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Phone: 865.573.8188
Fax: 865.573.8133
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SITE LOGIC Report

Bio-Trap In Situ Microcosm Study

Contact: Bill Toran
Address: Aztech Technologies, Inc.
5 McCrea Hill Road
Balliston Spa, NY 12020

Phone: (518) 855-5383

Email: btoran@aztechtech.com

MI Identifier: 039NC

Report Date: March 29, 2016

Project: Dec/Jacks Drycleaners, 734112

Comments:

Executive Summary

A Bio-Trap® *In Situ* Microcosm (ISM) study was performed in well MW-7 to investigate whether the addition of an electron donor or bioaugmentation culture would enhance the anaerobic biodegradation of tetrachloroethene (PCE), trichloroethene (TCE), and associated daughter compounds. The ISM assembly deployed in well MW-7 consisted of three units: (i) a MNA unit containing no exogenous amendment, (ii) a BioStim unit amended with SRS®-SD (Small Droplet Emulsified Vegetable Oil Substrate) as an electron donor, and (iii) a BioAug unit amended with SRS®-SD and a mixed consortium of dechlorinating bacteria (SDC-9™). Following an in-well deployment period of 62 days, the ISM units were recovered for CENSUS® analysis of dechlorinating bacteria and quantification of contaminant concentrations, dissolved gases, volatile fatty acids (VFAs), and anions. A summary of the results is provided in Table 1 and Figure 1. Following are key observations from the results obtained for each *in situ* microcosm.

MW-7 MNA, BioStim, and BioAug Units

- In the MNA unit containing no exogenous amendment, the *Dehalococcoides* concentration was measured at 10^5 cells/bead, which exceeded the 10^4 cells/mL density threshold proposed by Lu et al. (2006) as a screening criterion for generally useful rates of biological reductive dechlorination. In addition, all three key reductase genes (*i.e.*, *tceA*, *bvcA*, and *vcrA*) were detected in the MNA unit, suggesting that a genetic potential for complete reductive dechlorination of PCE and TCE to innocuous end products such as ethene exists under MNA conditions at well MW-7. Both the *tceA* and *vcrA* reductase genes were detected at 10^4 cells/bead, while the gene encoding BVC reductase was detected at a level that was three orders of magnitude lower (*i.e.*, 10^1 cells/bead).
- The BioStim unit amended with SRS®-SD was characterized by high concentrations of *Dehalococcoides* sp. (10^7 cells/bead), as well as substantial levels of reductase genes *tce*A (10^5 cells/bead) and *vcr*A (10^7 cells/bead). The *bvcA* reductase gene was detected in the BioStim unit, but was below the practical quantitation limit. The *Dehalococcoides* population in the BioStim unit was not only two orders of magnitude higher compared to the MNA unit, but three orders of magnitude higher than the BioAug unit deployed in MW-7. Similarly, the key reductase genes *tceA* and *vcrA* were detected at substantially higher levels in the BioStim unit (10^5 and 10^7 cells/bead, respectively) than in the BioAug unit (both reductase genes measured at 10^2 cells/bead). The high concentration of *Dehalococcoides* sp. observed in the BioStim unit tends to normally be associated with ISM units containing an exogenous dechlorinating bacterial culture. The results for the BioStim unit, therefore, appear to have been influenced by a bioaugmentation culture. Furthermore, both the BioStim and BioAug units contained SRS®-SD as an electron donor, so any substantial increases in the *Dehalococcoides* population are likely due to the addition of a dechlorinating bioaugmentation culture.
- The BioAug unit contained the lowest levels of *Dehalococcoides* sp. and key reductase genes relative to the MNA and BioStim units. *Dehalococcoides* sp. were detected at a concentration of 10^4 cells/bead, while both the *tceA* and *vcrA* reductase genes were detected at a low level of 10^2 cells/bead. The *bvcA* reductase gene was below the detection level.
- Contaminant analysis indicated that *cis*-1,2-dichloroethene (*cis*-1,2-DCE) was the primary chlorinated ethene detected in all three ISM units, with concentrations ranging from 643 µg/L (in the BioStim unit) to 892 µg/L (in the MNA unit). However, increased production of vinyl chloride (73.7 µg/L) and ethene (5.9 µg/L) in the BioStim unit relative to the MNA and BioAug units was clearly evident, suggesting that enhanced reductive dechlorination had occurred in the BioStim unit during the 62-day deployment period. Again, these results are more commonly observed under conditions permitting the *in situ* maintenance of high concentrations of exogenously added halorespiring bacteria.
- By contrast, the *cis*-1,2-DCE concentration in the BioAug unit (887 µg/L) was similar to that observed in the MNA unit (892 µg/L), and vinyl chloride concentrations were below the detection level in both the MNA and BioAug units. Dissolved ethene levels were comparable in these two ISM units as well. These results suggest that reductive dechlorination to vinyl chloride and ethene were not appreciably enhanced under bioaugmentation conditions during the deployment period.

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- The dissolved methane concentration was higher in the BioStim unit (350 µg/L) relative to the MNA (260 µg/L) and BioAug (280 µg/L) units by a small amount, suggesting that reducing conditions were relatively comparable in all three ISM units.
- Acetic acid was detected in the BioStim and BioAug units deployed, indicating that microorganisms were fermenting the SRS®-SD substrate. No other volatile fatty acids were detected in any of the ISM units.
- For all three ISM units, sulfate was detected at concentration levels ranging from 29 to 41 mg/L, suggesting that competition with sulfate-reducing bacteria for available electron donors may be possible but may not pose a serious hindrance to reductive dechlorination based on the sulfate levels present at well MW-7.

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The *In Situ* Microcosm Approach

Site managers have frequently turned to laboratory microcosms or small pilot studies to evaluate bioremediation. However, duplication of *in situ* conditions in the laboratory is difficult and the results often do not correlate to the field. Pilot studies are performed in the field, but are often prohibitively expensive as an investigative tool. Bio-Trap studies serve as cost-effective, *in situ* microcosms providing microbial, chemical, and geochemical evidence to evaluate biodegradation as a treatment mechanism and to screen remedial alternatives.

Typically each Bio-Trap Unit will contain samplers to evaluate the following:

Geochemical Fingerprint (GEO)	•20 mL amber VOA vial with a nylon screened cap designed for assessment of a variety of geochemical parameters including anions and metabolic acids.
Contaminant of Concern (COC)	•A low density polyethylene (LDPE) passive diffusion bag designed for analysis of a variety of COCs including chlorinated solvents and petroleum hydrocarbons.
Microbial Populations (MICRO)	•PVC cassette containing Bio-Sep beads which provide a large surface area for microbial attachment and were designed for analysis by a variety of molecular biological tools (MBTs).

How do ISMs work?

The MICRO sampler (microbial populations) contains Bio-Sep® beads, an engineered composite of Nomex® and powdered activated carbon which provides an incredibly large surface area (~600 m^2/g) that is readily colonized by subsurface microorganisms. In addition to a matrix for microbial growth, the Bio-Sep® beads can be "baited" with amendments including electron donors (e.g. hydrogen-releasing compounds) to investigate biostimulation approaches to enhance biodegradation. The ISM units also contain a COC (contaminant of concern) sampler to measure contaminant concentrations, daughter product formation, and dissolved gases and a GEO (geochemical fingerprint) sampler for quantification of geochemical parameters (nitrate, iron, sulfate, etc.), and volatile fatty acids (pyruvic, lactic, acetic, propionic, etc.).

Bio-Trap® *In Situ* Microcosm studies at chlorinated solvent sites typically include three types of Bio-Trap Units deployed within a monitoring well. Each Bio-Trap Unit corresponds to one of the three most common remedial options: monitored natural attenuation (MNA), Biostimulation (BioStim), and Bioaugmentation (BioAug). All three Bio-Trap Units contain COC and GEO samplers for chemical and geochemical analyses. The key difference between the Bio-Trap Units is in the MICRO sampler.

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Types of ISM Units typically deployed and MICRO sampler configurations:

Control (MNA)	•Bio-Sep® beads contain no additional electron donor and represent current aquifer conditions.
Biostimulation (BioStim)	•Bio-Sep® beads are baited with a specified electron donor (sodium lactate, EOS, HRC, molasses, etc) or an Amendment Supplier is used to release the desired amendment.
Bioaugmentation (BioAug)	•Bio-Sep® beads are pre-inoculated with a *Dehalococcoides* culture. These units can also be baited with an additional electron donor.

MNA Unit: The purpose of the Control ISM Unit is to quantify contaminant-degrading bacteria and daughter product formation under monitored natural attenuation (MNA) conditions and to serve as a baseline for comparison to BioStim and/or BioAug Units.

Following in-well deployment, DNA or phospholipid fatty acids can be extracted from the Bio-Sep beads for CENSUS or PLFA analyses. For example, DNA extracted from the Bio-Sep beads can be used in CENSUS analysis of *Dehalococcoides* (qDHC) and vinyl chloride reductase (qVC) genes to evaluate the potential for complete reductive dechlorination of PCE to ethene under MNA conditions. The VOC and anion samplers can be used to determine concentrations of contaminants, daughter products, dissolved gases, terminal electron acceptors, and chloride.

BioStim Unit: The Biostimulation ISM Unit is designed to test the hypothesis that electron donor addition will stimulate growth of dechlorinating bacteria and enhance biodegradation. As with the MNA Unit, the BioStim Unit contains COC and GEO samplers for chemical analyses. The BioStim Unit may contain either a MICRO sampler that contains Bio-Sep beads "baited" with the specified electron donor or an amendment supplier to release the desired amendment over the incubation time. If an Amendment Supplier is used the MICRO sampler will contain standard Bio-Sep beads for the growth matrix.

BioAug Unit: The Bioaugmentation ISM Unit is designed to evaluate bioaugmentation as a treatment technology. The MICRO sampler contains Bio-Sep beads pre-inoculated with the desired commercial culture and also contains an electron donor of choice. As with the MNA and BioStim Units, the BioAug Unit also contains a COC and GEO samplers for chemical analyses.

CENSUS®

Based on quantitative polymerase chain reaction (qPCR), CENSUS® is a nucleic acid-based approach to quantify specific microorganisms, groups of microorganisms, or functional genes involved in bioremediation or other biological processes. CENSUS® targets include bacteria and functional genes responsible for biodegradation of chlorinated solvents and petroleum products among others.

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Results

Table 1. Summary of the results obtained for *In Situ* Microcosm Units.

Sample Information	MW-7 MNA	MW-7 BioStim	MW-7 BioAug
Microbial Populations (cells/bead)			
Dehalococcoides (DHC)	**4.22E+05**	**5.72E+07**	**1.34E+04**
tceA Reductase (TCE)	**1.61E+04**	**9.91E+05**	**5.97E+02**
bvcA Reductase (BVC)	**2.62E+01**	**2.30E+01 (J)**	<2.50E+01
vcrA Reductase (VCR)	**3.33E+04**	**1.13E+07**	**4.58E+02**
Contaminant of Concern (µg/L)			
Tetrachloroethene	<10	<10	<10
Trichloroethene	<10	<10	<10
cis-1,2-Dichloroethene	**892**	**643**	**887**
trans-1,2-Dichloroethene	**9.4**	**6.8**	**11.6**
Vinyl chloride	<10	**73.7**	<10
Dissolved Gases (µg/L)			
Ethene	**0.22**	**5.9**	**0.21**
Ethane	**4.8**	**5.2**	**5.3**
Methane	**260**	**350**	**280**
Volatile Fatty Acids (mg/L)			
Acetic Acid	<5.0	**19**	**15**
Propionic Acid	<5.0	<5.0	<5.0
Pyruvic Acid	<5.0	<5.0	<5.0
Butyric Acid	<5.0	<5.0	<5.0
Lactic Acid	<10	<10	<10
Anions (mg/L)			
Chloride	**110**	**110**	**120**
Nitrite	<0.50	<0.50	<0.50
Nitrate	<0.50	<0.50	<0.50
Ortho Phosphate	<1.5	**4.5**	<1.5
Sulfate	**41**	**29**	**37**

Legend:
NA = Not analyzed due to headspace in the vial NS = Not sampled J = Estimated result below PQL but above LQL I = Inhibited
< = Result not detected

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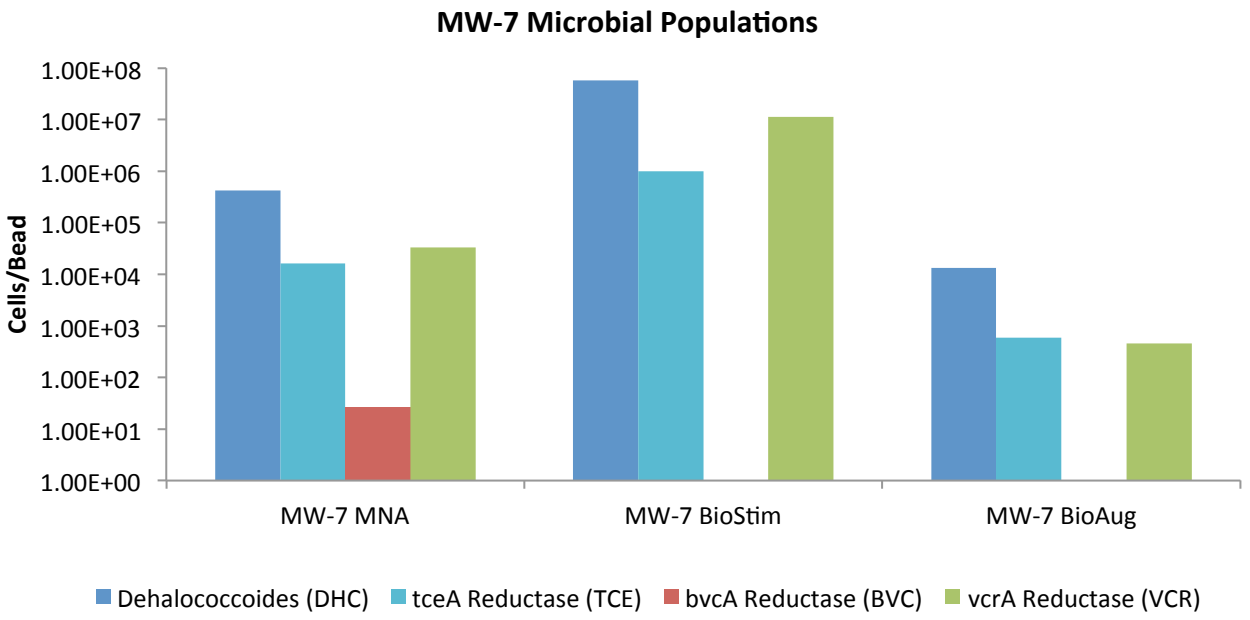

Figure 1. CENSUS® results for selected microbial populations (cells/bead).

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Interpretation

Bio-Trap® *In Situ* Microcosm studies are designed to provide the chemical, geochemical, and microbiological lines of evidence required to evaluate remediation options in a single, cost-effective field study. To aid in the decision-making process, comparisons should generally focus on differences in results between *In Situ* Microcosm units. For example, comparison of the *Dehalococcoides* populations in the Control and BioStim units can be used to assess whether electron donor addition would stimulate growth of this key group of halorespiring bacteria. While results for individual analyses should be compared between units, overall interpretation should integrate all lines of evidence with due consideration of site conditions, site activities, and the desired treatment mechanism. The following discussion describes interpretation of results in general terms and is meant to serve as a guide.

Microbial Populations: CENSUS® analysis allows site managers to quantify targeted members of the microbial community deemed critical for site remediation. Total Eubacteria provides an index of the total bacterial biomass and is generally greater than 10^6 cells/bead in the absence of factors inhibiting microbial growth. While a number of bacterial cultures capable of utilizing PCE and TCE as growth-supporting electron acceptors have been isolated[1-5], *Dehalococcoides* sp. may be the most important because they are the only bacterial group that has been isolated to date which is capable of complete reductive dechlorination of PCE to ethene[6]. In fact, the presence of *Dehalococcoides* sp. has been associated with the full dechlorination to ethene at sites across North America and Europe[7]. Thus, CENSUS® quantification of *Dehalococcoides* in each Bio-Trap *In Situ* Microcosm unit can be used to evaluate the likelihood of complete reductive dechlorination of PCE and TCE under MNA conditions, the ability of electron donor addition alone to stimulate growth of halorespiring bacteria (BioStim), and the survival of commercial *Dehalococcoides* cultures in the field (BioAug). The accumulation of the daughter products *cis*-DCE and vinyl chloride termed "DCE stall" is relatively common at PCE/TCE sites especially under MNA conditions. Accumulation of vinyl chloride, generally considered more carcinogenic than the parent compounds, is particularly problematic. CENSUS® quantification of vinyl chloride reductase genes (*bvc*A and *vcr*A) was developed to more definitively confirm the potential for biodegradation of vinyl chloride. Again, comparison of vinyl chloride reductase copies between units can be used to assess the efficacy of enhanced bioremediation approaches (biostimulation and bioaugmentation) to enhance populations of organisms specifically capable of reductive dechlorination of vinyl chloride.

Dissolved Gases: When comparing concentrations of dissolved gases between *In Situ* Microcosm units, particular care should be afforded to the dissolved ethene concentration. While ethene can volatilize, can be further metabolized, or be further reduced to ethane in some environments, greater concentrations of ethene generally indicate complete reductive dechlorination of PCE and TCE. In addition to quantifying the end products of reductive dechlorination, analysis of dissolved gases includes determination of dissolved methane. Combined with results of geochemical analysis (See Anions), elevated methane concentrations are indicative of highly reducing conditions conducive to reductive dechlorination. However, methanogens also compete with dechlorinating bacteria including *Dehalococcoides* for available hydrogen.

Anions: Although increases in chloride ion concentrations are often coupled with reductive dechlorination and daughter product formation, the main purpose of the GEO sampler is to measure concentrations of competing electron acceptors and assess the redox status. Elevated concentrations of nitrate, for example, would suggest anoxic conditions less conducive to reductive dechlorination. Production of ferrous iron combined with elevated sulfate concentrations generally indicates iron-reducing conditions. Lower concentrations of sulfate combined with sulfide production (but low methane production) suggests sulfate-reducing conditions. The production of methane (Table 1 - dissolved gases) suggests highly reducing, methanogenic conditions. While dechlorination of TCE to *cis*-DCE occurs under iron-reducing conditions (and in more strongly reducing environments), further reduction to vinyl chloride and ethene may require more anaerobic conditions (sulfate reduction and methanogenesis).

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Glossary

Amendment Supplier: a component that fits inside the MICRO-Trac/Bio-Trap unit at the bottom. This component is designed to slowly diffuse a desired amendment within a BioStim and/or a BioAug Unit during the incubation time.

Sampler: Individual components consisting either of a geochemical (GEO), contaminant of concern (COC) or microbial (MICRO) sampler. Geochemical samplers are essentially VOA vials with special septa that facilitate transfer. The microbial samplers are made from a smaller PVC pipe ~1" x 3 ½" and contains Bio-Sep® beads which serve as a microbial growth matrix.

> **COC Sampler:** 40 mL amber VOA with a low density polyethylene membrane permitting passive diffusion of volatile organic compounds (VOCs).
>
> **GEO Sampler:** a 20 mL amber VOA with a nylon based membrane permitting passive diffusion of anionic species.
>
> **MICRO Sampler:** a polyvinylchloride cassette containing Bio-Sep® beads which provide a large surface area for microbial growth. In addition to a matrix for microbial growth, the Bio-Sep® beads can be "baited" with amendments including ^{13}C labeled chlorobenzene as used in this study. Bio-Sep® beads were designed to allow extraction of phospholipids fatty acids and DNA for analysis of microbial communities.

Unit: 1.25" x 15" PVC housing that all of the samplers are placed into for deployment. Units will have baffled end caps to separate different zones within the monitoring well. Typically, each unit will correspond to a treatment approach.

Assembly: Collections of Units for a particular monitoring well. Samplers (GEO, COC, and MICRO) are placed in each unit. Units are linked to form an Assembly. An entire Assembly (consisting of multiple units) is deployed in each well.

CENSUS: CENSUS is based on a technique called quantitative polymerase chain reaction (qPCR) whereby many copies of a specific gene are generated. As each gene copy is made, a fluorescent marker is released, measured, and used to quantify the number of target genes present in a sample.

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