KB-1® and KB-1® Plus for Remediation of Chlorinated Solvents

1. Phil Dennis, SiREM
2. Anaerobic bioaugmentation cultures containing the dechlorinating bacteria *Dehalococcoides*, *Dehalobacter*, *Dehalogenimonas and Geobacter*
3. MSDS/technical information attached
4. Number of field scale applications to date: hundreds of sites
5. Case studies attached

KB-1® and KB-1® Plus are natural, non-pathogenic, anaerobic microbial consortiums (mixed cultures) proven to rapidly and completely degrade chlorinated solvents such as tetrachloroethene (PCE), trichloroethene, cis-1,2-dichloroethene, 1,1-dichloroethene and vinyl chloride, 1,1,1-trichloroethane, 1,1-dichloroethane 1,2-dichloroethane, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, chloroform, and dichloromethane to non-toxic, environmentally acceptable, end products such as ethane, ethane and acetate. These cultures and were derived from naturally occurring bacterial populations found in soil and groundwater at chlorinated solvent sites located in North America and are not genetically modified.

The KB-1® and KB-1 Plus cultures are produced in SiREM's facility in Guelph, Ontario, under sterile conditions following stringent quality assurance/quality control (QA/QC) procedures. The cultures are routinely screened for pathogens and pathogens have not been detected since large scale production commenced in 2002. The cultures are shipped to the application site in stainless steel vessels by express courier and are applied under anaerobic conditions to prevent the exposure of oxygen sensitive microbes to air.

KB-1® and KB-1® Plus have been applied at more than 60 sites in California including several sites in the Los Angeles region. The cultures have received waste discharge requirement (WDR) approval from California Regional Water Quality Boards in 7 of 9 regions. KB-1® has also been approved for injection in other jurisdictions, KB-1® was added to Environment Canada's Domestic Substances List in 2008 (DSL) for use in groundwater remediation in Canada. KB-1®and selected KB-1® Plus cultures are approved as groundwater injectants by the North Carolina Department of Water Quality. KB-1® and KB-1® Plus were approved in 2012 for import into Australia and have a history of safe use in and in 39 US states, Canada, 5 European countries and Malaysia.

130 Research Lane, Ste 2
Guelph ON N1G 5G3
(519) 822-2265

KB-1® Material Safety Data Sheet

Section 1: Material Identification

Trade Name: KB-1®
Chemical Family: bacterial mixture
Chemical name: No IUC name for mixture is known to exist
Manufacturer/Supplier: SiREM
130 Research Lane, Suite 2,
Guelph, Ontario,
Canada N1G 5G3

For Information call: 519-822-2265 / 1-866-251-1747 x236
Emergency Number: 519-822-2265
Description: Microbial inoculum (non-pathogenic, non-hazardous)
Trade Name: KB-1®
Product Use: Bioremediation of contaminated groundwater.
Date Prepared: 2 February 2005

Section 2: Composition, Information on Ingredients

KB-1® is a microbial culture grown in an aqueous dilute mineral salt solution media containing no hazardous ingredients.

The microbial composition of KB-1® (as determined by phylogenetic analysis) is listed in Table 1. Identification of organisms was obtained by matching 16S rRNA gene sequence of organisms in KB-1® to other known organisms. The characteristics of related organisms can be used to identify potential or likely characteristics of organisms in KB-1®.

Table 1. Genus' Identified in KB-1® Microbial Inoculum

Genus
Dehalococcoides sp.
Geobacter sp.
Methanomethlovorans sp.

Section 3: Hazards Identification:

A review of the available data does not indicate any known health effects related to normal use of this product.

Section 4: First Aid Measures:

Avoid direct contact with skin and eyes. In any case of any exposure which elicits a response, a physician should be consulted immediately.

Eye Contact: Flush eyes with water for at least 15 minutes, occasionally lift upper and lower eyelids, if undue irritation or redness occurs seek medical attention.

Skin Contact: Remove contaminated clothing and wash skin thoroughly with water and antibacterial soap. Seek medical attention if irritation develops or open wounds are present.

Ingestion: Do not induce vomiting, drink several cups of water, seek medical attention.

Inhalation: Remove to fresh air. If not breathing give artificial respiration. In case of labored breathing give oxygen. Call a physician.

Section 5 - Fire Fighting Measures:

Non-flammable
Flash Point: not applicable
Upper flammable limit: not applicable
Lower flammable limit: not applicable

Section 6 – Accidental Release Procedures

Spilled KB-1® should be soaked up with sorbant and saturated with a 10% bleach solution (prepared by making a one in ten dilution of diluted standard bleach [normally sold at a strength of 5.25% sodium hypochlorite] to disinfect affected surfaces. Sorbant should be double bagged and disposed of as indicated in section 12. After removal of sorbant, area should be washed with 10% bleach solution to disinfect. If liquid from the culture vessel is present on the fittings, non-designated tubing or exterior of the stainless steel pressure vessel liquid should be wiped off and the area washed with 10% bleach solution.

Section 7 - Handling and Storage

KB-1® is shipped in stainless steel pressure vessels and connected to injection lines and inert gas is used to pressurize the vessel to displace the contents. KB-1® should be handled with care to avoid any spillage. Vessels are shipped with 1 pound per square inch (psi) pressure; valves should not be opened until connections to appropriate lines for subsurface injection are in place.

Storage Requirements: Avoid exposing stainless steel pressure vessels to undue temperature extremes (i.e., temperatures less than 0°C or greater than 30°C may result in harm to the microbial cultures and damage to the vessels). All valves should be in the closed position when the vessel is not pressurized or not in use to prevent the escape of gases and to maintain anaerobic conditions in the vessel. Avoid exposure of the culture to air as the presence of oxygen will kill dechlorinating microorganisms.

Section 8 - Exposure Controls/Personal Protection

Personal protective equipment:

Skin: Protective gloves (latex, vinyl or nitrile) should be worn.
Eye Protection: Wear appropriate protective eyeglasses or goggles when opening pressure vessels, valves, or when pressurizing vessels to inject contents into the subsurface.
Respiratory: No respiratory protection is required.
Engineering Controls: Good general room ventilation is expected to be adequate.

Section 9: Physical and Chemical Properties:

Physical State: liquid
Odour: skunky odour
Appearance: dark grey, slightly turbid liquid under anaerobic conditions, pink if exposed to air (oxygen).
Specific gravity: not determined
Vapor pressure: not applicable
Vapor density: not applicable
Evaporation rate: not determined
Boiling point: ~100° C
Freezing point/melting point: ~ 0°C

pH: 6.5-7.5
Solubility: fully soluble in water

Section 10 – Stability and Reactivity Data

Stable and non-reactive.
Maintain under anaerobic conditions to preserve product integrity.
Materials to avoid: none known

Section 11 - Toxicological Information

Potential for Pathogenicity:

KB-1® has tested negative (i.e., the organisms are not present) for a variety of pathogenic organisms listed in Table 2. While there is no evidence that virulent pathogenic organisms are present in KB-1®, there is potential that certain organisms in KB-1® may have the potential to act as opportunistic (mild) pathogens, particularly in individuals with open wounds and/or compromised immune systems. For this reason standard hygienic procedures such as hand washing after use should be observed.

Table 2, Results of Human Pathogen Screening of KB-1® Dechlorinator

Organism	**Disease(s) Caused**	***Test result***
Salmonella sp.	Typhoid fever, gastroenteritis	Not Detected
Listeria monocytogenes	Listerioses	Not Detected
Vibrio sp.,	Cholera, gastroenteritis	Not Detected
Campylobacter sp.,	Bacterial diarrhea	Not Detected
Clostridia sp.,	Food poisoning, Botulism, tetanus, gas gangrene	Not Detected
Bacillus anthracis	Anthrax	Not Detected
Pseudomonas aeruginosa	Wound infection	Not Detected
Yersinia sp.,	Bubonic Plague, intestinal infection	Not Detected
Yeast and Mold	Candidiasis, Yeast infection etc.	Not Detected
Fecal coliforms	Indicator organisms for many human pathogens diarrhea, urinary tract infections	Not Detected
Enterococci	Various opportunistic infections	Not Detected

Section 12. Disposal Considerations

Material must be disinfected or sterilized prior to disposal. Consult local regulations prior to disposal.

Section 13 – Transport Information

Non-hazardous, non-pathogenic microbial inoculum – Biosafety Risk Group 1.

Chemicals, Not Otherwise Indexed (NOI), Non-hazardous

Not subject to TDG or DOT guidelines.

Disclaimer:

The information provided on this MSDS sheet is based on current data and represents our opinion based on the current standard of practice as to the proper use and handling of this product under normal, reasonably foreseeable conditions.

Last revised: 2 August 2011

130 Research Lane, Ste 2
Guelph ON N1G 5G3
(519) 822-2265

KB-1® Plus Material Safety Data Sheet

Section 1: Material Identification

Trade Name: KB-1® Plus
Chemical Family: bacterial mixture
Chemical name: No IUC name for mixture is known to exist

Manufacturer/Supplier: SiREM
130 Research Lane, Suite 2,
Guelph, Ontario,
Canada N1G 5G3

For Information call: 519-822-2265 / 1-866-251-1747 x236
Emergency Number: 519-822-2265

Description: Microbial inoculum (non-pathogenic, non-hazardous)
Trade Name: KB-1® Plus
Product Use: Bioremediation of contaminated groundwater.
Date Prepared: 23 October 2008

Section 2: Composition, Information on Ingredients

KB-1® Plus is a microbial culture grown in a dilute aqueous mineral salt solution media containing no hazardous ingredients.

The microbial composition of KB-1® Plus is listed in Table 1.

Table 1. Major Microbial Groups Identified in KB-1® Plus Microbial Inoculum

Dehalococcoides sp.
Geobacter sp.
Methanomethylovorans sp.
Dehalobacter sp.
Dehalogenimonas sp.

Section 3: Hazards Identification:

A review of the available data does not indicate any known health effects related to normal use of this product.

Section 4: First Aid Measures:

Avoid direct contact with skin and eyes. In any case of any exposure which elicits a response, a physician should be consulted immediately.

Eye Contact: Flush eyes with water for at least 15 minutes, occasionally lift upper and lower eyelids, if undue irritation or redness occurs seek medical attention.

Skin Contact: Remove contaminated clothing and wash skin thoroughly with water and antibacterial soap. Seek medical attention if irritation develops or open wounds are present.

Ingestion: Do not induce vomiting, drink several cups of water, seek medical attention.

Inhalation: Remove to fresh air. If not breathing give artificial respiration. In case of labored breathing give oxygen. Call a physician.

Section 5 - Fire Fighting Measures:

Non-flammable
Flash Point: not applicable
Upper flammable limit: not applicable
Lower flammable limit: not applicable

Section 6 – Accidental Release Procedures

Spilled KB-1® Plus should be soaked up with sorbant and saturated with a 10% bleach solution (prepared by making a one in ten dilution of diluted standard bleach [normally sold at a strength of 5.25% sodium hypochlorite] to disinfect affected surfaces. Sorbant should be double bagged and disposed of as indicated in section 12. After removal of sorbant, area should be washed with 10% bleach solution to disinfect. If liquid from the culture vessel is present on the fittings, non-designated tubing or exterior of the stainless steel pressure vessel liquid should be wiped off and the area washed with 10% bleach solution.

Section 7 - Handling and Storage

KB-1® Plus is shipped in stainless steel pressure vessels in a protective over pack. KB-1® Plus should be handled with care to avoid any spillage. Vessels are shipped with 1 pound per square inch (psi) pressure; valves should not be opened until connections to appropriate lines for subsurface injection are in place.

Storage Requirements: Avoid exposing stainless steel pressure vessels to undue temperature extremes (i.e., temperatures less than 0°C or greater than 30°C may result in harm to the microbial cultures and damage to the vessels). All valves should be in the closed position when the vessel is not pressurized or not in use to prevent the escape of gases and to maintain anaerobic conditions in the vessel. Avoid exposure of the culture to air as the presence of oxygen will kill dechlorinating microorganisms.

Section 8 - Exposure Controls/Personal Protection

Personal protective equipment:

Skin: Protective gloves (latex, vinyl or nitrile) should be worn.
Eye Protection: Wear appropriate protective eyeglasses or goggles when opening pressure vessels, valves, or when pressurizing vessels to inject contents into the subsurface.
Respiratory: No respiratory protection is required.
Engineering Controls: Good general room ventilation is expected to be adequate.

Section 9: Physical and Chemical Properties:

Physical State: liquid
Odour: skunky odour
Appearance: dark grey, slightly turbid liquid under anaerobic conditions, pink if exposed to air (oxygen).
Specific gravity: 1
Vapor pressure: not applicable
Vapor density: not applicable
Evaporation rate: not determined
Boiling point: ~100° C
Freezing point/melting point: ~ 0°C

pH: 6.5-7.5
Solubility: fully soluble in water

Section 10 – Stability and Reactivity Data

Stable and non-reactive.
Maintain under anaerobic conditions to preserve product integrity.
Materials to avoid: none known

Section 11 - Toxicological Information

Potential for Pathogenicity:

KB-1® Plus has tested negative (i.e., the organisms are not present) for a variety of pathogenic organisms listed in Table 2. While there is no evidence that virulent pathogenic organisms are present in KB-1® Plus, there is potential that certain organisms in KB-1® Plus may have the potential to act as opportunistic (mild) pathogens, particularly in individuals with open wounds and/or compromised immune systems. For this reason standard hygienic procedures such as hand washing after use should be observed.

Table 2, Results of Human Pathogen Screening of KB-1® Plus

Organism	**Disease(s) Caused**	***Test result***
Salmonella sp.	Typhoid fever, gastroenteritis	Not Detected
Listeria monocytogenes	Listerioses	Not Detected
Vibrio sp.,	Cholera, gastroenteritis	Not Detected
Campylobacter sp.,	Bacterial diarrhea	Not Detected
Clostridia sp.,	Food poisoning, Botulism, tetanus, gas gangrene	Not Detected
Bacillus anthracis	Anthrax	Not Detected
Pseudomonas aeruginosa	Wound infection	Not Detected
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Fecal coliforms	Indicator organisms for many human pathogens diarrhea, urinary tract infections	Not Detected
Enterococci	Various opportunistic infections	Not Detected

Section 12. Disposal Considerations

Material must be disinfected or sterilized prior to disposal. Consult local regulations prior to disposal.

Section 13 – Transport Information

Non-hazardous, non-pathogenic microbial inoculum

Chemicals, Not Otherwise Indexed (NOI), Non-hazardous

Not subject to TDG or DOT guidelines.

Disclaimer:

The information provided on this MSDS sheet is based on current data and represents our opinion based on the current standard of practice as to the proper use and handling of this product under normal, reasonably foreseeable conditions.

Last revised: 12 June 2012

SiREM
Leading Science · Lasting Solutions

130 Research Lane, Ste 2
Guelph ON N1G 5G3
(519) 822-2265

Chemical Components in KB-1® Growth Media

KB-1® consists of a microbial culture grown in a mineral salts media containing the ingredients listed in Table 1.

Table 1: Chemical Ingredients of KB-1® growth media

Chemical Name	Formula	CAS#	Concentration grams/Liter
Potassium Phosphate Dibasic	KH_2PO_4	7758-11-4	0.27
Potassium Phosphate Monobasic	K_2HPO_4	7778-77-0	0.34
Ammonium Chloride	NH_4Cl	12125-02-9	0.535
Calcium Chloride	$CaCl_2$	10035-04-8	0.07
Magnesium Sulfate	$MgSO_4$	10034-99-8	0.125
Ferrous Chloride	$FeCl_2$	13478	0.02
Sodium bicarbonate	$NaHCO_3$	144-55-8	2.0
Ferrous Ammonium Sulfate	$(NH_4)_2Fe(SO_4)_2$	7783-85-9	0.4
Sodium sulfide	Na_2S	1313-84-4	0.12
Resazurin	$C_{12}H_6NNaO_4$	62758-13-8	0.001
Boric Acid	H_3BO_3	10043-35-3	0.0006
Zinc Chloride	$ZnCl$	7646-85-7	0.0002
Sodium Molybdate	Na_2MoO_4	10102-40-6	0.0002
Nickel II Chloride	$NiCl_2$	7791-20-0	0.0015
Manganese Chloride	$MnCl_2$	13446-34-9	0.002
Copper II Chloride	$CuCl_2$	10125-13-0	0.0002
Cobalt Chloride	$CoCl_2$	7791-13-1	0.003
Disodium Selenite	Na_2SeO_3	10102-18-8	0.00004
Aluminum Trisulfate	$Al_2(SO_4)_3$	10043-01-3	0.0002
Vitamins	Various	Various	0.01 maximum

Abstract I-22, in: A.R. Gavaskar and C.F. Silver (Symposium Chairs), *In Situ and On-Site Bioremediation—2007*. Proceedings of the Ninth International In Situ and On-Site Bioremediation Symposium (Baltimore, Maryland; May 7–10, 2007). ISBN 978-1-57477-161-9, published by Battelle Press, Columbus, OH, www.battelle.org/bookstore

Full-Scale Bioremediation of Chlorinated Ethenes in a High-Sulfate Aquifer at NAS North Island

Lisa Smith (lsmith@geosyntec.com), Mike Berman, Neal Durant, Eric Hood, Jamey Rosen, Marlaina Auger, and Peter Dollar
(GeoSyntec Consultants, Columbia, Maryland, USA)
Charles Perry (Base Realignment and Closure, San Diego, California, USA)
Michael Pound (Naval Facilities Engineering Command Southwest, San Diego, California, USA)

A combination of active (forced gradient) and passive (natural gradient) enhanced in situ bioremediation (EISB) treatment systems has been installed as the final removal action for a shallow aquifer impacted by *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC) at Operable Unit 24, Naval Air Station (NAS) North Island in Coronado, California. Specific challenges associated with this site include close proximity to San Diego Bay, high sulfate concentrations, the presence of a shallow saltwater interface beneath the site, low hydraulic conductivity of the target aquifer, and the need for bioaugmentation in addition to biostimulation.

A freshwater-saltwater interface occurs at approximately 35 feet below ground surface at the site and appears to effectively impede vertical migration of the chloroethene plume. However, sulfate is present in concentrations ranging from 50 to 1800 mg/L and will compete with chlorinated ethenes for electron donor added to the system. While conditions at the site are naturally anaerobic and support biological reductive dechlorination, the rate of natural attenuation is insufficient to prevent the horizontal migration of cDCE and VC to the San Diego Bay. *Dehalococcoides* (*Dhc*) bacteria were observed at several locations across the site, but *Dhc* containing the VC reductase gene (*Dhc-vcrA*) were detected at only one location. Biotreatability test results demonstrated that sulfate concentrations were rapidly reduced after the addition of electron donor, that bioaugmentation with a *Dhc-vcrA* culture (KB-1®), in combination with biostimulation, is necessary to achieve complete dechlorination of cDCE and VC to ethene, and that complete dechlorination was achieved in approximately one month, suggesting that a target two-year timeframe for full-scale remediation is reasonable.

Given the results of a series of pre-design investigations, an active recirculation system to amend groundwater with lactate and KB-1® was designed for the source area and a passive biobarrier system using emulsified soybean oil (ESO) and KB-1® was designed for the downgradient plume. To minimize entrainment of saltwater from beneath the freshwater-saltwater interface the recirculation system will operate at a relatively low flow rate of 1.5 gallons per minute. Lactate will be injected in weekly pulses to minimize biofouling of the injection wells and the recirculation system includes capacity to deliver chloride dioxide as a biofouling control agent. In the downgradient plume, seven ESO barriers have been installed, each spaced at a distance equivalent to a two-year groundwater travel time. Construction and start-up of both full-scale EISB systems will be complete by May 2007. Detailed performance monitoring will be conducted over the subsequent two-year operating period.

A-57, in: H.V. Rectanus and R. Sirabian (Chairs), *Bioremediation and Sustainable Environmental Technologies—2011.* International Symposium on Bioremediation and Sustainable Environmental Technologies (Reno, NV; June 27–30, 2011). ISBN 978-0-9819730-4-3, Battelle Memorial Institute, Columbus, OH. **www.battelle.org/biosymp**

EISB with Emulsified Vegetable Oil and Bioaugmentation at a Former Industrial Site in California

Leo Lehmicke (Independent Consultant, Redmond, Washington, USA)
Ken Puentes (Hargis + Associates, Inc., San Diego, California, USA)

Enhanced in situ biodegradation (EISB) was applied at a former manufacturing facility in California impacted with tetrachloroethene (PCE) and trichloroethene (TCE). Because of the presence of ketones and other anthropogenic electron donors, the daughter products *cis*-dichloroethene (cDCE) and vinyl chloride (VC) were also present in high concentration both on and offsite. Lower concentrations of ethene were also present. In 2005, a pilot test was performed at an onsite source area to evaluate emulsified vegetable oil (EVO) as a long term electron donor for EISB. The objectives of the pilot test were to evaluate well spacing, injection methods, radius of influence (ROI) of injected fluid, concentration of EVO and its longevity, and the efficacy of a bioaugmentation culture to improve complete dechlorination to ethene.

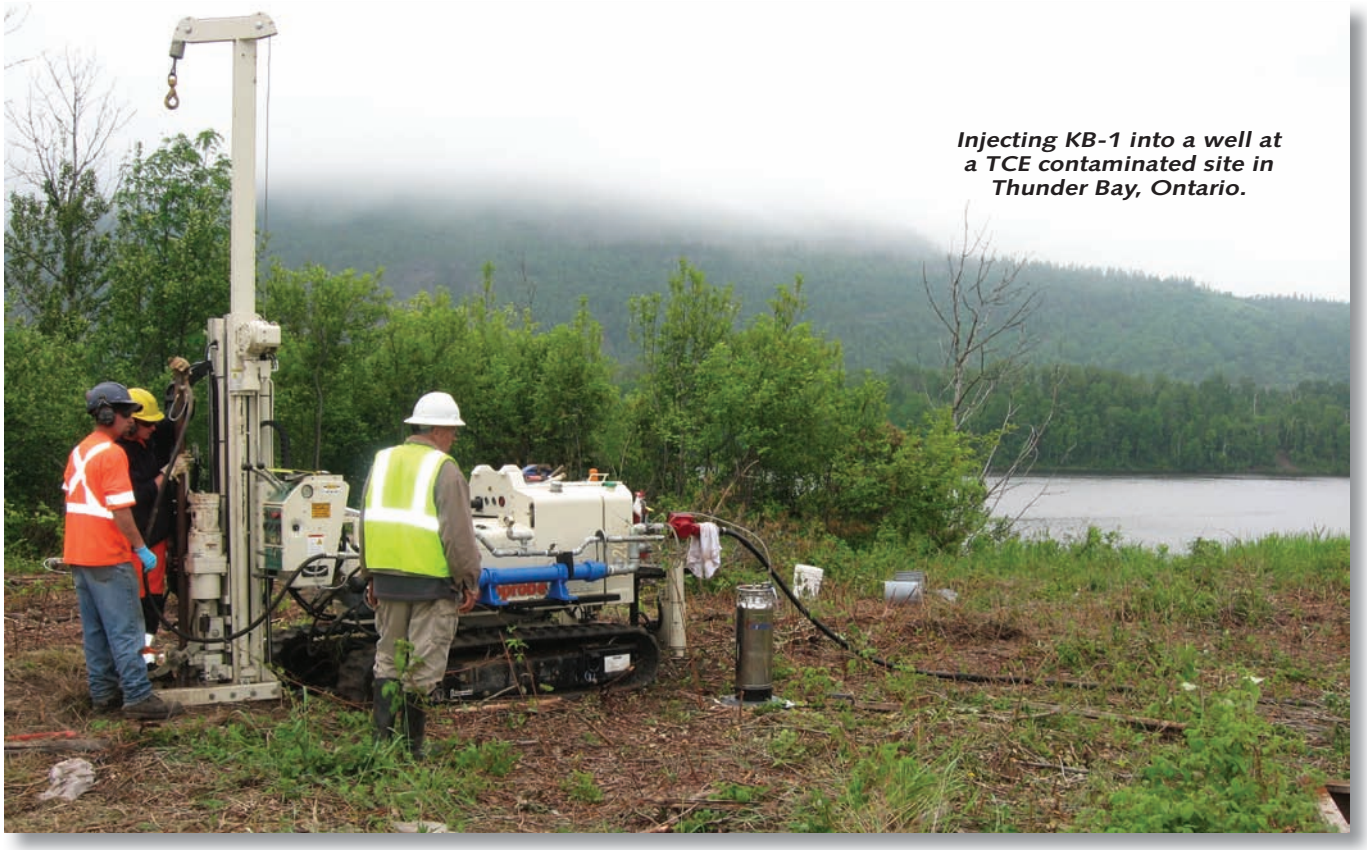
The pilot test included three injection points in two separate sand units at depths between 35 and 65 feet. Injections were performed using two 2-inch wells and one open borehole advanced by direct push methods. EVO was injected at concentrations between 1 and 3.2% after the injection wells were bioagumented with KB-1. Bromide was added as a tracer. Observed ROIs agreed with calculated ROIs. Baseline PCE, TCE, cDCE and VC concentrations in MW2, located 10 feet down gradient of an injection well, were roughly 5, 1, 40 and 2 mg/l. During the first year following injection, PCE and TCE declined below 1 mg/l and cDCE increased to 90 mg/l indicating that additional PCE and TCE sorbed to the soil matrix were also being degraded. By August 2007 (2.5 yrs), concentrations in MW2 had declined to 0.2 mg/l for PCE and TCE, and 0.5 mg/l for cDCE. Vinyl chloride increased slightly and ethene was observed at greater than 1 mg/l. Donor (as total organic carbon [TOC]) was still 500 mg/l at a year but declined by 2007.

A full-scale biobarrier was planned along the site boundary to reduce VOC mass flux offsite. At the baseline sampling event for full-scale implementation in 2009, ethene concentration in MW2 was more than 80% of the total chlorinated ethene mass. Additional donor was injected around MW2 during full-scale implementation. Currently PCE, TCE and cDCE concentrations in MW2 are non-detect, VC is less than 0.01 mg/l and ethene is more than 95% of the total mass. Two other monitoring wells have ethene at greater than 80% of the concentration of the total chlorinated ethene mass, and two additional wells have 5–10% ethene

Overall, the pilot test was successful and showed that bioaugmentation coupled with EVO addition could substantially increase complete dechlorination to ethene at the site. The client no longer owns the site, and installation of ex-situ infrastructure was not feasible or desired. The passive in situ biobarrier that was successfully implemented and remains in place obviated the need for ex situ infrastructure and pumping or recirculation of groundwater. The long lifespan of EVO (2+ years) resulted in minimizing re-injection costs and disturbance of the current site owner's operations. However, at sites with chlorinated VOC concentrations this high, reinjection of donor is likely to be a necessity.

THE CASE FOR USING IT AT CONTAMINATED SITES

BIOAUGMENTATION



Injecting KB-1 into a well at a TCE contaminated site in Thunder Bay, Ontario.

With an estimated 10,000 contaminated sites in Canada, there are plenty of opportunities to use a variety of treatment methods to clean them up. Bioaugmentation is one method that has received mixed reviews over the years.

The approach is thought of as "pixie dust" by some consultants and others in the clean-up industry that were burned by the claims of salesmen that their product could clean up a site within days merely by injecting the "snake oil" down some wells. When the promised results never came, there was always some explanation as to why (e.g., not enough wells, different flow regime, improper application, etc.).

by John Nicholson

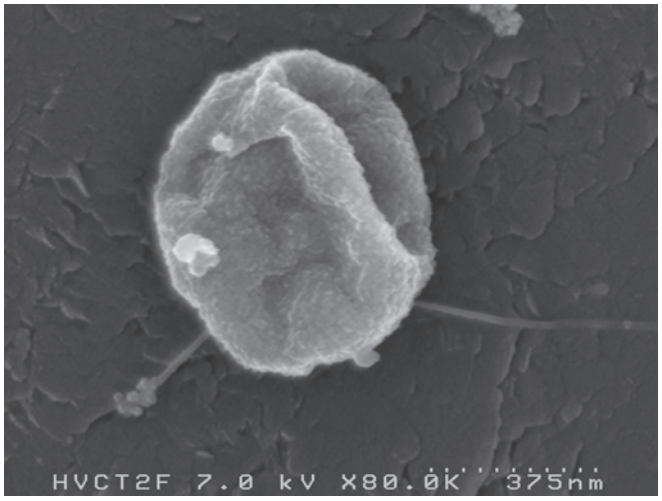
"In the case of the Thunder Bay project, a total of 65 litres of KB-1 was injected into 65 locations on the contaminated site."

Although there were (and likely still are) pretenders out there, there are legitimate products that actually can clean up a site.

Bioaugmentation involves the addition of beneficial microorganisms to improve the rate or extent of biodegradation. Theoretically, any soil or groundwater contaminant containing carbon can be biodegraded by microorganisms. The key is to find the right microbial culture and ensure the conditions are optimal to allow them to eat their food — the contaminant of concern.

Canadian success story

In Canada, an anaerobic culture of beneficial microorganisms called Dehalococcoides (Dhc) was developed at the University of Toronto (UofT) under the direction of professor Elizabeth Edwards. Dhc are anaerobic bacteria that



Dehalococcoide bacteria commercially developed as KB-1

are very good at degrading tetrachloroethene (PCE) and tricholorethene (TCE) in groundwater.

Anaerobic cultures grow in the absence of oxygen (in fact, oxygen in poisonous to them). This allows remediation specialists to inject the cultures directly into the soil and/or groundwater without the hassle of ensuring adequate oxygen supply as well.

The disadvantage of anaerobic microorganisms is that there growth rate is slower than aerobic cultures. Nonetheless, their application can be very effective depending on the site.

The Dhc developed at UofT are now produced commercially and sold as KB-1® by SiREM, based in Guelph, Ontario. More than 180 sites in the USA and Europe have used KB-1® as the bioaugmentation culture. Geological conditions at these sites have ranged from clay to fractured rock. Groundwater temperatures at these sites have ranged from 8°C to 30°C. The number of injection wells used has ranged from one to over 800.

KB-1 was used extensively at contaminated sites in the United States and Europe before SiREM completed the costly and time consuming process of registering the product under the New Substances Notification (NSN) Regulation governed by Environment Canada and Health Canada.

The NSN Regulation is a doubled-edged sword for any company looking to commercialize a new microbial culture in Canada. For SiREM, the process of registering KB-1® was a challenge. However, the regulatory hurdle it crossed in registering the product is now a competitive advantage as it is the only provider of such a product in Canada.

First commercial site in Canada

The first successful application of KB-1 in Canada was in Thunder Bay, Ontario during the summer of 2009. Located in the northwest shore of Lake Superior, Thunder Bay is known for its grain elevators and pulp and paper production. It may also become known as the first place where the application of a dechlorinating microbial culture was used to enhance the cleanup of TCE.

TCE is a chlorinated hydrocarbon commonly used industrial solvent, including the degreasing of metal parts. In the past, its widespread use and poor waste disposal (who hasn't heard stories about the practice of throwing the bucket of dirty solvent out the back door), TCE is a very common contaminant at brownfield sites across North America.

One challenge of treating TCE anaerobically is that it can be transformed into vinyl chloride, which is far more toxic than TCE. The Dhc found in KB-1 converts TCE to ethene, a non-toxic gas. (Ethene is the same gas given off by bananas as they age in a fruit bowl.)

In the case of the Thunder Bay project, a total of 65 litres of KB-1® was injected into 65 locations on the contaminated site. Within 12 weeks there was up to 60 per cent reduction in TCE in the wells. Once all the TCE is consumed, the microorganisms die off as there is no more food for them.

John Nicholson, M.Sc., P.Eng. is based in Toronto, Ontario. Contact John at john.nicholson@ebccanada.com

HELPING Nature

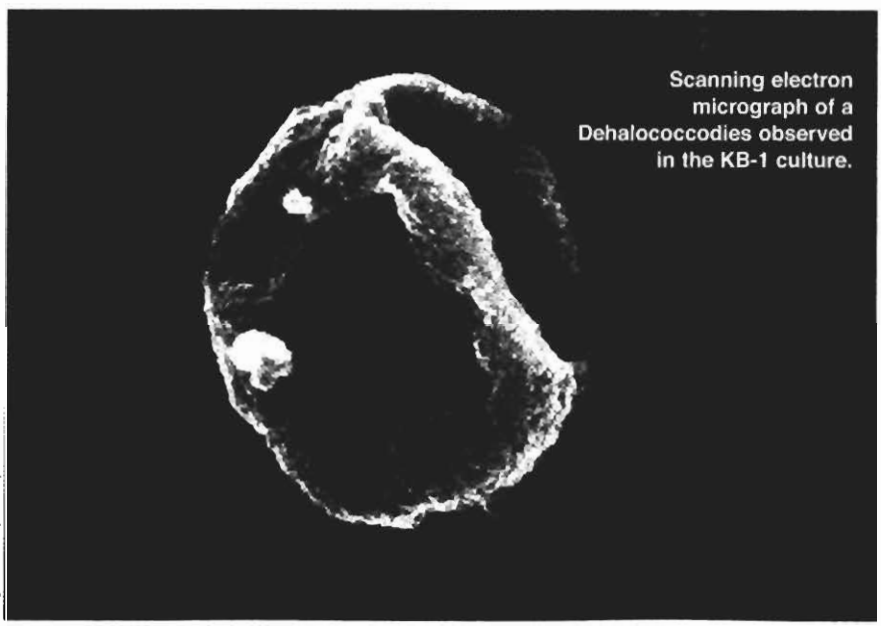
Biostimulation and bioaugmentation can give Mother Nature a boost in remediating chlorinated solvents in groundwater.

By Thomas Krug, David Major and Philip Dennis

Enhanced in situ bioremediation (EISB) for chlorinated ethenes in groundwater includes both biostimulation and bioaugmentation. This combination of methods is proving to be an effective remediation strategy at an increasing number of military and industrial sites in the United States. One reason is cost; EISB has been demonstrated to be the least expensive technology for remediation of dense non aqueous phase (DNAPL) source zones.

A study by Newell et al. in 2005, compared cost and performance data at 36 source-depletion sites and indicated that enhanced bioremediation had the lowest cost per treatment volume, $29-per-cu-yd, followed by thermal ($88-per-cu-yd), chemical oxidation ($125-per-cu-yd) and surfactant and co-solvent flushing ($385-per-cu-yd).

Around 10 years ago the first bacteria that completely dechlorinated tetrachloroethene (PCE) and trichloroethene (TCE) was isolated by researchers at Cornell University. The bacteria was named *Dehalococcoides ethenogenes*. The researchers observed that *D. ethenogenes* dechlorinated PCE to TCE to *cis*-1,2-dichloroethene (cDCE) to vinyl chloride (VC) and then to non-toxic ethene. Also significant was the observation that *D. ethenogenes* was unable to grow without specific chlorinated compounds, suggesting these bacteria would die after successful remediation of a site. *D. ethenogenes* is one species of a growing grouping of bacteria that can dehalogenate a variety of compounds and known more generally as *Dehalococcoides (Dhc)*.



Scanning electron micrograph of a Dehalococcodies observed in the KB-1 culture.

Images courtesy GeoSyntec Consultants

The Two Pillars of EISB

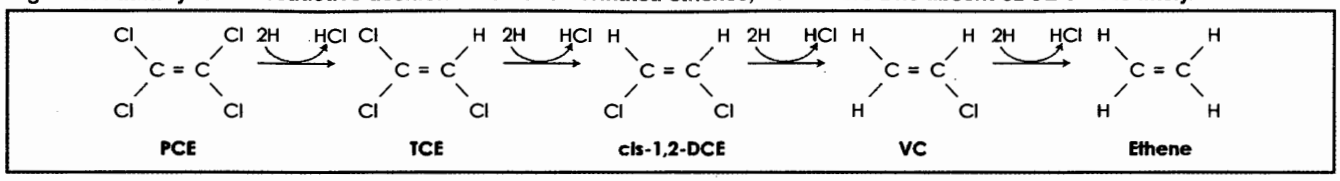
Biostimulation is the addition of electron donor, i.e., food for microorganisms, to subsurface environments to stimulate biodegradation activity. Biostimulation is most commonly performed through the addition of lactate, alcohols, or commercial formulations such as emulsified vegetable oils. If biostimulation is performed at a PCE or TCE site where naturally occurring *Dhc* are present they can then dechlorinate these compounds to ethene. If native *Dhc* are absent, bioaugmentation is required to prevent dechlorination from stalling at cDCE, or if they are the wrong type of *Dhc* from stalling at VC. Bioaugmentation is the injection of microorganisms into the subsurface which are effective at degrading contaminants. Bioaugmentation with *Dhc*, ensures that PCE, TCE and cDCE are completely dechlorinated to ethene and avoids a cDCE stall caused by incomplete dechlorinators.

The use of indigenous *Dhc* under biostimulation alone, or biostimulation combined with bioaugmentation, is increasingly common. SiREM, a division of GeoSyntec Consultants Inc., produces a bioaugmentation product, KB-1® Dechlorinator (KB-1), that contains more than 100 billion *Dhc* cells/L and has been used at close to 60 sites in 19 states and in Europe. In combination with KB-1, SiREM also performs genetic tests for *Dhc* in groundwater, which are used to determine the concentration of *Dhc* prior to bioremediation, and their growth and spread under EISB.

Proof of Concept

The first field application of KB-1 was performed in May 2000 at Kelly AFB in San Antonio, Texas. The site was contaminated with PCE, historically used at a jet engine shop for degreasing. The study compared biostimulation alone versus biostimulation and bioaugmentation with KB-1 in adjacent 30-ft-long re-circulating test plots. The study indi-

Figure 1. Pathway for the reductive dechlorination of chlorinated ethenes, note where Dhc absent cDCE stall is likely.



cated that indigenous microorganisms were capable of only partial dechlorination of PCE (present at 1-mg/L) and TCE to cDCE with biostimulation. Genetic tests indicated that *Dhc* were absent prior to bioaugmentation.

After bioaugmentation with 13-L of KB-1, *Dhc* was initially detected in the injection point, but spread throughout the entire test plot within 105 days. Estimates of the total *Dhc* present at the conclusion of the study indicated that the number of *Dhc* had increased at least 10-fold beyond the number cells introduced directly by bioaugmentation, confirming growth of the introduced *Dhc*.

Concurrent with *Dhc* growth complete stoichiometric dechlorination to ethene occurred with concentrations of PCE, TCE and cDCE all below 5 µg/L, within 200 days after bioaugmentation. Degradation half lives for all chloroethenes were in the order of only a few hours. In contrast, the adjacent biostimulation test plots exhibited only partial dechlorination to cDCE.

Case Studies

Seal Beach Naval Weapons Facility. Historical releases of PCE and TCE at a former locomotive maintenance shop at Seal Beach Naval Weapons Installation Restoration Site 40 resulted in a 300-ft-long plume. A plot test of biostimulation with lactate was conducted for more than 18 months resulting in the dechlorination of PCE and TCE to cDCE with no further breakdown products detected. The data from the initial phase of the pilot test were consistent with the absence of *Dhc*. Bioaugmentation with 40-L of KB-1 was conducted at the site in April 2003. This was the first KB-1 bioaugmentation to be performed in a passive groundwater system at a U.S. military facility.

Following bioaugmentation, genetic tests for *Dhc* indicated high concentrations of *Dhc* in the injection wells, and within several months high concentrations were reported in wells as far as 20-ft to 30-ft down gradient. Significant concentrations of ethene were detected for the first time one month after bioaugmentation. About seven months after bioaugmentation, concentrations of PCE and TCE were below California MCLs in all wells, and cDCE was below California MCLs in the injection wells and one down gradient well. Based on the positive results of the pilot study, full-scale biostimulation with bioaugmentation is currently underway at the site.

Kennedy Space Center (KSC) Launch Complex 34. Launch Complex 34 (LC34) at KSC had large historical releases of TCE as a result of maintenance activities. A field demonstration of bioaugmentation for treatment of a TCE dense non-aqueous phase liquid (DNAPL) source area was initiated in 2003 by GeoSyntec and independently evaluated by the U.S. Environmental Protection Agency's Superfund Innovative Technologies Evaluation (SITE) program. The demonstration was performed in a test plot with groundwater recirculation and biostimulation followed by KB-1 bioaugmentation in April 2004. During biostimulation, TCE was degraded to cDCE and VC with relatively low ethene production. Genetic tests indicated the native *Dhc* detected were deficient in an enzyme called vinyl chloride reductase, which is required for the efficient dechlorination of VC to ethene.

The lack of VCR in the native *Dhc* may explain the relatively low amounts of ethene detected. Following bioaugmentation with KB-1, large amounts of ethene (up to 92-mg/L) were detected. This amount of ethene formed on a molar basis was greater than the initial aqueous TCE concentration of 350-mg/L, suggesting that the DNAPL was actually dissolved by the combination of biostimulation and bioaugmentation. The EPA SITE program evaluation indicated that the overall decrease in the mass of TCE in the target treatment zone exceeded 98 percent, indicating that high concentration sites, even those with DNAPL, are compatible with EISB including bioaugmentation.

Benefits and Challenges

The use of biostimulation combined with bioaugmentation has been successfully demonstrated and found to be cost effective compared to other technologies. Studies show that there are clear benefits to enhanced bioremediation using bioaugmentation including prevention of cDCE and vinyl chloride stall, increased speed and dependability of remediation, and more efficient use of electron donor with associated cost savings.

As with most other remedial technologies, the greatest challenge with EISB is the efficient distribution of amendments. EISB requires biostimulation to provide energy as well as producing and maintaining suitably anaerobic and reducing conditions. Sites must have a relatively neutral pH and in some situations buffering of pH may also be required. Certain contaminants such 1,1,1-trichlroethane (1,1,1-TCA) can inhibit dechlorination of chlorinated ethenes. Sites with high concentrations of 1,1,1-TCA may require a more complex EISB approach including the use of bioaugmentation cultures effective at dechlorinating 1,1,1-TCA. In most cases suboptimal site conditions can be managed effectively so that the cost benefits of using bioremediation can be realized. TME

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SiREM
Leading Science · Lasting Solutions

KB-1 plus®

Case Study

Client:
GSI Water Solutions
Portland, Oregon

Site Location:
Oregon

Services Provided:
- KB-1® Bioaugmentation
- Gene-Trac® *Dehalococcoides* and *Dehalobacter* testing

"Within 8 months of KB-1® Plus bioaugmentation, 1,1,1-TCA and TCE concentrations decreased to below 5 µg/L in 4 of 6 monitoring wells."

1,1,1-TCA/TCE Remediation Using KB-1® Plus

Project Highlights

- Effective bioremediation of a comingled 1,1,1-TCA/TCE plume
- All chlorinated VOCs were below or near ROD specified limits within 18 months
- KB-1® Plus and electron donor applied in a single mobilization into an aerobic aquifer using anaerobic chase water
- Successful pilot study led to two full scale KB-1® Plus bioaugmentation implementations at the Site

Problem Definition

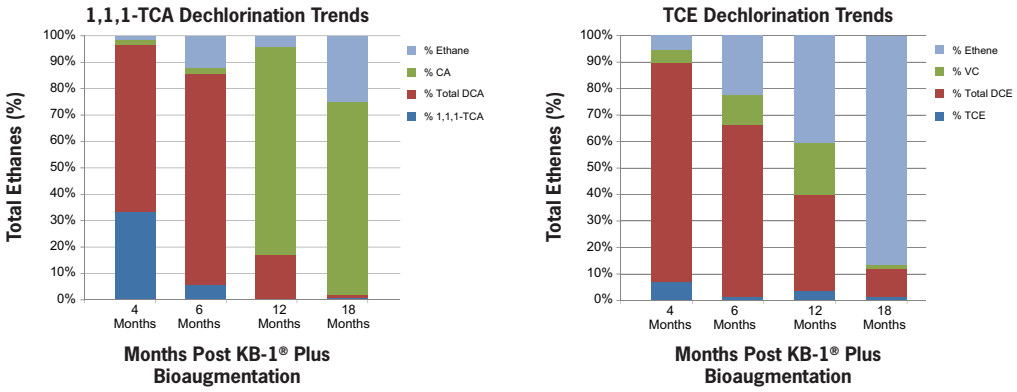
Three Site areas were impacted with 1,1,1-trichloroethane (1,1,1-TCA) and trichloroethene (TCE) associated with historical solvent use. Notably, 1,1,1-TCA is known to inhibit the reductive dechlorination of TCE if the requisite dechlorinating microorganisms are absent. Gene-Trac® testing indicated that dechlorinating *Dehalococcoides (Dhc)* and *Dehalobacter (Dhb)* microbes were absent prior to bioaugmentation. The Site groundwater was aerobic and not conducive to growth of dechlorinating microorganisms prior to enhanced *in situ* bioremediation (EISB). A pilot study was conducted to determine if: (1) EISB would promote degradation of TCE and 1,1,1-TCA below EPA Record of Decision (ROD) limits; and (2) would EISB reduce overall remediation costs.

Solution

The pilot study, initiated in June 2008, included 17 temporary injection points which were used to apply emulsified vegetable oil (EVO) and 1 liter of KB-1® Plus per point to introduce *Dhc* and *Dhb* microorganisms. Anaerobic water was injected before and after KB-1® Plus to limit the culture's exposure to aerobic groundwater. This allowed the electron donor and KB-1® Plus to be applied in the same mobilization, minimizing application costs.

Notable Results

Within 8 months of KB-1® Plus bioaugmentation, 1,1,1-TCA and TCE concentrations decreased to below 5 µg/L in 4 of 6 monitoring wells. After 18 months, all chlorinated VOCs were below or near ROD limits. The pilot test was deemed sufficiently effective that full-scale application in this area was not required. Full-scale KB-1® Plus bioaugmentation was implemented at two other Site areas in 2009 and 2010.



Monitoring results from a typical performance monitoring well for 1,1,1-TCA dechlorination (left) and TCE dechlorination (right). 1,1,1-TCA dechlorination through 1,1-DCA to chloroethane (CA) was observed with greater than 90% of the reported mass as CA and ethane after 18 months. TCE dechlorination through cDCE and VC to ethene was observed with greater than 80% of the mass as ethene after 18 months. Courtesy of GSI Water Solutions.