LESSONS LEARNED FROM IMPLEMENTATION OF MONITORED NATURAL ATTENUATION FOR AN AEROBIC TRICHLOROETHENE PLUME AT THE IDAHO NATIONAL ENGINEERING AND ENVIRONMENTAL LABORATORY

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ABSTRACT

The Test Area North (TAN) Facility of the Idaho National Engineering and Environmental Laboratory (INEEL) is the site of a 3-km-long trichloroethene (TCE) plume resulting from past waste injections into the deep fractured basalt aquifer. An in-depth natural attenuation field evaluation showed that aerobic TCE degradation was occurring with a half-life of 9 to 21 years (1) in the plume's distal zone. Given the lack of precedent for the significance of this degradation mechanism under intrinsic conditions, activity-dependent enzyme probe assays were performed on TAN field samples. Results showed that microbial enzymes known to cometabolize TCE are present and active in the TAN aquifer, both inside and outside of the TCE plume. In addition, a multi-level sampling program was implemented to investigate the three-dimensional contaminant distribution in the distal zone. These results confirmed the original conceptual model for the distal zone by demonstrating that no previously uncharacterized zones of TCE flux exist in the distal zone.

Despite the fact it was initially unexpected that intrinsic aerobic cometabolism would be an important degradation mechanism at TAN, the hypothesis was rigorously tested until sufficient evidence was collected to convince both the technical and regulatory members of the project team that intrinsic aerobic cometabolism of TCE was occurring. Ultimately, demonstration of this mechanism was the most important component of obtaining regulatory approval of the monitored natural attenuation (MNA) remedy. Given that this is the largest TCE plume for which the United States Department of Energy (DOE) has achieved regulatory acceptance of MNA, other DOE sites could benefit from these lessons learned.

INTRODUCTION

TAN is located in the northern portion of the INEEL. Industrial activities at TAN generated wastewater that was introduced into the groundwater via a waste injection well from 1953 to 1972 to dispose of all liquid waste streams generated at TAN. This included low-level radioactive wastewater, industrial wastewater (including organic liquids), and sanitary sewage.

The result of this waste injection was the evolution of a nearly 3 km-long TCE plume (Figure 1). Due to the size of the plume and complexity of the site conditions, the plume was divided into three areas:

Fig. 1. TAN TCE plume showing distal zone monitoring wells

- Hotspot—defined as that portion of the plume with TCE concentrations >20,000 μg/L,

- Medial zone—defined as that portion of the plume with TCE concentrations between 1,000 and 20,000 μg/L, and

- Distal zone—defined as that portion of the plume with TCE concentrations less that 1,000 μg/L.

The original Record of Decision (2) selected pump-and-treat as the default remedy for the TAN groundwater contamination for all three plume sections. However, it allowed for evaluation of innovative technologies for their potential to enhance or replace pump and treat. Through a series of field investigations, enhanced in situ bioremediation was selected as the hotspot remedy, replacing the default pump and treat system. In addition, a pump and treat facility was constructed and is currently operating as the remedy for the plume's medial zone.

An in-depth MNA field evaluation, which studied all attenuation mechanisms for TCE in groundwater, was conducted for the large aerobic distal zone of the plume. The application of the tracer-corrected method, which separates effects of degradation from dispersion, showed that aerobic TCE degradation was occurring with a half-life of 9 to 21 years (1). A September 2001 ROD Amendment (3) documented regulatory acceptance of MNA as the selected remedy for the distal zone of the plume. Based on field and laboratory data, the degradation mechanism was believed to be aerobic cometabolism by indigenous methanotrophs, propanotrophs, or phenol-oxidizers. This had not been described previously as a significant mechanism for intrinsic TCE degradation in the field because of competitive inhibition and the specific environmental conditions needed for significant degradation. Given these issues, it was somewhat surprising that cometabolism was sufficient to support significant TCE degradation in an aerobic oligotrophic aquifer with minimal biological activity.

Although the MNA field evaluation provided convincing evidence regarding the observed effects of this degradation mechanism, a thorough investigation was conducted after the completion of the field evaluation in order to determine whether this mechanism could be documented. Specifically, two data gaps identified during the MNA field evaluation that required further investigation were 1) a detailed understanding of the three-dimensional plume anatomy and 2) direct evidence for the degradation mechanism.

MATERIALS AND METHODS

A network of six Flexible Liner Underground Technology (FLUTe™) multi-level sampling systems was installed and sampled from 2001 through 2003 to address the first data gap. To address the second data gap, a field trial of enzyme activity-dependent probes was conducted. Each of these activities is described further below.

Multi-Level Sampling

The data included in the MNA field evaluation and the initial application of the tracer-corrected method were generated from samples collected from one interval in each well that was evaluated. Furthermore, most of the sampling intervals in these wells were located in the upper 100 ft of the contaminated thickness. It was possible, although unlikely, that large flow zones with high contaminant flux were missed by these initial sampling activities. Therefore, as a part of the MNA remedy, a commitment was made to the regulatory agencies to further investigate the three-dimensional anatomy of the plume by collecting vertically discrete samples from several open boreholes in the distal zone. A multi-level sampling program was undertaken to meet this commitment. Field testing of several multi-level sampling systems and approaches showed that a commercially available multi-level sock sampler, the FLUTe™ sampling system, was best suited to the multi-level sampling application at TAN (4). A FLUTe™ system consists of an

airtight membrane that is hydraulically emplaced inside a well by applying positive pressure to the inside of the flexible liner using water. This causes the liner, which is attached to a tether, to evert into the well. The system is equipped with sampling ports at specified depths that are attached to tubes that extend above ground surface to allow for discrete-interval sampling of the subsurface using gas drive principles. FLUTe™ systems were installed into six wells at TAN, resulting in a total of 51 discrete multi-level sampling locations (4).

Activity-Dependent Enzyme Probes

Activity-dependent enzyme probes are research tools that have the potential to provide direct evidence for aerobic cometabolic oxidation of chlorinated ethenes, most notably TCE. The probes react with enzymes that are known to degrade TCE. If an appropriate enzyme is present and active within a given sample, then application of the probes will result in a fluorescent product that can easily be seen under a microscope. If the appropriate enzyme is not present (or present by not active in a given sample), then the probes will not be transformed into a fluorescent product. Activity-dependent enzyme probes have been developed that are specific to the soluble methane monooxygenase (sMMO) enzyme system (5) as well as various toluene oxygenase enzyme systems (6, 7, 8).

It is important to understand the context of activity-dependent enzyme probes in terms of molecular tools currently available for assessing the presence and activity of microbial species and populations. DNA probes can provide information as to whether bacteria in a sample carry the genes that encode for enzymes known to degrade TCE. If the correct genes are not present, then no TCE degradation is possible without addition of the correct bacteria (bioaugmentation). If the correct genes are present, mRNA probes can show whether the genes are being expressed. However, it is possible for both of these techniques to yield positive results and still not have direct evidence that a cometabolic degradative process is active in a given system.

This is illustrated further in Table I, which is a matrix showing hypothetical responses of DNA, mRNA, and enzyme probe assays given the presence/absence of various combinations of an inducer (i.e., primary substrate) and an inhibitor. The hypothetical responses shown in this table assume that bacteria with the correct enzyme system are present in all cases. If the appropriate primary substrate is absent from a given sample (rows 1 and 2 in Table I), then the enzyme will be present but not active in the sample. This would lead to a positive response from the DNA probes but not from the mRNA probes (the genes are not being expressed) or the activity-dependent enzyme probes. However, if the primary substrate is present along with an inhibitor of the enzyme system (row 3), then the enzyme would still be present but not active in that sample. In this case, both DNA and mRNA probes would yield positive responses but activity-dependent enzyme probes would yield a negative response. Finally, if the primary substrate were present without an inhibitor, then all three assays would yield a positive response. It can be seen from Table I that activity-dependent enzyme probes most accurately reflect the presence and activity of the appropriate enzyme systems, thus providing the most direct evidence of active TCE cometabolism currently available.

Table I. Hypothetical Enzyme Probe Response (assumes presence of correct enzymes)

Experimental Conditions		Response		
Primary Substrate (Inducer) Present	Inhibitor Present	DNA Probe	mRNA Probe	Enzyme Probe
No	No	Yes	No	No
No	Yes	Yes	No	No
Yes	Yes	Yes	Yes	No
Yes	No	Yes	Yes	Yes

In addition to the activity-dependent enzyme probe assays, a series of molecular probes have been developed or adapted to investigate the genetic potential of toluene and methane-oxidizing microbial populations. As discussed above, the enzyme probes measure actual enzymatic activity and provide data that are otherwise not obtainable. However, the molecular techniques can be used in combination with the probe data to provide context for the activity-dependent enzyme probes by detecting functional genes and assessing levels of enzyme production from these genes.

Sampling and Analytical Methods

The purposes of this investigation were to collect additional groundwater chemistry data to confirm previous trends of contaminant attenuation and to provide direct evidence for the degradation mechanism using activity-dependent enzyme probe and molecular assays. This was accomplished through the collection of samples from both FLUTe™ and non-FLUTe™ wells. Analytes included in this investigation were standard groundwater chemistry parameters, activity-dependent enzyme probe assays, and molecular techniques. Groundwater chemistry parameters included volatile organic compound (VOC) contaminants, redox-sensitive parameters, biological activity indicators, biological nutrients, and water quality parameters; all of which were analyzed using a combination of fixed and field laboratory measurements (Table II). In addition, dissolved gas samples were collected following the bubble strip method (9) and analyzed for methane, hydrogen, and carbon monoxide by reduction gas analysis. Samples for activity-dependent enzyme probe and molecular assays for both sMMO and toluene oxygenase enzymes were collected as filters and whole water samples. The filters were collected by pumping 20 to 50 L of groundwater through an inline 142 mm, 0.2 micron filter (Supor membranes, Pall Gelman) housed in a stainless steel filter stand (Fisher Scientific). The entire filter stand assembly, including the filter, was autoclaved prior to sample collection. Immediately upon sample collection, the filter stand was placed on ice and was stored at 4°C until transport to the laboratory. In all cases, the samples were analyzed the same day that they were collected. The whole water samples were collected as approximately 10 L of unfiltered

groundwater in acid washed high-density polyethylene (HDPE) bottles or carboys and were stored at 4°C until transport to the laboratory.

Table II. Analytical Parameters and Methods

Parameter	Analytical Method and Laboratory
Chlorinated Ethenes	EPA SW-846 8260B – Fixed Laboratory
Dissolved Gases (CH_4, H_2, CO, and C_2H_6)	Reduced Gas Analysis –Fixed Laboratory
Chloride	Field Test Kit – Field Laboratory
Nitrate (as Nitrogen)	Field Test Kit – Field Laboratory
Sulfate	Field Test Kit – Field Laboratory
Iron	Field Test Kit – Field Laboratory
Carbon Dioxide	Field Test Kit – Field Laboratory
Alkalinity	Field Test Kit – Field Laboratory
DOC	EPA Method 415.1 – Fixed Laboratory
Metals (Al, Ca, Mg, K, Na, and Cu)	SW-846 Methods 3010A and 6010B – Fixed Laboratory
Tritium	Liquid Scintillation Counting – Fixed Laboratory
Ammonia (as Nitrogen)	Field Test Kit – Field Laboratory
Phosphate	Field Test Kit– Field Laboratory
Temperature/pH/ Conductivity/DO/ ORP	Flow-through cell during groundwater sampling

Figure 1 shows the wells included in this investigation. The round symbols represent FLUTe™ sampling locations and the square symbols represent wells sampled using low-flow submersible pumps. Samples were collected from the FLUTe™ systems following the protocol in Wymore et al. (4). The submersible pumps were sampled consistent with low-flow principles (10). The sampling strategy employed led to a total of 24 activity-dependent enzyme probe and molecular sampling locations, as well as extensive sampling for groundwater chemistry parameters from wells throughout the fringe of the plume and across the entire contaminated thickness.

RESULTS AND DISCUSSION

Results from multi-level sampling indicated that despite variations in contaminant concentrations within a given well, contaminant ratios were relatively constant with depth, potentially indicating a well-mixed system on a plume-wide scale. An example of this is shown in Figure 2, which shows vertical profiles of several parameters from a monitoring well equipped with a FLUTe™ system. Although concentrations were not uniform with depth, the remarkably consistent trends

in the profiles of contaminants and several other parameters indicate that the observed variations are likely a result of local hydrogeologic heterogeneities.
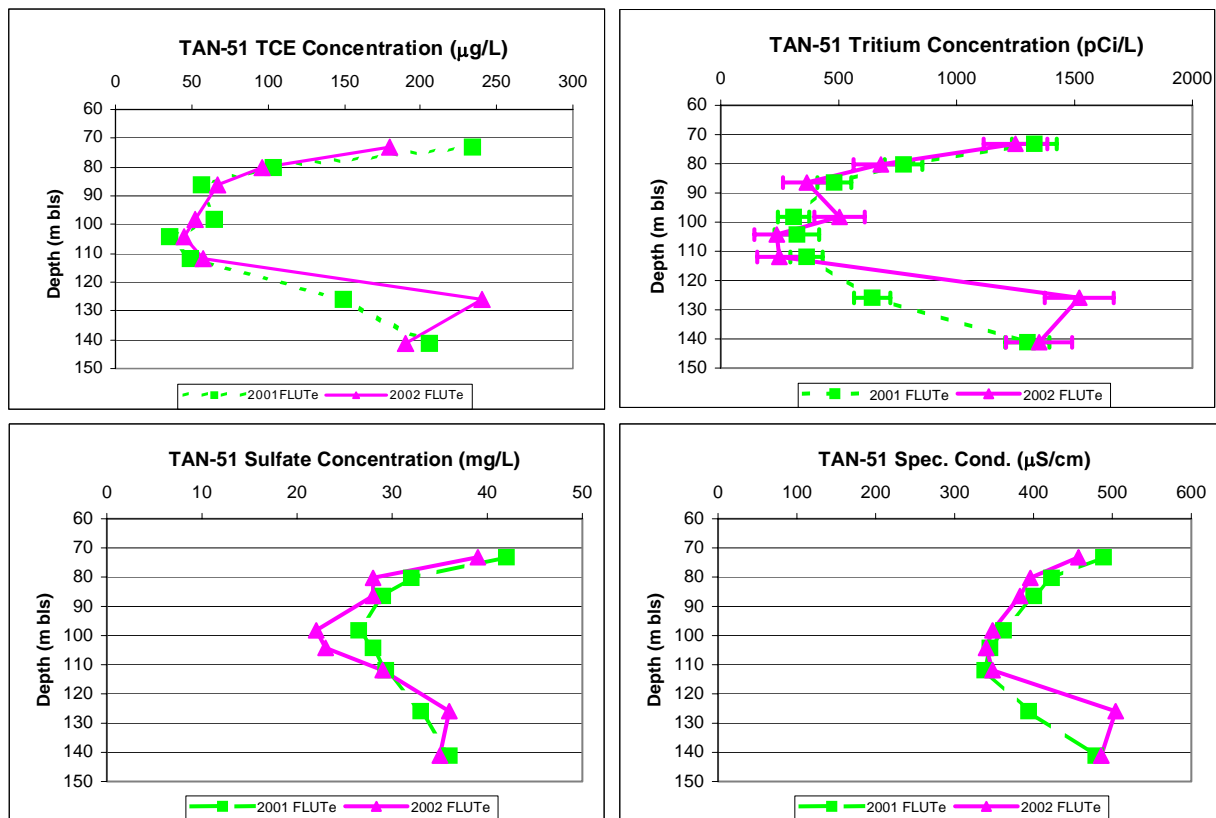

Fig. 2. Vertical profiles from well TAN-51

In addition, data from all sampling intervals within a given well were in reasonable agreement with the historical point samples collected from similar locations in the plume (i.e., the data included in the initial application of the tracer-corrected method). For example, historical TCE concentrations from Well TAN-51 (Figure 1) were approximately 200 µg/L, and the maximum concentration measured in the vertical profile was approximately 240 µg/L (Figure 2). Overall, these results confirmed the conceptual model for three-dimensional plume anatomy of the distal zone by demonstrating that no previously uncharacterized zones of TCE flux exist in the distal zone.

The results of the activity-dependent enzyme probe assays are shown in Table III. For the sMMO enzyme, a positive signal was observed following application of the probes to all samples. Table III also shows that positive responses to at least one of the four toluene enzyme probes were observed for 21 of 24 sampling locations. These results show that sMMO is present and active both inside and outside the plume and that toluene oxygenase enzymes are also active in the TAN aquifer.

Table III. Activity-Dependent Enzyme Probe Results

Well	Sample Location (m below land surface)	Toluene Probes[1]	Methane Probes[2] (intensity/min)
TAN-51	73	++	+
	80	++	+
	98	+	+
	104	+++	+
	126	-	+
	141	-	+
TAN-55	97	++++	+
	123	++++	NA
	141	++++	+
TAN-52	74	+++	+
	81	+	+
	110	+++	+
	114	+	NA
	134	++	+
	139	+	+
TAN-16	94	++	+
TAN-56	74	-	+
TAN-57	70	+	+
	87	+	+
	134	+	+
TAN-58	90	++++	+
TAN-7	91 (#1)	+++	+
	91 (#2)	+++	+
	91 (#3)	++++	+

1 - Each plus sign represents a positive response for one of four different toluene enzyme probes.
2 - A plus sign indicates a fluorescent response was observed; a minus sign indicates no response.

Figure 3 shows an example of both a negative and positive response to application of an activity-dependent enzyme probe. The blue slides on the left represent a total cell count for a given sample, as measured by the DAPI method. The green slides on the right show the number of cells in which the fluorescent signal was detected. The upper pair of slides shows a negative response to the enzyme probe because although several cells were present in the sample, none of them fluoresced. In contrast, the lower pair of slides shows a positive response to the enzyme probes in that many of the cells present in the sample were labeled with the fluorescent signal.
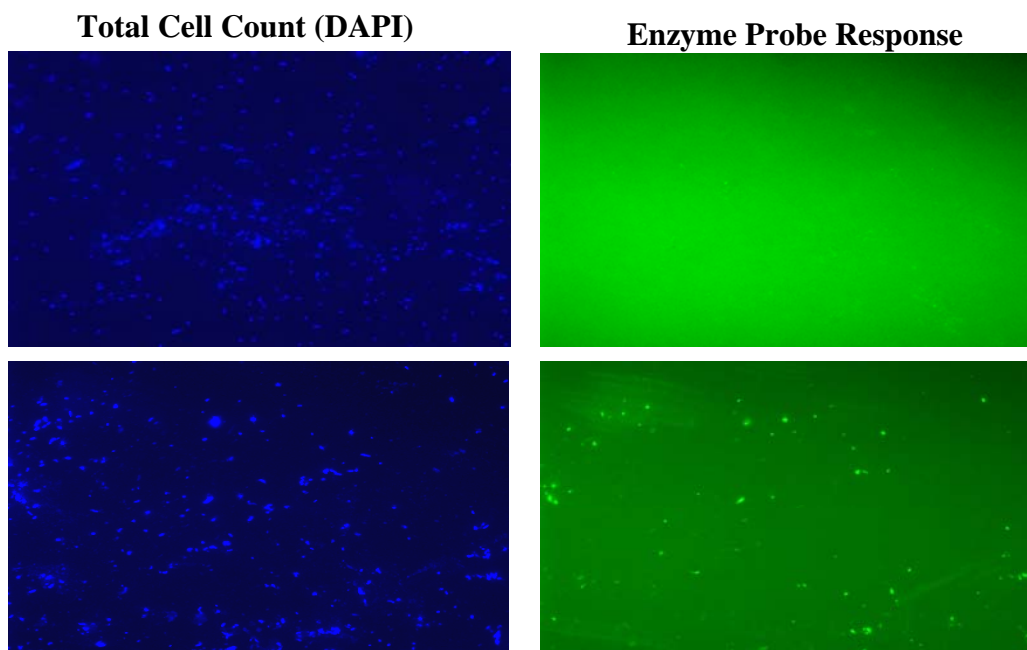
Total Cell Count (DAPI)　　　　　**Enzyme Probe Response**

Fig. 3. Negative (upper) and positive (lower) toluene enzyme probe response

In addition to the enzyme probe results, methane was detected at low levels throughout the aquifer, and methanotrophs were successfully enriched from TAN samples. Also, the presence of the genes encoding for the sMMO enzyme was confirmed through DNA extractions from 10 separate sampling locations at TAN.

Because of the widespread positive detections in response to application of the enzyme probes, several control studies were conducted in order to verify the field results. These studies included the naphthalene assay (confirmation of sMMO presence), scans of product from the sMMO enzyme probe assay, studies of enzyme activity in the presence of formate (a reductant), phenylacetylene (an irreversible inhibitor of sMMO), and methane inhibition followed by oxygen recovery. The results of all control studies supported the field results by implicating sMMO. Given that sMMO has been repeatedly shown to degrade TCE via aerobic cometabolism in both field and laboratory settings, these results conclusively document what is believed to be the first reported case of intrinsic aerobic cometabolism of TCE at environmentally significant rates.

CONCLUSION

The work presented herein has substantial implications for remediation at TAN and for the field of groundwater remediation in general. First, FLUTe™ systems represent the technically superior and most cost effective sampling systems for the deep, fractured basalt aquifer that was the subject of this investigation. This finding could be important at other sites with similar hydrogeologic settings at which multi-level sampling is required. This study showed that FLUTe™ systems can provide data not available using other methods or equipment for multi-level sampling in deep fractured rock aquifers.

More importantly, it was initially unexpected that intrinsic aerobic cometabolism would be an important degradation mechanism at TAN. The existing body of scientific literature, as well as published MNA protocols, dismissed this mechanism as being unimportant under intrinsic conditions. Despite this, because the preliminary evaluation suggested that this mechanism might be important, it was rigorously tested until sufficient evidence was collected to convince both the technical and regulatory members of the project team that intrinsic aerobic cometabolism of TCE was occurring at TAN. Ultimately, demonstration of this mechanism was the most important component of obtaining regulatory approval of the MNA remedy. Given that this is the largest TCE plume for which DOE has achieved regulatory acceptance of MNA, other DOE sites could benefit from these lessons learned.

REFERENCES

1. Sorenson, K. S., L. N. Peterson, R. L. Ely, and R. E. Hinchee, 2000, "An Evaluation of Aerobic Trichloroethene Attenuation Using First-Order Rate Estimation," *Bioremediation Journal*, 4(4): 337-358.

2. DOE-ID, August 1995, *Record of Decision for the Technical Support Facility Injection Well (TSF-05) and Surrounding Groundwater Contamination (TSF-23) and Miscellaneous No Action Sites Final Remedial Action*, DOE/ID-10139, U.S. Department of Energy Idaho Operations Office, Idaho Falls, Idaho.

3. DOE-ID, 2001, *Record of Decision Amendment, Technical Support Facility Injection Well (TSF-05) and Surrounding Groundwater Contamination (TSF-23) and Miscellaneous No Action Sites, Final Remedial Action*, DOE/ID-10139, Revision 0, U. S. Department of Energy Idaho Operations Office, Idaho Falls, Idaho.

4. Wymore, R.A., K.S. Sorenson, Jr., and P. Steven Porter, 2004. "Evaluation of Multi-level Sampling Systems for Assessment of Contaminant Distribution in a Deep, Fractured Basalt Aquifer," *Journal of Contaminant Hydrology,* submitted.

5. Miller, A.R., F. Roberto, W. Keener, and M.E. Watwood, 2002, "Development of a fluorescence-based assay for soluble methane monooxygenase," *Applied Microbial Biotechnology,* 58: 183-188.

6. Keener, W.K., and M.E. Watwood, 1997, "Probes for enzyme-dependent fluorescent labeling of bacteria degrading trichloroethylene," In: Alleman, B.C. and Leeson, A. (eds), *In Situ and On-Site Bioremediation*, Vol. 5, Proceedings of the: *Fourth International In Situ and On-site Bioremediation Symposium*, Batelle Press, Columbus, Ohio. pp. 327-332.

7. Keener, W.K., M.E. Watwood, and W.A. Apel, 1998, "Activity-dependent fluorescent labeling of bacteria that degrade toluene via toluene 2,3-dioxygenase," *Applied Microbial Biotechnology,* 49:455-462.

8. Kauffman, M.E., W.K. Keener, S.R. Clingenpeel, M.E. Watwood, M. Lehman, and D. Reed, 2003, "Activity-dependent fluorescent labeling of bacteria that degrade toluene via 3-methylcatechol)," *Journal of Microbial Methods,* 55(3): 801-805.

9. Chapelle, F. H., D. A. Vroblesky, J. C. Woodward, and D. R. Lovley, 1997, "Practical Considerations for Measuring Hydrogen Concentrations in Groundwater," *Environmental Science and Technology*, Vol. 31, No. 10, pp. 2873-2877.

10. Puls, R. W. and M. J. Barcelona, 1996, *Low-Flow (Minimal Drawdown) Groundwater Sampling Procedures*, Office of Research and Development and Office of Solid Waste and Emergency Response, EPA/540/S-95/504, U.S. Environmental Protection Agency, Washington D.C.