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Enclosure 18

Response to Request for Additional Information

Acetone and 2-Butanone Creation Associated with Biological and Chemical Remediation of Environmental Contamination

Acetone and 2-Butanone Creation Associated with Biological and Chemical Remediation of Environmental Contamination

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Remediating environmental contamination by either biological or chemical methods typically results in the generation of temporary chemical intermediates as part of the process. These intermediate compounds may be related to either contaminant degradation pathways or reactions generated from the amendment itself. This article summarizes previously researched pathways and representative case studies discussing the authors' experience in generating relatively high concentrations of acetone and 2-butanone (also referred to as methyl ethyl ketone [MEK]) during both biological and chemical treatments. Experience shows that even relatively high concentrations of acetone and MEK intermediates are quickly attenuated and prove not to be a hazard outside of the treatment area. © 2011 Wiley Periodicals, Inc.

INTRODUCTION

Biological and chemical treatment of various contaminants of concern (COCs) has developed as a viable and sometimes preferable method for cleanup of environmental contamination. Ideally, the end products of this process are nonhazardous or have a much lower level of toxicity compared to the original COC. In some cases, the absence of the appropriate microbial communities or insufficient contact with reactive chemical agents results in the accumulation of chemical intermediates known to exhibit toxicity. For example, reductive dechlorination of chlorinated ethenes or polychlorinated biphenyls (PCBs) can result in the generation of higher-toxicity compounds. Incomplete biological dechlorination of tetrachloroethene (PCE) or trichloroethene (TCE) can result in the accumulation of lesser chlorinated compounds such as *cis*-1,2-dichloroethene (cDCE) or vinyl chloride (VC). While cDCE is not considered a carcinogen, VC is considered a carcinogen with groundwater cleanup levels lower than PCE or TCE (US EPA, 2011). Some lesser chlorinated PCB congeners are more toxic with lower cleanup levels than fully chlorinated versions (Suter & Tsao, 1996).

In addition to the COC-related intermediates, the remediation amendments used as part of the treatment process can also generate intermediates of potential concern (IPCs). The authors have observed significant and temporary accumulation of acetone and

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2-butanone (also referred to as methyl ethyl ketone [MEK]) IPCs during the fermentation of electron donors and as side reactions during chemical oxidation. This article summarizes previously researched pathways and experience related to these IPCs during the course of environmental remediation projects.

BIOLOGICAL PRODUCTION OF ACETONE AND MEK

Due to sharply rising petroleum prices in the late 1970s, there was renewed interest in biological acetone production using microbes from the *Clostridium* species and in optimizing solvent production at industrial scales.

The biological production of ketone and alcohol solvents began around 1900 as a way to generate compounds useful for industrial processes, including acetone and butanol from starch using the *Bacillus* genus of bacteria (Speakman, 1920). Fermentative processes were the primary method for generating acetone until the Cumene Process was developed in 1944 (Hock & Lang, 1944). However, due to sharply rising petroleum prices in the late 1970s, there was renewed interest in biological acetone production using microbes from the *Clostridium* species and in optimizing solvent production at industrial scales (Monot, Martin, Petitdemange, & Gay, 1982). Monot's group was able to achieve solvent production efficiencies as high as 28 to 33 percent using glucose as a fermentable substrate in combination with optimal magnesium, iron, and potassium levels. Other researchers have been able to blend *Clostridium* species to degrade cellobiose (partially hydrolyzed cellulose) to produce a blend of ethanol and acetone (Ng, Ben-Bassat, & Zeikus, 1981). Therefore, it is realistic to expect that rapid fermentation of carbohydrate-based amendments, including glucose, dextrose, and cellobiose, would result in the production and temporary accumulation of acetone and butanol intermediates.

The biological production of MEK is generally associated with the dehydrogenation of 2-butanol, whereby the OH group is oxidized into a ketone. The production of MEK is well documented in the manufacturing of cheese, in which 2,3-butylene generated by *Pediococcus cerevisiae* is transformed by *Lactobacillus plantarum* into MEK as the cheese ages (Keen, Walker, & Peberdy, 1974). Yeast, using NAD-dependent alcohol dehydrogenase enzymes, has been documented to generate MEK from 2-butanol (Braenden, Joenvall, Eklund, & Furugren, 1975). Others have found that C1-utilizing methylotrophs in common lake and soil samples are able to produce MEK during oxidation of *n*-propane and 2-butanol, with the highest extracellular accumulation rates associated with yeast species *Candida*, *Hansenula*, and *Pichia* and bacterial species *Pseudomonas*, *Methylococcus*, and *Methylosinus* (Hou, Patel, Laskin, Barnabe, & Marczak, 1979). In abiotic experiments discussed in Hou's publication, lysed *Pseudomonas* and *Hansenula* produce MEK the fastest based on available protein levels. Similar to acetone, it is reasonable to expect the temporary accumulation of MEK in anaerobic groundwater during carbohydrate fermentation.

PRODUCTION OF ACETONE AND MEK DURING REMEDIATION

Acetone and MEK are widely used in various cleaning and solvent applications. In some cases, they may be a site COC. MEK is one of the most common volatile organic ketone compounds and has been widely used as an industrial solvent for paints, lacquers, and varnishes (Lurie, 1967). Due to their high water solubility and insignificant sorption to organic carbon (low retardation), these compounds may form and migrate with

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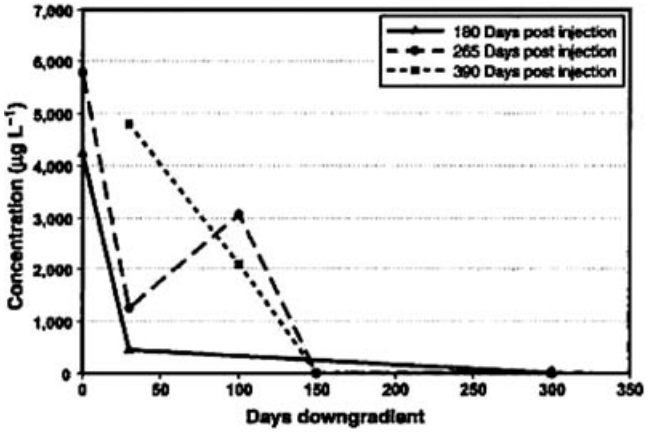

Exhibit 1. MEK concentration versus distance over three sampling events
Source: Figure 2.28 from Suthersan and Payne (2005).

groundwater from upgradient releases. Therefore, detections of acetone or MEK may not be directly related to site activities that resulted in the initial contamination.

However, when an impacted aquifer is amended with an electron donor, treated with chemical oxidants, or heated during the course of a remedial action, a small portion of the organic matter in that system may be converted to ketones, such as acetone or MEK. These processes may temporarily raise groundwater concentrations for these IPCs above site-specific cleanup levels and trigger regulatory concern unless the processes of ketone production and its fate and transport are understood. When these processes are understood, short-term monitoring should be sufficient to alleviate regulatory concern by verifying that impacts are localized, temporary, and pose negligible risk.

Reductive Bioremediation

In one literature case study, the injection of corn syrup, cheese whey, and bioaugmentation culture resulted in the short-term accumulation of non-COC MEK (Suthersan & Payne, 2005). At this site, MEK was initially noted at a concentration of 3,200 micrograms per liter (μg/L), but concentrations dropped below the detection limit (100 μg/L) during the subsequent monitoring period. The authors hypothesized the MEK detection to be a consequence of highly effective dechlorination reactions and possibly associated with the enhanced activities of *Clostridium* sp. or other ketone-producing fermenters that also dechlorinate ethenes. In another case study reported by the same authors, elevated acetone and MEK concentrations were detected in the injection well and several downgradient wells, with initial levels as high as 6,000 μg/L. These concentrations rapidly decreased with time and distance as shown in Exhibit 1.

Chemical Oxidation

In an unpublished bench study using activated persulfate, acetone was generated as an IPC during the treatment of a solution containing both gasoline and various chlorinated solvents (R. A. Brown, data transmittal and personal communications, 2011). The studied persulfate activators included various combinations of zero-valent iron (ZVI), ferrous

iron, ferrous ammonium sulfate, and alkalinity. Carbonate anion was also introduced as a variable to assess free-radical quenching in some cells. The highest concentrations of acetone were produced using either ferrous ammonium sulfate or ZVI with an elevated pH of 10 as activators, both in the absence of carbonate.

Abiotic Mechanisms

Acetone may also be generated abiotically as a result of electrical resistance heating treatment degrading native organic carbon (Beyke & Fleming, 2005). At five United States Naval facilities where electrical resistance heating was employed to treat nonaqueous phase liquid and dissolved chlorinated volatile organic compounds (cVOCs), the authors observed the production and accumulation of acetone during the heating process. Acetone groundwater concentrations were typically less than 500 milligrams per liter (mg/L) at these sites, and acetone concentrations declined to nondetectable levels as the sites cooled to ambient conditions.

Simultaneous presence of high concentrations of acetate along with oxidized iron could produce acetone until the iron is biologically reduced to the ferrous form.

Other abiotic mechanisms identified include iron-mediated reactions. In iron literature, there are reports of small amounts of butane (C4H10) and butene (C4H8) formed during degradation of TCE and TCA using ZVI. Oxidation from butane to MEK could also occur, although the authors found no published reports demonstrating this mechanism directly. Additionally, there is also some evidence that certain metal oxides, including iron oxide, can abiotically oxidize acetate to generate acetone. Thus, simultaneous presence of high concentrations of acetate along with oxidized iron could produce acetone until the iron is biologically reduced to the ferrous form.

Longevity

When acetone and MEK are generated, these IPCs also provide a readily biodegradable food source for microbes performing reductive dechlorination. Typical half-lives for acetone in groundwater range from 19 to 197 days (Aaronson & Howard, 1997). MEK is known to be highly degradable (Devinny, Deshusses, & Webster, 1999) with reported environmental half-lives ranging from 13 to 128 days (Aaronson & Howard). The formation, accumulation, and persistence of these fermentation products are a widely recognized potential limitation of substrate addition for enhanced bioremediation (Henry, 2010).

GENERATION OF KETONES: OTHER POSSIBLE SOURCES

There are several circumstances in which acetone and MEK may be detected in samples collected at remediation sites even though acetone and MEK are not physically present in groundwater or soil.

Preservation Artifacts

Acetone can be chemically produced in soil samples as a result of sodium bisulfate preservation under Method 5035 (Clausen et al., 2004). The proposed mechanism is that volatile fatty acids (VFAs), such as propionic acid, generated during preservation can be biologically reduced to isopropyl alcohol. Isopropyl alcohol undergoes photocatalytic

oxidation to acetone. This photocatalytic oxidation can occur during the sample hold time (between sample collection and analysis).

Storage and Transport Artifacts

While not a common practice in the environmental field anymore, both cleaners and ambient air can be sources of acetone and MEK detected at low concentrations in samples. These could include the use of acetone to clean laboratory glassware or MEK present in products used to clean coolers.

False Positives

Certain VFAs can be transformed into acetone during sample analytical processing. It is possible to convert acetic acid or other VFAs to acetone by high temperature and the packing in a column during gas chromatography analysis. It may even be possible to make this conversion during certain sample cleanup steps that are employed prior to gas chromatography analysis.

CASE STUDIES

Case Study 1: Confidential Client, Mercer Island, Washington

Background

An active drycleaner began remediation of dense, nonaqueous phase liquid and dissolved cVOC plume in 1999. The primary cVOC of concern is PCE. Much lower concentrations of lesser chlorinated compounds were also present due to trace levels of organic carbon in the aquifer. Source area excavation was performed. Twenty-two thousand pounds (lb) Fenton's reagent chemical oxidant were injected in 1999, which reduced the maximum PCE concentrations down to 88 mg/L by 2006. The site plan is presented as Exhibit 2. Bioremediation injection locations are shown with IP-1 with a dot or 1S with a crossed dot. Bioremediation activities are discussed in detail later in the article. The acetone and MEK detected at this site were likely associated with the enhanced fermentation of modified cellulose.

Geology and Hydrogeology

The site and surrounding vicinity consists of mostly paved surfaces overlying 1 to 3 feet (ft) of compacted gravel fill. Under the fill, the shallow perched aquifer consists of 4 to 10 ft of silty sand above dense glacial till deposits. Shallow groundwater moves to the north-northeast with a gradient ranging between 0.07 to 0.10 ft per ft.

Bioremediation Activities

From April through October 2008, approximately 2,000 gallons (gal) commercially prepared emulsified vegetable oil (EOS® 598B42) were injected into the shallow aquifer using push probes. A total of 28 locations received EOS injections over the course of three

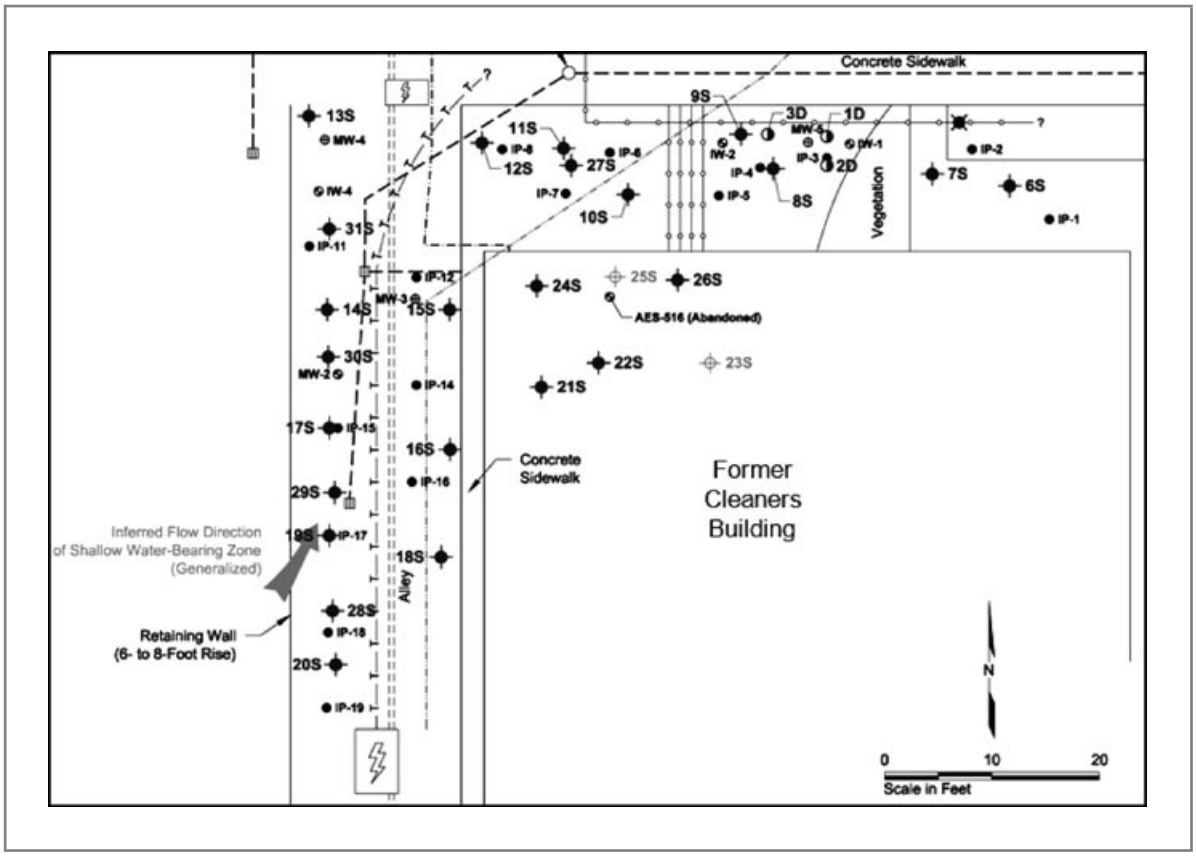

Exhibit 2. Confidential client site plan

mobilizations to the site. Injections had to be terminated due to emulsion migrating into utility corridors and ultimately into storm drains. By July 2009, the primary site constituents included dechlorination products cDCE and VC at concentrations ranging up to 41.0 mg/L and 6.9 mg/L, respectively. Ethene was detected at low concentrations in one monitoring well; no methane was detected at the site. Groundwater pH was suppressed as low as 5.8 by July 2009 but typically ranged between 6.1 and 6.2 in the treated areas. Source area oil injection locations are labeled IP-1 through IP-19 in Exhibit 2.

Source area data collected approximately 1 year following the final oil injection round suggested that the shallow aquifer was very reductive, based on measured oxidation-reduction potentials (ORPs) as low as −195 millivolts (mV). The shallow aquifer also generally contained sufficient organic carbon to fuel both complete dechlorination and methanogenesis (Exhibit 3). However, groundwater data indicated that complete dechlorination was very limited and methanogenesis was negligible. Additionally, neither acetone nor MEK was detected at any point following the oil injections. Bold values in Exhibit 3 indicate detected compounds. For compounds that were not detected (U), the detection limit is posted.

Due to apparent "*cis*-stall" occurring at the site, approximately 2,525 lb EHC® amendment and 710 lb BounTA™ nutrient amendment were injected into the shallow aquifer during February 2010 using push probes. EHC® is an electron donor providing modified cellulose and ZVI to fuel reductive bioremediation. BounTA™ is a macro- and

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Monitoring Well	Sampling Date	pH	ORP (mV)	TOC	PCE	TCE	cDCE	VC	Ethene	Ethane	Methane	Acetone	MEK
				[milligrams per liter (mg/L)]									
MW–2	7/28/2009	6.15	–129	**130**	**0.17**	**0.22**	**41**	**6.9**	0.50 U	0.50 U	3.0 U	0.50 U	0.50 U
IW–1	7/28/2009	6.23	–188	**2.0**	**2.5**	**0.42**	**8.7**	**1.5**	**0.10**	0.10 U	1.1 U	0.50 U	0.50 U
IW–2	7/28/2009	6.11	–190	**83**	0.10 U	0.02 U	**11**	**5.9**	1.0 U	1.0 U	8.0 U	0.50 U	0.50 U
IW–4	7/28/2009	6.23	–195	**21**	**0.03**	**0.04**	**18**	**2.9**	0.10 U	0.10 U	1.1 U	0.50 U	0.50 U

Exhibit 3. Source area groundwater data (1 year post-EOS)

Monitoring Well	Sampling Date	pH	ORP (mV)	TOC	PCE	TCE	cDCE	VC	Ethene	Ethane	Methane	Acetone	MEK
				[milligrams per liter (mg/L)]									
MW–2	7/28/2009	6.15	–129	**130**	**0.17**	**0.22**	**41**	**6.9**	0.50 U	0.50 U	3.0 U	0.50 U	0.50 U
MW–2	5/26/2010	6.67	–187	**330**	0.010 U	0.010 U	0.010 U	**0.017**	0.065 U	0.360 U	8.3 U	**1.3**	**2.4**
MW–2	11/17/2010	6.65	–214	**27**	0.004 U	0.004 U	**0.07**	**0.29**	**0.21**	**0.042**	**12**	0.10 U	0.10 U
MW-2	5/25/2011	7.16	–188	**10**	**0.0002**	**0.28**	**1.0**	**0.31**	**0.005**	**0.026**	**19**	0.005 U	0.005 U
IW–1	7/28/2009	6.23	–188	**2.0**	**2.5**	**0.42**	**8.7**	**1.5**	**0.10**	0.1 U	1.1 U	0.5 U	0.5 U
IW–1	5/25/2010	6.39	–94	**360**	0.05 U	0.050 U	**6.6**	**1.9**	**1.0**	0.5 U	7.6 U	**4.0**	**3.0**
IW–1	11/16/2010	6.06	–127	**530**	0.050 U	0.050 U	**3.1**	**6.8**	**0.75**	**0.10**	**8.1**	**2.1**	**7.5**
IW–1	5/24/2011	6.68	–123	**67**	0.004 U	0.004 U	**0.027**	**0.14**	**0.74**	**0.040**	**19**	**0.36**	**0.6**
IW–2	7/28/2009	6.11	–190	**83**	0.10 U	0.02 U	**11**	**5.9**	1.0 U	1.0 U	8.0 U	0.50 U	0.50 U
IW–2	5/25/2010	6.74	–112	**4800**	0.05 U	0.05 U	**0.77**	**0.46**	**0.25**	0.20 U	4.7 U	**27**	**81**
IW–2	11/16/2010	7.04	–144	**180**	0.30 U	0.30 U	0.30 U	0.30 U	**0.003**	**0.017**	**4.4**	**9.6**	**32**
IW–2	5/25/2011	6.98	–184	**65**	0.002 U	0.002 U	0.002 U	0.002 U	**0.006**	**0.067**	**12**	**0.19**	**0.33**
IW–4	7/28/2009	6.23	–195	**21**	**0.025**	**0.041**	**18**	**2.9**	0.10 U	0.10 U	1.1 U	0.50 U	0.50 U
IW–4	5/25/2010	6.15	–112	**6.2**	**0.041**	0.020 U	**2.5**	**1.9**	**0.096**	0.04 U	1.0 U	0.50 U	0.50 U
IW–4	11/17/2010	6.36	–144	**2.4**	0.004 U	**0.009**	**0.25**	**0.4**	**0.22**	**0.012**	**4.8**	0.10 U	0.10 U
IW–4	5/24/2011	7.07	52	**3.9**	0.010 U	**0.021**	**0.71**	**1.1**	**0.36**	**0.006**	**2.4**	0.25 U	0.25 U

Exhibit 4. Pre- and post-EHC®/BounTA™ source area data

micronutrient source. A total of 31 locations received the combined amendment slurry. Injections were generally located between prior oil injection locations to improve electron donor homogeny in the source area. Injection quantities had to be reduced at most locations due to amendment surfacing through cracks up to 5 ft away from the probe location. EHC®/BounTA™ injection locations are shown in Exhibit 2 as bold dots with cross-hairs and are labeled 1S through 31S.

It is the authors' experience that failure to achieve complete dechlorination is a function of insufficient or unbalanced nutrient availability. The primary purpose of the February 2010 injections was to provide strong nutrient support to develop a robust anaerobic bacterial population, including dehalococcoides. As is evident by the data presented in Exhibit 4, this process was successful in generating strong anaerobic metabolic activity, including stimulating complete microbial dechlorination, methanogenesis, and production of appreciable concentrations of both acetone and MEK in all but one source area well.

Acetone and MEK Observations

Following the EHC® and BounTA™ injections, concentrations of metabolic intermediates acetone and MEK increased up to 27 mg/L and 81 mg/L, respectively. The highest concentrations of these compounds were noted 3 months after the injections were completed. Subsequent monitoring indicates that concentrations of both acetone and MEK have substantially attenuated over time. We have noted an interesting correlation between increasing methanogenesis and concentration reductions in acetone and MEK at this site. It is unclear, based on this data set, whether the methanogens could be utilizing these compounds as electron donors or simply outcompeting the microbes producing acetone and MEK as dissolved organic carbon becomes less readily available. While not included earlier, it is important to note that acetone and MEK were not detected in any monitoring wells outside of the injection area.

Case Study 2: NuWay II Drycleaner Site, Lebanon, Oregon

Background

This former drycleaner began remediation of a dissolved cVOC plume in 1998. COCs include PCE, TCE, cDCE, VC, and Stoddard Solvent as a light nonaqueous phase liquid. A groundwater pump-and-treat system operated from 1998 through 2004. In 2005, a 5-month reductive bioremediation pilot test was completed. Based on those results, an interim removal action measure (IRAM) using reductive bioremediation was conducted for additional areas from October 2007 through September 2008. The site is currently in monitored natural attenuation. The acetone and MEK detected at this site were potentially associated with the anaerobic degradation of an anomalous slug of dissolved toluene moving through the site.

Geology and Hydrogeology

The geology beneath the site consists of 5 to 11 ft of medium stiff, brown and gray silt overlying dense, silty, sandy gravel. The gravel unit is underlain by a second silt and clay layer at a depth of about 25 ft below ground surface (bgs). This second silt and clay layer acts as a partial confining unit for the shallow aquifer. The site-monitoring wells and the extraction wells are completed in the shallow gravel unit. The depth to groundwater varies from 5 to 10 ft bgs with a gradient generally toward the northwest at approximately 0.004 to 0.007 ft per ft. Localized mounding along the east side of the site suggests that a vitrified clay sewer line running under the alley along the east side of the site is leaking.

Bioremediation Activities

Reductive bioremediation treatment at the site was achieved using a closed-loop groundwater recirculation system combined with targeted slug injections. Electron donors used include CarBstrate™, ethanol, and lactic acid. CarBstrate™ is a dextrose-based amendment with micro- and macronutrient fortification. During the pilot test, IN-1 and IN-2 were used for injection and P-1 was used as the sole extraction point (Exhibit 5). For the IRAM, monitoring well (MW) NII-1S was used as the injection point to enhance

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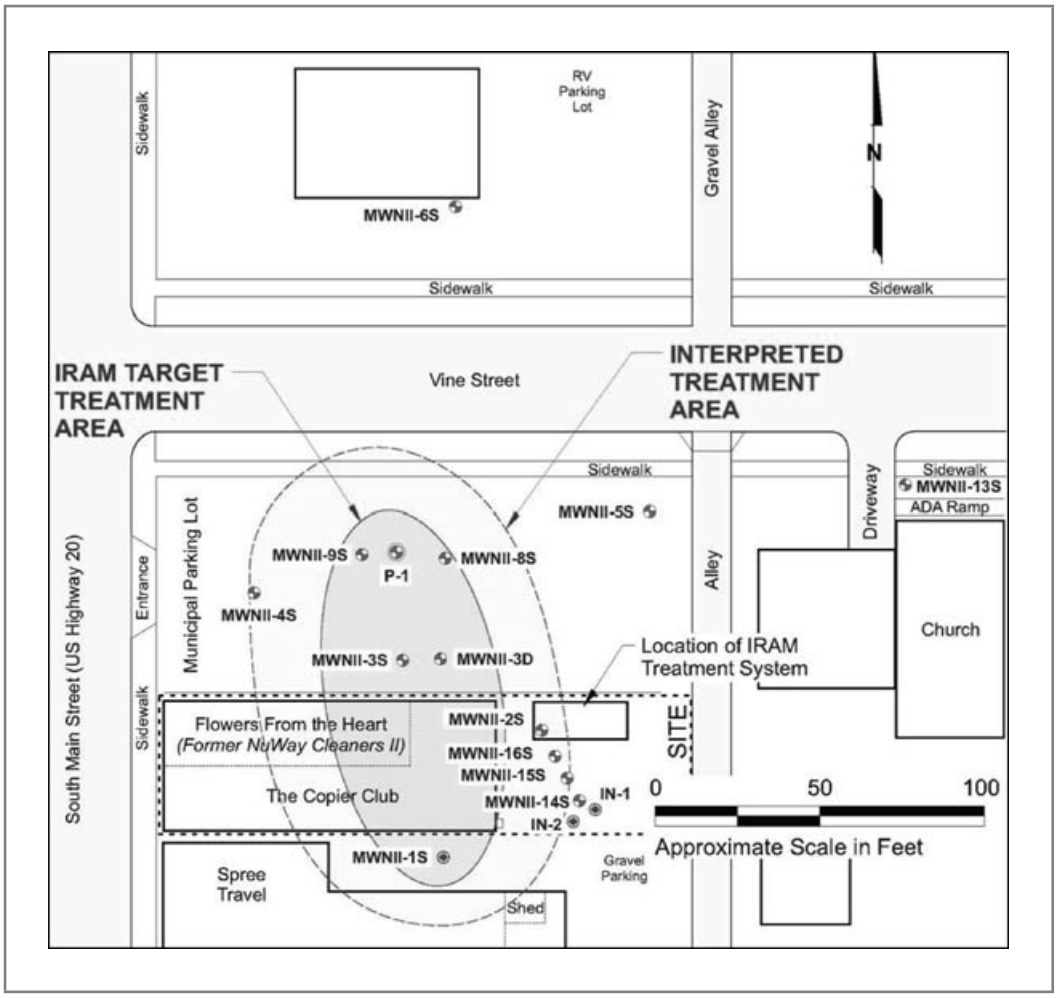

Exhibit 5. NuWay II site plan

treatment under the adjacent building. To enhance electron donor distribution, MW-NII-4S, -5S, and -6S all received three quarterly slug injections of amendment.

Over the course of the two system operational periods, 4,425 lb. CarBstrate™ and approximately 2,900 lb ethanol and lactate were introduced. No "*cis*-stall" was noted during either remediation phase, and both complete dechlorination and methanogenesis were evident. Ethene and ethane were detected at concentrations up to 150 μg/L and 160 μg/L, respectively. Dissolved methane was detected up to 24,000 μg/L beginning March 2008 across the IRAM treatment area. However, prior to March 2008, methane concentrations were much lower and typically ranged between 3,000 μg/L and 5,000 μg/L.

Acetone and MEK Observations

During the initial pilot test using CarBstrate™ only, no acetone or MEK was noted in groundwater at the site. During the expanded IRAM, acetone and MEK were detected in multiple wells beginning January 2008. Detections were closely associated with a slug of toluene moving through the site, which was detected at dissolved concentrations up to

Monitoring Well MWNII-5S	PCE (µg/L)	TCE (µg/L)	c-DCE (µg/L)	t-DCE (µg/L)	VC (µg/L)	Other Detected VOCs (µg/L)
23-Mar-07	**108**	**34.6**	**3.44**	**1.72**	< 0.5	ND
29-Jun-07	**105**	**38.9**	**4.60**	**1.83**	< 0.5	ND
28-Sep-07	**54.9**	**19.2**	**2.44**	**0.840**	< 0.5	ND
8-Nov-07	< 10	< 10	**31.4**	< 10	< 10	**Toluene (1,350)**
12-Dec-07	< 100	< 100	< 100	< 100	< 100	**Toluene (7,650)**
10-Jan-08	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	**Acetone (184), MEK (122)**
15-Feb-08	< 100	< 100	< 100	< 100	< 100	**Toluene (16,800)**
14-Mar-08	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5	**Toluene (1,890)**
9-Apr-08	< 25	< 25	< 25	< 25	< 25	**Toluene (3,270)**
8-May-08	< 25	< 25	< 25	< 25	< 25	**Toluene (4,650)**
24-Sep-08	< 25	< 25	< 25	< 25	< 25	**Toluene (1,440)**
9-Dec-08	< 25	< 25	< 25	< 25	< 25	**Toluene (523)**
15-Jun-09	<0.50	<0.50	**0.61**	<0.50	<0.50	ND
1-Dec-09	<0.50	<0.50	<0.50	<0.50	<0.50	ND

Exhibit 6. NuWay II well MW-NII-5S groundwater quality data

16,800 µg/L. Based on the pattern of detections, it is believed that disposal of toluene into the sewer system made its way into the aquifer through cracks in the sewer pipe. Exhibit 6 presents data from MW-NII-5S, the well believed to be closest to the source of the leak. In this well, acetone and MEK detections were very brief and transient. It was noted during monthly sampling of MW-NII-5S that the toluene concentrations were gradually increasing but disappeared when acetone and MEK were detected. The slug of toluene detected during February 2008 may have obscured acetone or MEK due to elevated detection limits. Dissolved methane was first analyzed in this well during March 2008 (not tabulated) and was detected at 14,000 µg/L.

Downgradient from MW-NII-5S, MEK was detected in MW-NII-6S at much higher concentrations (1,520 µg/L) than those noted in MW-NII-5S (Exhibit 7). Acetone was never detected in the MW-NII-6S. The leading edge of the toluene slug, detected in MW-NII-5S beginning November 2007, may have been have been attenuated by existing microbes by the time it reached MW-NII-6S. Alternatively, MEK simply may have been migrating faster with groundwater compared to the more hydrophobic toluene. Dissolved methane was routinely analyzed in MW-NII-6S, and concentrations prior to February 2008 were all less than 500 µg/L. Methane concentrations jumped to 4,200 µg/L in February 2008 and 10,000 µg/L by March 2008. This increase in dissolved methane concentrations was concurrent with the disappearance of MEK from MW-NII-6S.

MW-NII-4S, located on the west side of the hydraulic mounding associated with ongoing groundwater injection, also experienced a similar detection of MEK in January 2008 associated with the leading edge of the toluene slug. As the data are very similar to MW-NII-6S, tabulated data are not included. MEK was detected in MW-NII-4S only during one event (January 2008) at 78.7 µg/L. Toluene was detected in January 2008 at

Remediation DOI: 10.1002/rem

Monitoring Well MWNII-6S	PCE (µg/L)	TCE (µg/L)	c-DCE (µg/L)	t-DCE (µg/L)	VC (µg/L)	Other Detected VOCs (µg/L)
29-Jun-07	**565**	**172**	**342**	**7.25**	**3.55**	ND
28-Sep-07	**726**	**178**	**330**	**6.04**	**2.92**	ND
8-Nov-07	**9.4**	**9.2**	**751**	<5.0	< 5.0	ND
12-Dec-07	< 5.0	< 5.0	**716**	**6.7**	< 5.0	ND
10-Jan-08	< 50	< 50	< 50	< 50	< 50	**MEK (1,520)**
15-Feb-08	**7.68**	**5.72**	**71.3**	< 1.0	< 1.0	**Toluene (248)**
14-Mar-08	**5.83**	**6.51**	**208**	**2.56**	**12.6**	**Toluene (298)**
9-Apr-08	< 25	< 25	**156**	< 25	**26.5**	**Toluene (878)**
8-May-08	< 25	< 25	**75.0**	< 25	< 25	**Toluene (6,790)**
24-Sep-08	< 50	< 50	< 50	< 50	< 50	**Toluene (3,140)**
9-Dec-08	< 50	< 50	< 50	< 50	< 50	**Toluene (1,820)**
15-Jun-09	<25	<25	<25	<25	<25	**Toluene (1,100)**
1-Dec-09	< 0.50	**0.60**	**1.09**	< 0.50	< 0.50	**Toluene (2.13)**

Exhibit 7. NuWay II well MW-NII-6S groundwater quality data

4.5 µg/L and subsequently increased to 2,300 µg/L in February 2008. Concurrently, methane increased from 5,700 µg/L in January 2008 to 9,900 µg/L in February 2008, and 16,000 µg/L in March 2008.

Case Study 3: Springvilla Drycleaner Site, Springfield, Oregon

Background

This former drycleaner began remediation of dissolved cVOC plume in 2004. COCs include PCE, TCE, cDCE, and VC. Source area excavation and permanganate chemical oxidation were completed during 2004 and 2005. Reductive bioremediation IRAM was selected to remediate the groundwater plume from 2007 through 2009. The site is currently in monitored natural attenuation. The acetone detected at this site was likely associated with a change in the location of electron donor and the rapid fermentation of ethanol and/or lactate.

Geology and Hydrogeology

The geology beneath the site consists of unconsolidated alluvial deposits to at least 100 ft bgs. Shallow alluvial deposits consist of 9 to 11 ft of clayey silt to silty clay overlying gravel with varying amounts of sand and silt. Due to observed differences in hydraulic response, the gravel unit was divided into shallow and intermediate zones. The shallow zone extends to approximately 25 ft bgs, and the intermediate zone extends to greater than 100 ft bgs. Depth to groundwater in both aquifer zones varies from 8 to 12 ft bgs with a gradient generally toward the west at approximately 0.005 to 0.006 ft per ft.

Bioremediation Activities

Reductive bioremediation treatment at the site was achieved using a closed-loop groundwater recirculation system combined with targeted amendment slug injections. Following extraction of groundwater, some oxygen could diffuse into the holding tank prior to reinjection. Electron donors used include CarBstrate™, ethanol, lactate, emulsified vegetable oil products (Remediation and Natural Attenuation Service's Newman Zone® buffered, nonionic product and JRW Bioremediation's LactOil®), and micro- and macronutrient fortification. Amendments were distributed across the approximately 5 million gal aquifer using multiple flow cells created by different groupings of injection and extraction locations.

Reductive bioremediation treatment at the site was achieved using a closed-loop groundwater recirculation system combined with targeted amendment slug injections.

Over the 24 months of the bioremediation IRAM activities, 13,700 lb CarBstrate™, 25,140 lb ethanol and lactate, 15,450 lb emulsified vegetable oil products, and 960 lb nutrients were introduced. No "*cis*-stall" was noted, and both complete dechlorination and methanogenesis were evident. Ethane was the end product typically detected in the shallow zone, with concentrations up to 15 μg/L. Ethene was the primary end product in the intermediate zone, with concentrations ranging up to 86 μg/L. Dissolved methane was detected up to 16,200 μg/L in the shallow zone and 9,900 μg/L in the intermediate zone. However, typical methane concentrations ranged between 3,000 and 7,000 μg/L in both aquifer zones. See Fowler and Dockter (2010) for more detailed information regarding site activities.

Acetone and MEK Observations

Acetone was detected as a fermentation intermediary following conversion of previously extracting shallow well MW-3 (EX-2s) into an injection well (Exhibit 8) in early February 2009. Extraction continued in other shallow wells nearby, including cross-gradient MW-16 (EX-3s) and downgradient MW-22 (EX-1s). During the 35-day injection operation, approximately 85,000 gal groundwater amended with 980 lb complex lactates was introduced into MW-3; MW-16 and MW-22 extracted 51,000 and 7,700 gal groundwater, respectively. Injection and extraction operation of MW-3 was discontinued for 25 days prior to sampling conducted during April 2009.

As shown in Exhibit 9, acetone was detected in shallow zone well MW-3 and cross-gradient MW-16 during April 2009 at concentrations of 281 μg/L and 678 μg/L, respectively. Acetone was not detected in downgradient MW-22, and MEK was not detected in any shallow wells during April 2009. Unfortunately, many of the sampling events included only the short-list cVOCs instead of the full list of VOCs (labeled "NS" on the exhibit), so more comprehensive analysis of acetone persistence is not possible.

Case Study 4: Confidential Superfund Site, Northeast United States

Background

The site is a former waste oil recycling facility that has undergone various remedial actions since 1982. A hydraulic containment system was installed to manage a cDCE plume (about 300 μg/L) emanating from residual materials beneath a mass of lagoon bottoms that were solidified/stabilized and covered with a Resource Conservation and Recovery Act "B" cap.

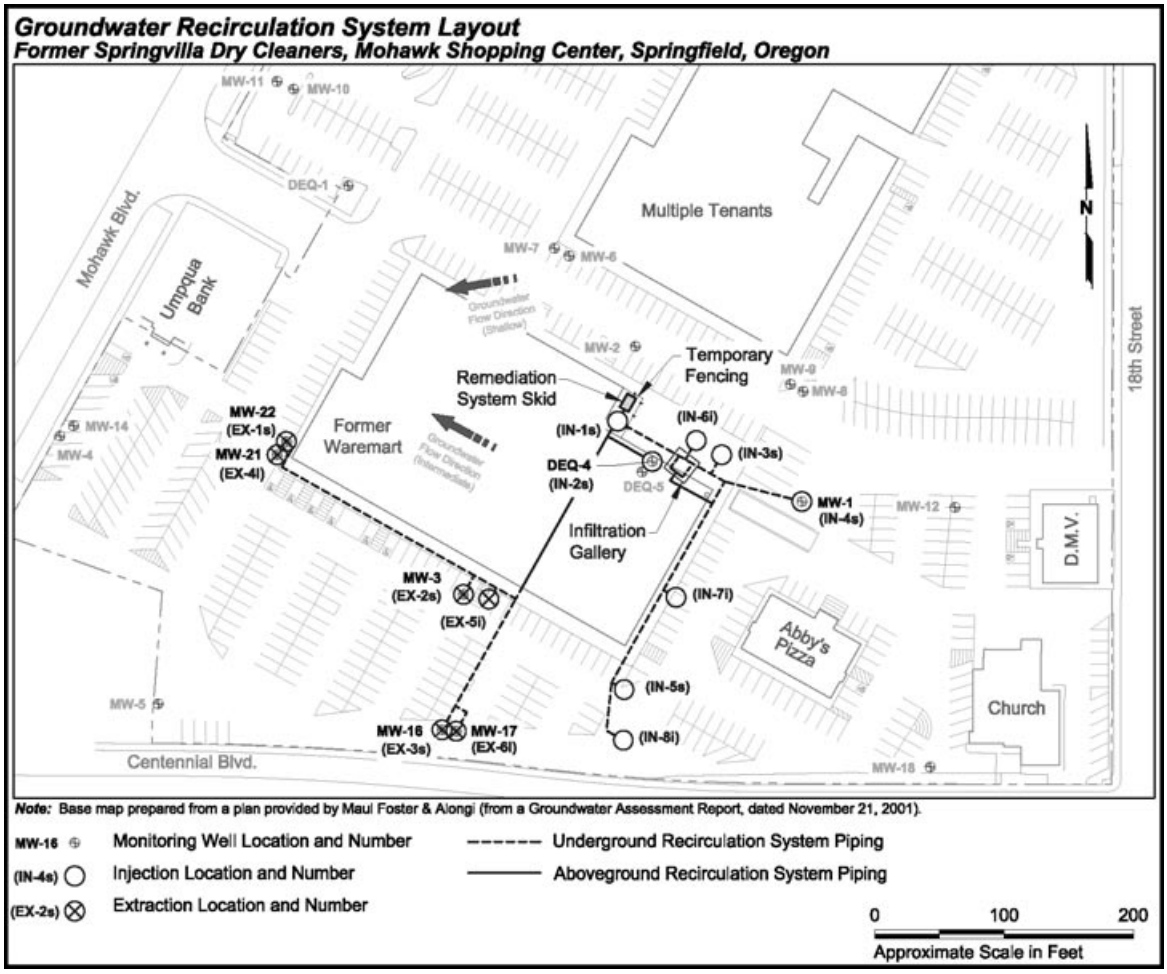

Exhibit 8. Springvilla site plan

Geology and Hydrogeology

The overburden aquifer consists of coarse to fine sand and silts, with varying amounts of gravel and clay. Groundwater was located at 6 to 8 ft bgs and flowed through the unconsolidated glacial materials at a velocity of 25 to 50 ft per year (ft/yr). The reported groundwater velocity is about 60 ft/yr. The pH is circum-neutral, Eh values were below +100 mV, dissolved oxygen levels were below 1.0 mg/L, and sulfate concentrations ranged from 10 to 50 mg/L. Appreciable concentrations of methane and ethene were detected in most of the shallow wells, indicating that the aquifer was anaerobic and amenable to bioremediation approaches (i.e., no bioaugmentation was required).

Bioremediation Activities

During September/October 2009, a 200-ft-long EHC® -amended zone situated perpendicular to the groundwater flow direction was created to control the cDCE plume

Well	Sample Date	PCE	TCE	cDCE	VC	Ethene	Ethane	Methane	Acetone	MEK
		(micrograms per liter [µg/L])								
MW-3	10-Apr-08	**584**	<20	<20	<20	<0.5	<0.5	**32**	NS	NS
	12-May-08	**593**	**16.0**	**10.8**	<2.5	--	--	--	NS	NS
	9-Jul-08	**494**	**13.8**	**13.2**	<10	--	--	--	NS	NS
	10-Sep-08	**310**	<5.00	<5.00	<5.00	<0.5	<0.5	**6,200**	NS	NS
	2-Dec-08	**428**	**13.2**	**18**	<2.50	<0.5	<0.5	**14,700**	NS	NS
	7-Apr-09	<5.00	<5.00	<5.00	**7.25**	<0.5	<0.5	**16,200**	**281**	<50
	1-Apr-10	<1.0	<1.0	<1.0	<1.0	<13	**15**	**8,400**	<50	<10
	21-Oct-10	<1.0	<1.0	<1.0	<1.0	<57	<40	**3,000**	NS	NS
	5-Apr-11	<1.0	<1.0	<1.0	<1.0	<1.3	**15**	**5,200**	NS	NS
MW-16	10-Apr-08	**647**	<20	<20	<20	<0.5	<0.5	**3.9**	NS	NS
	12-May-08	**588**	**16.2**	<10	<10	NS	NS	NS	NS	NS
	9-Jul-08	**487**	**12.0**	**21.0**	<10	--	--	--	NS	NS
	10-Sep-08	**626**	**27.8**	**39.2**	<10	**0.35**	<0.5	**2,500**	NS	NS
	2-Dec-08	**492**	**16.2**	**25.3**	**10.7**	<0.5	<0.5	**9,430**	NS	NS
	7-Apr-09	**41.2**	**10.5**	**104**	**65.9**	<0.5	**13.8**	**5,890**	**679**	<100
	1-Jul-09	**33**	**14**	**150**	**52**	<52	<52	**880**	NS	NS
	7-Oct-09	<1.0	<1.0	<1.0	<1.0	--	--	--	NS	NS
	1-Apr-10	<1.0	<1.0	<1.0	**3.3**	<13	<13	**7,000**	<50	<10
	21-Oct-10	<1.0	<1.0	<1.0	<1.0	<40	<57	**5,400**	NS	NS
	5-Apr-11	<1.0	<1.0	2.8	**6.2**	<25	<25	**4,800**	NS	NS

Exhibit 9. Springvilla MW-3 and MW-16 groundwater quality data

and replace the hydraulic containment system. The targeted depth interval was from 6 to 35 ft bgs. The area received 30,050 lb EHC® applied to 8 injection points (Exhibit 10). It should also be noted that supplemental guar (a biodegradable carbon source) was used by the injection contractor during the EHC® mixing and suspension process.

Acetone and MEK Observations

Following EHC® injection via hydrofracturing, rapid and effective cDCE removal (without the accumulation of catabolites) was associated with the detection of MEK in wells YO-119 and YO-117D and, to a lesser degree, in wells YO-117S, YO-14ALX, YO-14X, and YO-12AX (Exhibit 11). Acetone generation was noted as a transient effect at YO-12AX in the March and June 2010 sampling events (Exhibit 12). Data from these two wells indicated that the occurrence of MEK coincided with increases in concentrations of TOC and dissolved iron. Therefore, it is likely that the production of MEK was due to enhanced microbial activity influenced by EHC, as desired.

Data from the July 2010 sampling event showed that the MEK concentration decreased in well YO-119, demonstrating the transient nature of the process. Specifically, MEK concentrations stabilized or decreased at well locations YO-119, YO-117D, and YO-12AX (Exhibit 12). However, based on the most recent data (samples collected on July 29, 2010), MEK concentrations were increasing at wells YO-14ALX and YO-117S.

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Exhibit 10. Site E: Design of EHC® *in situ* chemical reduction

EHC® PRB Dimensions	Value	Unit
PRB length	200	ft
PRB width	15	ft
Depth to top of plume	6	ft bgs
Depth to bottom of plume	35	ft bgs
PRB thickness	29.0	ft
PRB volume	87,000	ft^3
Mass of soil in PRB	5,003	U.S. tons
Estimated porosity	30%	
Volume pore space	26,524	ft^3
EHC® Mass Calculations		
Percentage EHC® by soil mass	0.30%	
Linear groundwater velocity	0.16	ft/day
Contact time	94	days
Contact time × application rate multiplier	28	days*%EHC®
Mass of EHC® required	30,050	lb
Mass of EHC® required per sq ft of face area	5.2	lb/ft^2

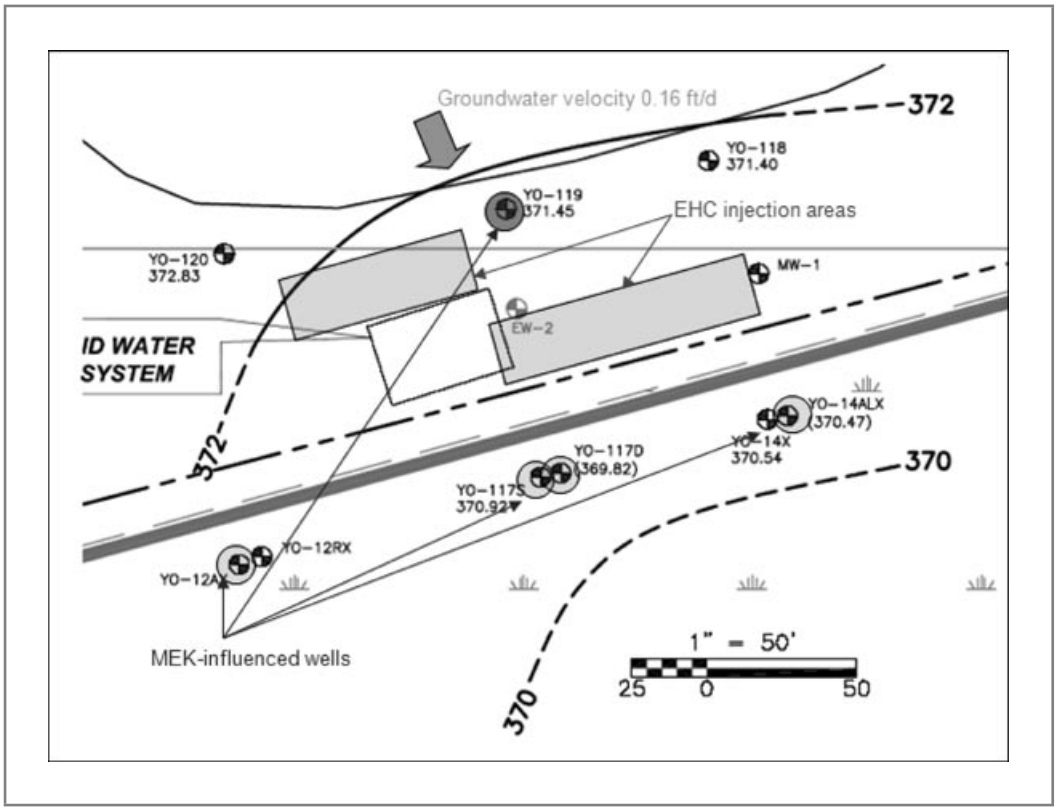

Exhibit 11. Site E: Location of monitoring wells and MEK impacts (dark gray circles indicate higher MEK; light gray circles indicate lower MEK concentrations)

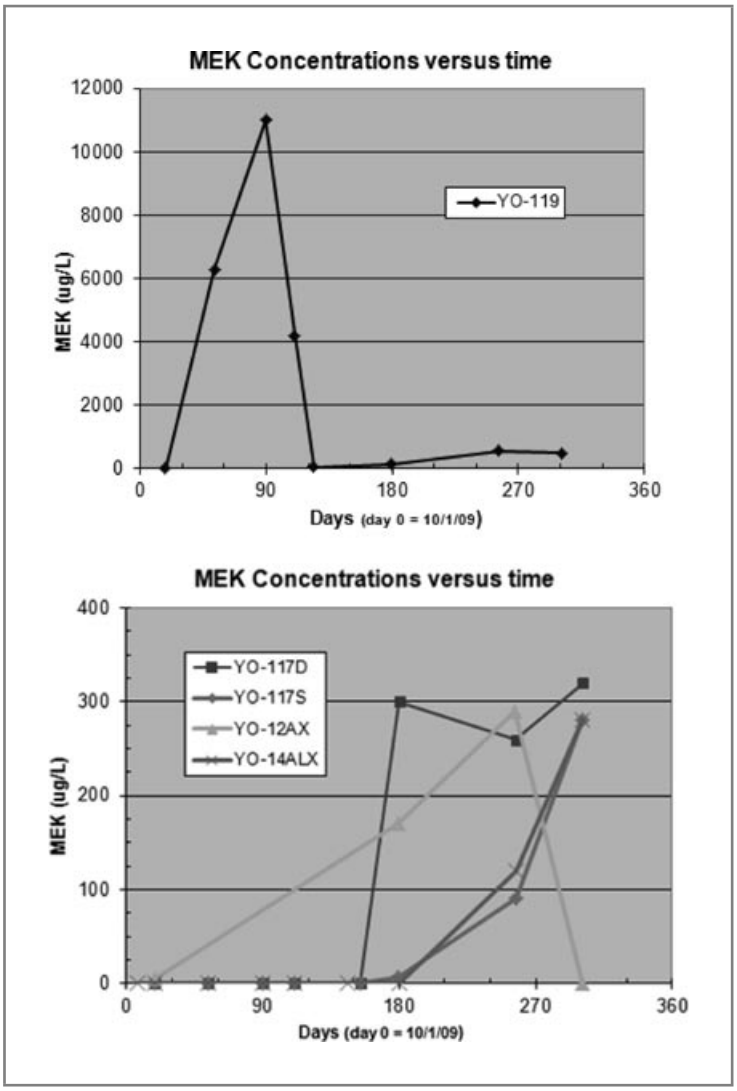

Exhibit 12. Site E: MEK time histories

Analysis of site-specific degradation rates is not yet practical, but based on the data in the downward slopes for MEK concentrations at YO-119 and YO-117D, it is possible to hypothesize half-lives ranging from 2 to 14 days (using YO-119D data) and up to 370 days (using YO-117D data). Using a range of half-lives of 20 to 200 days (which is consistent with literature values discussed earlier)—and assuming no further increases of MEK concentrations (consistent with concentration trends at YO-119 and YO-117D)—it would take between 3 months and 3 years for the June 2010 MEK concentrations to drop below cleanup criteria (Exhibit 13). However, based on observations and experiences at other sites, it is anticipated that the presence of MEK will rapidly decrease and will not be an issue within 12 to 18 months.

Predictably, the MEK concentrations at this site were closely correlated with the TOC levels (Exhibits 14 and 15). With respect to acetone detections on-site, the data are mixed. The historic acetone data at downgradient wells showed sporadic hits above the 5 μg/L performance standards, with a maximum of 50 μg/L detected in April 2001 at YO-12A. More typical detections are in the range of 5 to 16 μg/L. While these

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Exhibit 13. Site E: Hypothetical time for MEK to drop from July 2010 observed concentrations to below standards

			Time to Drop Below 5 μg/L (Days)	
Well	MEK μg/L in June 2010	Number of Half-Lives to Drop Below 5 μg/L	20-Day Half-Life	200-Day Half-Life
YO-119	480	7	132	1,317
YO-117D	320	6	120	1,200

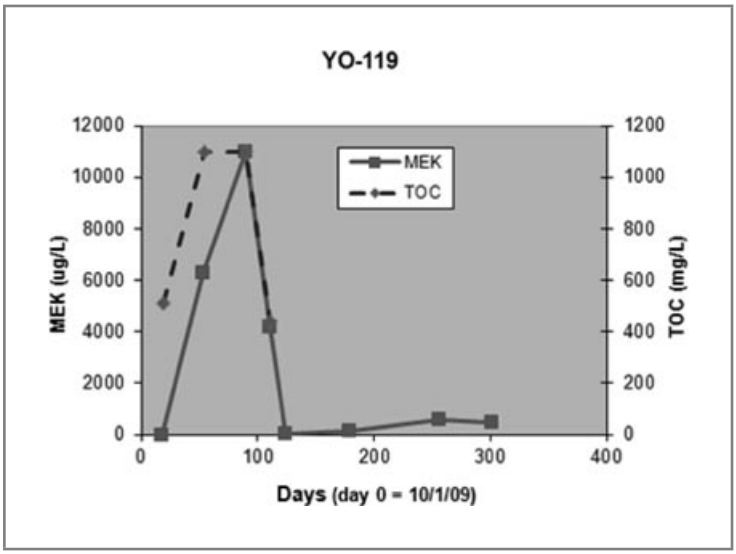

Exhibit 14. Site E: MEK and TOC in YO-119

detections are not conclusively linked to any one process, acetone was detected in several other wells in the April 2001 round of sampling, suggesting that those results may have been associated with laboratory contamination. The monitoring area consists of wetlands, which may provide sufficient TOC for naturally occurring processes to generate acetone. Nevertheless, it is presumed that the acetone detected in March and June 2010 at YO-12AX at concentrations of 71 μg/L and 80 μg/L, respectively, are the result of the EHC additions and subsequent fermentation processes.

DISCUSSION

The authors' experience has shown that acetone and MEK IPCs can be generated at relatively high concentrations during both biological and chemical remediation projects. Biologically derived acetone and MEK are observed primarily when using carbohydrate-based amendments. These amendments include glucose, dextrose, and cellulose-derived electron donors such as EHC®. Numerous bacterial and yeast species

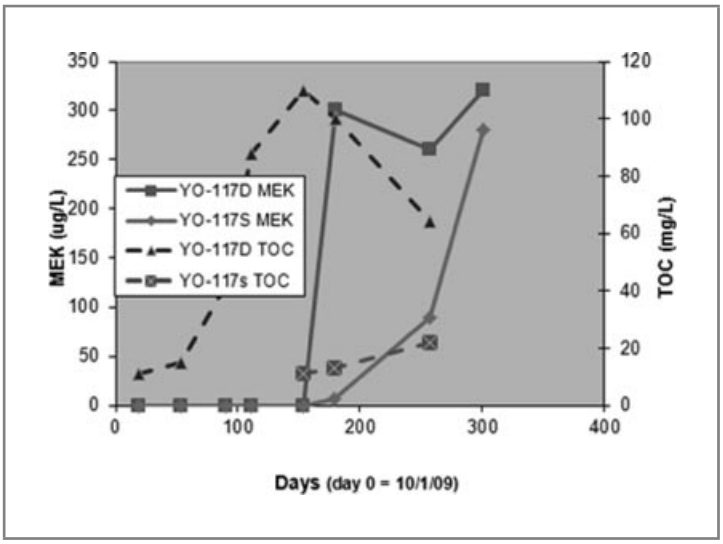

Exhibit 15. Site E: MEK and TOC in YO-117S and YO-117D

can convert carbohydrates, volatile fatty acids, and alcohols into solvents such as acetone and MEK.

The authors have also observed certain conditions under which high concentrations of acetone and MEK can accumulate due to biological activity. First, acetone and MEK are detected primarily after a sudden increase in bioavailable electron donor occurs. The NuWayII case study suggests that other ring compounds, such as toluene, may also be suitable for triggering the accumulation of these IPCs. Genera, such as spore-forming *Clostridium*, may have an initial advantage in fermenting newly available electron donors and subsequently secreting acetone and MEK as metabolic intermediates to maximize respiration of higher-energy compounds. However, it is most likely that other elements of the aquifer microflora are also associated and the generation of ketones is predictably not limited to *Clostridium*.

The accumulation of acetone and MEK in groundwater is not usually observed under strongly methanogenic conditions. Once methanogenesis pushes dissolved methane concentrations above 5,000 μg/L, acetone and MEK typically are observed only at very low levels, if at all. It is not clear why this occurs. It may be because methanogens, once well established, outcompete the microbes generating acetone and MEK for available carbon. It could also be related to methanogens consuming acetone and MEK as electron donors for the generation of their metabolic by-product, methane.

As noted in a variety of guidance documents (e.g., Henry, 2010), observed acetone and/or MEK following a biologically based remedial action may be related to the fermentation of relatively high amounts of carbon. The amount of acetone/MEK produced will likely be influenced by the amount and type of carbon amendment used and the site-specific microbial processes. There are cases where high concentrations of mobile electron donor sources generate acetone and MEK plumes near the leading edge of the electron donor slug. However, once the electron donor is consumed or methanogenesis is established, acetone and MEK no longer accumulate and rapidly disappear. In cellulose-based applications, where the source of electron donor does not move appreciably, acetone and MEK are not observed outside of the treated area.

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While acetone and MEK can accumulate in relatively high concentrations in areas meeting the criteria just discussed, the authors have never observed its migration beyond 50 meters from the treated area (Mueller, Moreno, Przepiora, Thompson, & Majer, 2011). Field evidence suggests that elevated levels of acetone and MEK in certain applications could be reported over a period of up to 18 months following injection in wells close to or within the treatment zone. At other sites, the similar production of these compounds should rapidly decline with time and attenuate within a short distance downgradient.

Finally, chemical reactions completed at cleanup sites can result in the accumulation of acetone and MEK through abiotic mechanisms. Many of these mechanisms seem to include iron, whether through ZVI, iron oxide reactions, iron-activated chemical oxidation in the absence of high carbonate concentrations, or reactions in iron-rich soils during thermal remediation.

REFERENCES

Aronson, D., Philip H., & Howard, P. H. (1997). Anaerobic biodegradation of organic chemicals in groundwater: A summary of field and laboratory studies. Prepared for: American Petroleum Institute, Chemical Manufacturer's Association, National Council of the Paper Industry for Air and Stream Improvement, Edison Electric Institute, and American Forest and Paper Association. North Syracuse, NY: Environmental Science Center, Syracuse Research Corporation.

Beyke, G., & Fleming, D. (2005). In situ thermal remediation of DNAPL and LNAPL using electrical resistance heating. Remediation Journal, 15, 5–22.

Braenden, C. I., Joenvall, H., Eklund, H., & Furugren, B. (1975). Alcohol dehydrogenases. In P. D. Boyer (Ed.), The Enzymes, Vol. 11 (pp. 103–190). New York, NY: Academic Press.

Brown, R. A., Mueller, J. G., Seech, A. G., Henderson, J. K., & Wilson, J. T. (2009). Interactions between biological and abiotic pathways in the reduction of chlorinated solvents. Remediation Journal, 20, 9–20.

Clausen, J. L., Hoyt, M., Wessling, E., Stearns, B., Ramirez, B., & Korte, N. (2004). Acetone production as a result of sodium bisulfate preservation of soil samples. Soil and Sediment Contamination: An International Journal, 13(3), 245–254.

Devinny, J. S., Deshusses, M. A., & Webster, T. S. (1999). Biofiltration for air pollution control. New York, NY: Lewis Publishers.

Fowler, T., & Dockter, C. (2010). Optimizing remediation strategies for a former dry-cleaner site. Remediation Journal, 20, 83–104.

Henry, B. (2010). Final report: Loading rates and impacts of substrate delivery for enhanced anaerobic bioremediation. ESTCP Project ER-0627. Arlington, VA: Environmental Security Technology Certification Program.

Hock, H., & Lang, S. (1944). Autoxydation von Kohlenwasserstoffen, IX. Mitteil.: Über Peroxyde von Benzol-Derivaten. Berichte der deutschen chemischen Gesellschaft (A and B ser.), 77, 257–264.

Hou, C. T., Patel, R., Laskin, A., Barnabe, N., & Marczak, I. (1979). Microbial oxidation of gaseous hydrocarbons: Production of methyl ketones from their corresponding secondary alcohols by methane- and methanol-grown microbes [Electronic version]. Applied and Environmental Microbiology, 38(1), 135–142.

Keen, A. R., Walker, N. J., & Peberdy, M. F. (1974). The formation of 2-butanone and 2-butanol in Cheddar cheese [Electronic version]. Journal of Dairy Research, 41, 249–257.

Lurie, A. P. (1967). Ketones. In Kirk-Othmer Encyclopedia of Chemical Technology (2nd ed.), Vol. 12 (pp. 101–169). New York, NY: John Wiley & Sons.

Monot, F., Martin J. R., Petitdemange, H., & Gay, R. (1982). Acetone and butanol production by Chlostridium acetobutylicum in a synthetic medium [Electronic version]. Applied and Environmental Microbiology, 44(6), 1318–1324.

Mueller, J., Moreno, J., Przepiora, A., Thompson, B., & Majer, T. (2011). Transient production of ketones during in situ treatment: Environmental fate of "fermentation" by-products. Battelle International Symposium on Bioremediation and Sustainable Environmental Technologies, Reno, Nevada.

Ng, T. K., Ben-Bassat, A., & Zeikus, J. G. (1981). Ethanol production by thermophilic bacteria: Fermentation of cellulosic substrates by cocultures of Clostridium thermocellum and Clostridium thermohydrosulfuricum [Electronic version]. Applied and Environmental Microbiology, 41(6), 1337–1343.

Speakman, H. B. (1920). Gas production during the acetone and butyl alcohol fermentation of starch. Journal of Biological Chemistry, 43, 401–411.

Suter, G. W., & Tsao, C. L. (1996). Toxicological benchmarks for screening potential contaminants of concern for effects on aquatic biota, 1996 Revision. ORNL publication ES/ER/TM-96/R2.

Suthersan, S. S., & Payne, F. C. (2005). In situ remediation engineering. Boca Raton, FL: RC Press.

U.S. Environmental Protection Agency. (2011). National primary drinking water regulation, list of contaminants and their MCLs. Retrieved August 9, 2011, from http://water.epa.gov/drink/contaminants/index.cfm#List.

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Bruce Thompson has more than 20 years of experience negotiating statements of work with regulatory agencies, managing investigations, remediation, and long-term operations and maintenance of Superfund sites on behalf of potentially responsible parties. He has negotiated and implemented cost-effective changes to remedies. He manages de maximis New England offices and serves on the board of directors. He received his BS in oceanography in 1985 from the United States Naval Academy.

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