appears to have been retained on the soil surfaces and has remained active for several years. Six months after injection, the TOC concentrations in MW-6, MW-7, and MW-12 were 130 mg/L, 320 mg/L, and 130 mg/L, respectively. Only 2 mg/L of TOC were detected in TMW-2, which is located near MW-7 but screened in the upper portion of the surficial aquifer. After a year, the TOC concentrations had decreased to trace levels in MW-7, but TMW-2, MW-6, and MW-12 continued to show elevated TOC concentrations with concentrations of 79 mg/L, 74 mg/L, and 75 mg/L, respectively. Similar results were observed three

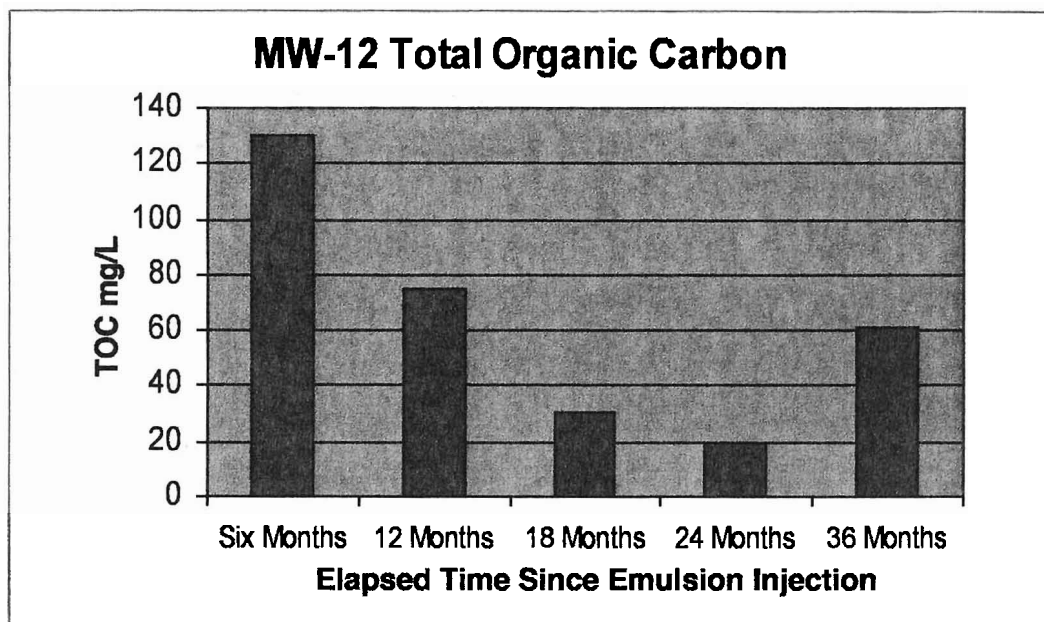


Exhibit 4. Total organic carbon concentration in MW-12 versus time

years after the emulsion injection, with concentrations of 110 mg/L, 20 mg/L, and 61 mg/L, respectively, for TMW-2, MW-6, and MW-12, but only trace levels of TOC were detected in MW-7. Exhibit 4 visually depicts the TOC concentrations in MW-12 over time.

The primary fermentation products of vegetable oil are molecular hydrogen and acetic acid. Monitoring VFAs provides evidence of continued vegetable oil fermentation. VFA concentrations six months after the injection ranged from 94 mg/L to 221 mg/L in the treatment area. The VFAs consisted almost entirely of acetic acid, with lesser amounts of propionic acid and butyric acid. As expected, microbes rapidly consumed the sodium lactate such that lactic acid was detected at the detection limit in only one well six months after injection, although the emulsion injection fluid contained over 3,000 mg/L of lactate. VFA concentrations in TMW-2, MW-6, and MW-12 remained at elevated levels one, two, three, and even four years after the emulsion injection. Three years after the emulsion injection, VFA concentrations in TMW-2, MW-6, and MW-12 ranged from 58 mg/L to 176 mg/L. Four years after the injection, the VFA concentrations ranged from 11 mg/L to 69 mg/L, indicating that vegetable oil fermentation continues to be active four years after the emulsion injection. Exhibit 5 visually depicts the VFA concentrations in MW-12 over time.

At some sites where injection of pure vegetable oil or vegetable oil emulsion has been implemented, a sudden decrease in chlorinated solvent concentrations has been observed. This is likely the result of contaminants partitioning from water into the vegetable oil (Borden et al., 2001; Boulicault et al., 2000). Immediately after treatment, contaminant concentrations remained essentially the same in MW-6, at the northern edge of the source area, and increased in both TMW-2 and MW-7, both located at the southwestern edge of the dissolved-phase plume. In MW-12, concentrations of 1,1,1-TCA and TCE increased dramatically by over an order of magnitude to 150,000 µg/L and 250,000 µg/L, respectively. MW-12 is located at the edge of the

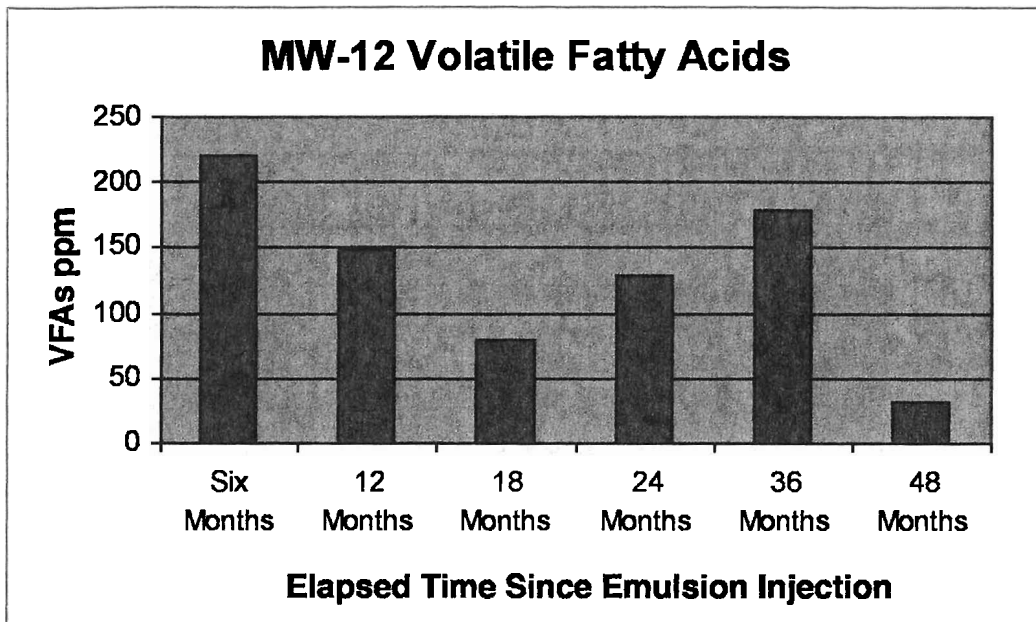


Exhibit 5. Volatile fatty acid concentration in MW-12 versus time

dense nonaqueous phase liquid (DNAPL) source, and the sudden increase in contaminant concentrations is likely the result of injection fluid displacement and mobilization of small droplets of DNAPL caused by the surfactants contained in the amendment.

The decrease in the primary contaminants 1,1,1-TCA and TCE in MW-6 and MW-12 was associated with concurrent increases in the daughter products, cDCE and VC. TCE concentrations in MW-6 decreased from 64,000 µg/L to below detection limits in 148 days. One year after the large initial increase in 1,1,1-TCA and TCE concentrations in MW-12, contaminant levels had been reduced from the initial concentrations by 73 percent and 83 percent, respectively, for 1,1,1-TCA and TCE. After two years of treatment, relatively high 1,1,1-TCA and TCE concentrations and elevated concentrations of daughter products suggest that residual soil contamination continues to supply dissolved contaminants in spite of the active reductive dechlorination. Three years after the emulsion injection, TCE and 1,1,1-TCA concentrations had been reduced to 1,100 µg/L and 870 µg/L, respectively, in MW-12, but after four years the concentrations had rebounded to 9,000 µg/L and 8,200 µg/L, respectively. Elevated concentrations of both chloroethane and chloroethene daughter products and ethene indicate that reductive dechlorination is still active in MW-12 four years after the emulsion injection.

VOC concentrations in MW-7 are subject to relatively sudden increases and decreases, because the well is located at the cross-gradient edge of the contaminant plume, and groundwater flow is subject to short-term flow direction changes from tidal effects and precipitation/recharge events. Total VOC concentrations in MW-7 decreased from 2,970 µg/L two days after the emulsion injection to nondetectable levels (< 2.5 µg/L) in 79 days, but a spike in cDCE and VC was observed three months later, and low levels of cDCE and VC persist in the well. Periodic spikes of VOCs will likely be observed in MW-7 as long as relatively high concentrations of cDCE and VC are present within the DNAPL source. TMW-2 is located near MW-7 but screened in the upper portion of the

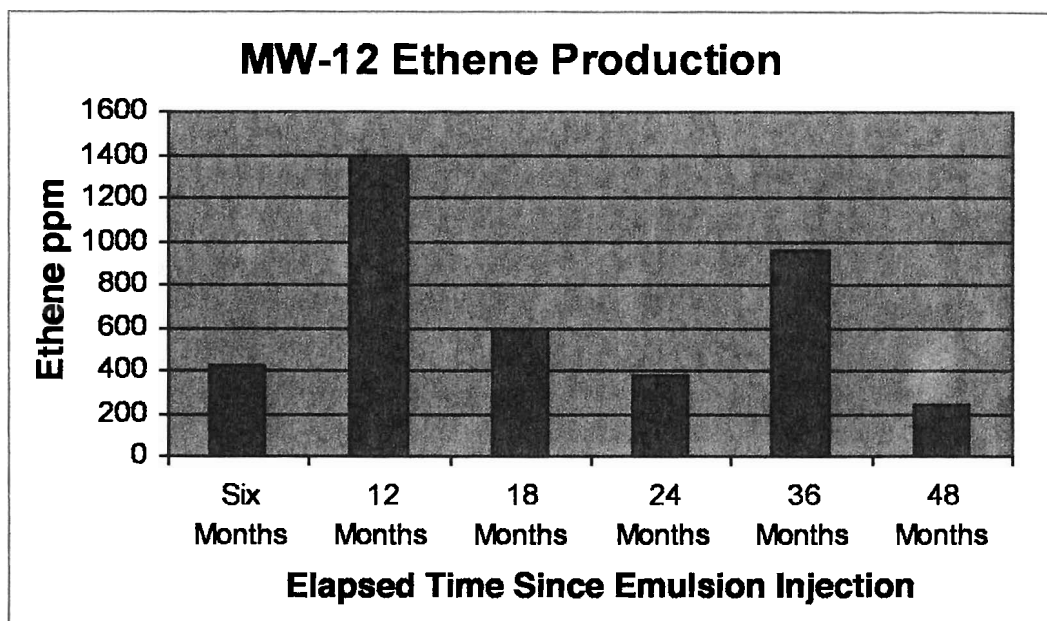


Exhibit 6. Ethene concentrations in MW-12 versus time

surficial aquifer. In TMW-2, two days after the emulsion injection, the total VOC concentrations were measured at 2,710 $\mu\text{g}/\text{L}$. After the emulsion injection, the VOC concentrations declined rapidly to 28 $\mu\text{g}/\text{L}$ after 175 days, but then increased after 352 days to 436 $\mu\text{g}/\text{L}$. TMW-2 is subject to similar VOC concentration fluctuations as MW-7 because of its location at the edge of the dissolved-phase plume. Four years after the injection, the total VOC concentration in TMW-2 had been reduced to 6 $\mu\text{g}/\text{L}$.

Ethene production is an excellent indicator of reductive dechlorination of chloroethenes, such as TCE and its degradation daughter products, *cis*-1,2-DCE and VC. Ethene was detected in both TMW-2 and MW-7, but the relatively low concentrations of VC did not allow for high concentrations of ethene to be produced. In MW-6, the ethene production peaked two years after the emulsion injection at a concentration of 980 $\mu\text{g}/\text{L}$ and has declined with decreasing VC concentrations to 120 $\mu\text{g}/\text{L}$ four years after the emulsion injection. Ethene concentrations increased after the emulsion injection in MW-12, with the peak ethene concentration of 1,400 $\mu\text{g}/\text{L}$ detected about one year after treatment. Four years after the emulsion injection, ethene production is still occurring in MW-12, but the ethene concentration has decreased to 240 $\mu\text{g}/\text{L}$. Exhibit 6 visually depicts the ethene concentrations in MW-12 over time.

CONCLUSIONS

The offsite production of a fine emulsion of soybean oil produced a low-cost electron-donor amendment. By using factory processing, a consistent submicron droplet size was produced, with a median droplet size (volume distribution) of 0.28 microns and 90 percent of the oil volume consisting of droplets smaller than 0.42 microns. The product provides a low-cost, off-the-shelf emulsion with a shelf life of several months without refrigeration and a shelf life of several years in cold storage. More important,

the submicron droplet size ensures that there will be little, if any, reduction of soil permeability, even in fine-grained soils.

The use of a proportional feed system allows better control of emulsified oil concentrations and subsurface distribution than other commonly used emulsion-injection methods, where small volumes of concentrated emulsion are first injected and then diluted with a second water injection. The feed system also allows for continuous injection of amendment into multiple injection points. With simultaneous injection through ten wells, 265,000 liters of dilute emulsion could be delivered into fine-grained soils in two weeks. The injection process would have required several months at this site if the single-point serial injection method commonly used by other edible-oil practitioners had been used.

Factory-processed vegetable oil emulsions combine the ease of injection and good subsurface distribution properties of a soluble electron donor with the added advantage of a slow-release electron donor. The Newman Zone amendment appears to be effective in both rapidly stimulating initial microbial activity (lactate) and supporting long-term reductive dechlorination with a slow-release electron donor (vegetable oil). A rapid reduction in 1,1,1-TCA and TCE and production of daughter products has been observed in the treatment area. Only one well, MW-12, which is located downgradient of the original DNAPL source, continues to have significant concentrations of the original contaminants. Ethene production and elevated levels of VFAs four years after the injection confirm that the emulsion can act as an electron donor for several years.

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William A. Newman is the founder and president of Remediation and Natural Attenuation Services (RNAS), Inc. RNAS, Inc., provides products and technical support for bioremediation. Mr. Newman's inventions/products include a low-cost oxygenation system for aerobic bioremediation (patent #6,561,497) and methods for manufacturing and injecting small-droplet edible-oil emulsions (patent #6,806,078) for anaerobic bioremediation.

Ronald C. Pelle, P.G., is president of P2 Environmental, Inc. He is a professional geologist with 18 years of experience in environmental consulting. His specialties include site assessments, soil and groundwater remediation, environmental project management, and due diligence assessments.
