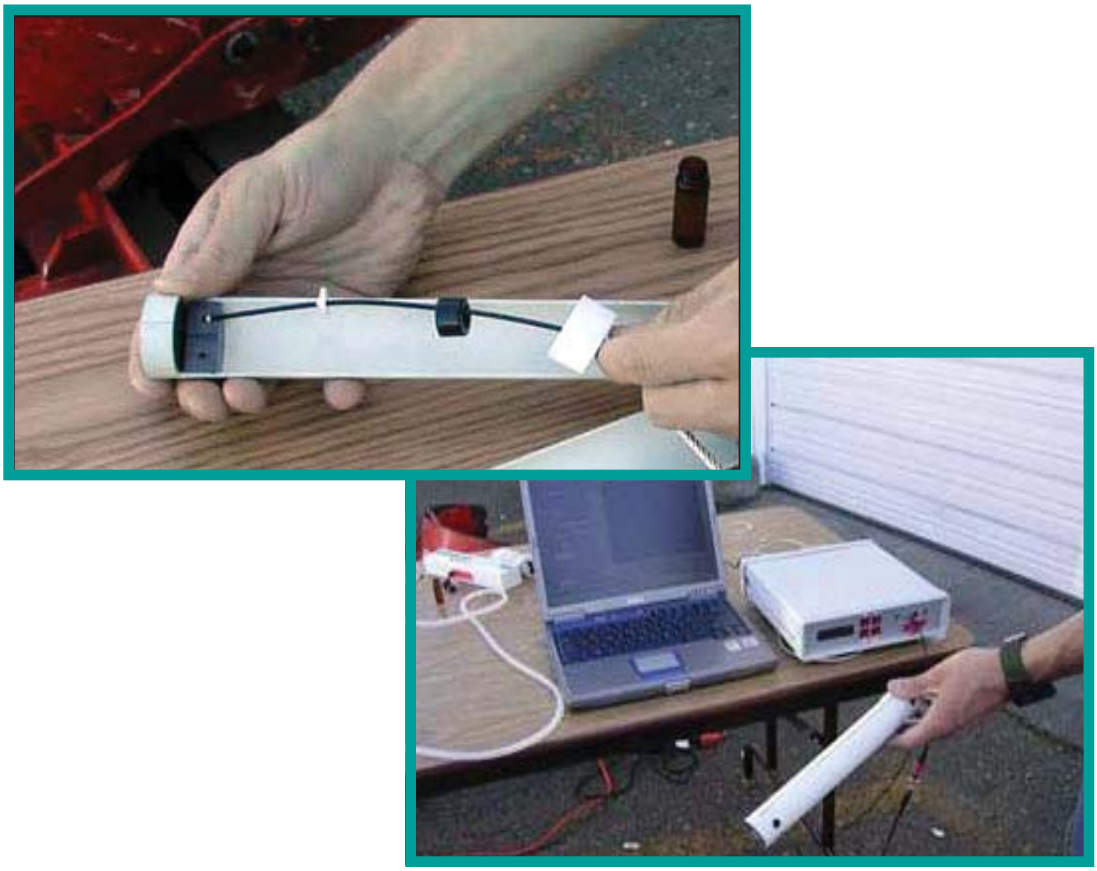
ESTCP
Cost and Performance Report
(ER-0115)



Fiber Optic Biosensors

December 2006

ENVIRONMENTAL SECURITY
TECHNOLOGY CERTIFICATION PROGRAM

U.S. Department of Defense

COST & PERFORMANCE REPORT
ESTCP Project: ER-0115

TABLE OF CONTENTS

		Page
1.0	EXECUTIVE SUMMARY	1
2.0	TECHNOLOGY DESCRIPTION	3
2.1	TECHNOLOGY DEVELOPMENT AND APPLICATION	3
2.2	PROCESS DESCRIPTION	5
2.3	PREVIOUS TESTING OF THE TECHNOLOGY	6
2.4	ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY	6
3.0	DEMONSTRATION DESIGN	9
3.1	PERFORMANCE OBJECTIVES	9
3.2	TEST SITE/FACILITY HISTORY/CHARACTERISTICS	10
3.3	PHYSICAL SETUP OPERATION	11
3.4	SAMPLING/MONITORING PROCEDURES	11
3.5	ANALYTICAL PROCEDURES	12
4.0	PERFORMANCE ASSESSMENT	13
4.1	PERFORMANCE DATA	13
4.2	PERFORMANCE CRITERIA	13
4.3	DATA ASSESSMENT	15
4.3.1	Vial Measurements	15
4.3.2	Flow-Through Cell Measurements	18
4.3.3	Down-Hole Profiling	21
4.3.4	Sentinel Well Measurements	25
4.4	TECHNOLOGY COMPARISON	25
5.0	COST ASSESSMENT	27
5.1	COST REPORTING	27
6.0	IMPLEMENTATION ISSUES	29
6.1	COST OBSERVATIONS	29
6.2	PERFORMANCE OBSERVATIONS	29
6.3	SCALE-UP	29
6.4	OTHER SIGNIFICANT OBSERVATIONS	29
6.5	LESSONS LEARNED	29
6.6	END-USER ISSUES	29
6.7	APPROACH TO REGULATORY COMPLIANCE AND ACCEPTANCE	30
7.0	REFERENCES	31
APPENDIX A	POINTS OF CONTACT	A-1

LIST OF FIGURES

Page

Figure 1. Schematic of the Fiber-Optic Biosensor System. 3
Figure 2. Schematic of the Two-Layer Detection Element of the CSU Biosensor, Illustrated for the Ethylene Dibromide Biosensor. 4
Figure 3. Biosensor Response as Photomultiplier Voltage Change Following a Change in Analyte Concentration. 4
Figure 4. Reactions Catalyzed by Hydrolytic Dehalogenases Produce Protons, Which Change the pH of the Environment Near the Enzyme. 5
Figure 5. Comparison of Fiber-Optic Sensor to a Penny to Demonstrate Small Size. 5
Figure 6. Fiber-Optic Biosensor. 6
Figure 7a. Correlation Between Biosensor and Laboratory Results. 17
Figure 7b. Biosensor and Laboratory Results for Vial Samples Shown with Percent Error Lines. 17
Figure 8. Flow-Through Cell Setup. 18
Figure 9. Flow-Through Cell Setup at 8MW47. 19
Figure 10. Flow-Through Cell Results at 8MW47—pH Versus Biosensor Readings. 19
Figure 11. Flow-Through Cell Results at 8MW47—ORP Versus Biosensor Readings. 20
Figure 12. Flow-Through Cell Results at 8MW47—DO Versus Biosensor Readings. 20
Figure 13. Flow-Through Setup at 8MW33. 22
Figure 14. Flow-Through Cell Readout Setup. 22
Figure 15. Flow-Through Cell Results at 8MW33—pH Versus Biosensor Readings. 23
Figure 16. Flow-Through Cell Results at 8MW33—ORP Versus Biosensor Readings. 23
Figure 17. Flow-Through Cell Results at 8MW33—DO Versus Biosensor Readings. 24
Figure 18. Down-Hole Profiling Setup. 24
Figure 19. Down-Hole Profiling Results 25
Figure 20. Sentinel Well Results for 8MW47. 26

LIST OF TABLES

Page

Table 1. Performance Objectives. 9
Table 2. Expected Performance and Performance Confirmation Methods. 14
Table 3. Comparison of Biosensor and Laboratory (GC/MS) Measurements of 1,2-DCA Concentrations Along with Laboratory Data on Co-Contaminants in Each Well. 16
Table 4. Comparison of Biosensor and Laboratory (GC/MS) Measurements of 1,2-DCA Concentrations Along with Field Parameter Results for Sampled Groundwater. 16

ACRONYMS AND ABBREVIATIONS

µg/L	micrograms per liter
1,2-DCA	1,2-dichloroethane
A/D	analog to digital
bgs	below ground surface
BMS	buffered measurement solution
CDM	Camp Dresser & McKee Inc.
CSU	Colorado State University
DCA	dichloroethane
DO	dissolved oxygen
DoD	Department of Defense
EA	EA Engineering, Science, and Technology
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
GC/MS	gas chromatography/mass spectroscopy
HCl	hydrochloric acid
LNAPL	light non-aqueous phase liquid
M	molar
mm	millimeter
mM	millimolar
nm	nanometer
ORP	oxidation/reduction potential
OU8	Operable Unit 8
PVA	polyvinyl alcohol
PWIA	Public Works Industrial Area
QA/QC	quality assurance/quality control
QC	quality control
Qva	Vashon advance outwash
Qvt	Vashon till
RPD	relative percent difference

SUBASE Bangor	Bangor Naval Submarine Base
UST	underground storage tank
VOA	volatile organic analyte
VOC	volatile organic compound

ACKNOWLEDGEMENTS

This report describes the demonstration of a novel analytical technology: fiber-optic-based biosensors for detecting groundwater contaminants in the field in near-real time. The report describes the demonstration of biosensors at a Department of Defense (DoD) site as well as supplemental development of additional biosensors.

Individuals who contributed to completion of this project and their affiliations: Ken Reardon, Ph. D., Colorado State University (CSU); Victor Acha, CSU; Brinson Willis, CSU; Cory Jenson, CSU; Roger Olsen, Ph.D. (PI), Camp Dresser & McKee Inc. (CDM); John Eisenbeis, Ph.D., CDM; Kristy Warren, CDM; Dan Adams, CDM; Michael Allen, Bangor Naval Submarine Base (SUBASE Bangor) (now with CDM); and Barb Chafin-Tissier, SUBASE Bangor.

Technical material contained in this report has been approved for public release.

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1.0 EXECUTIVE SUMMARY

Significant costs are associated with laboratory analyses of groundwater samples collected at Department of Defense (DoD) sites. Most of these samples are needed to characterize the nature and extent of contamination at a site, evaluate remedial system performance, and track contaminant plume migration via regularly scheduled monitoring events. There is need to replace laboratory analyses with reliable, easy-to-use field methods that produce real-time results. Colorado State University (CSU) has developed fiber-optic biosensors that are ideally suited for field monitoring of groundwater contaminants. Generally, a biosensor is a device that utilizes a biological recognition element (typically enzymes or antibodies) to sense the presence of an analyte and create a response that is converted by a transducer to an electrical or optical signal.

The primary issue regarding the use of biosensors is reliability, i.e., are biosensor results comparable to laboratory analyses? The end user also needs to know whether there are conditions that affect the reliability of biosensor performance. Biosensors also need to be easy to use and calibrate so that reproducible results can be obtained from different users. The demonstration described in this document was designed to address these issues. The overall objective of the biosensor demonstration was to provide a basis to justify the use of biosensors to augment or replace conventional analytical methods for measuring selected compounds in groundwater. Specific objectives included:

- Demonstrating the accuracy, reliability, and cost of biosensors
- Demonstrating the effectiveness of on-site field measurements using biosensors
- Determining operational limits associated with using the biosensors
- Transferring the biosensor technology to end users.

Biosensors were used to analyze groundwater sampled from several monitoring wells at Operable Unit 8 (OU8) of the Bangor Naval Submarine Base (SUBASE Bangor) in Kipsap County, Washington, to evaluate biosensor performance under a range of conditions. The target analyte was 1,2-dichloroethane (1,2-DCA). Groundwater samples were collected from monitoring wells spaced throughout the plume to analyze a wide range of 1,2-DCA and cocontaminant concentrations. The samples were analyzed by biosensors and gas chromatography/mass spectroscopy (GC/MS). A flow-through cell was also set up to allow biosensor readings in flowing water similar to the setup typically used to collect pH, conductivity, and turbidity readings prior to monitoring well sampling. Biosensors were lowered into monitoring wells to record down-hole in situ readings.

Performance of the biosensors was evaluated based on the following criteria:

- Accuracy, as demonstrated by a one-to-one correlation between the two analytical techniques (conventional GC/MS and biosensors)
- Range, as demonstrated by a response from less than 5 micrograms per liter (µg/L) to greater than 500 µg/L 1,2-DCA
- Precision, as demonstrated by a low relative percent difference (RPD) between duplicate analyses

- Sample throughput, as demonstrated by short analysis time in the field
- Mechanical reliability, as demonstrated by a low incidence of failure
- Versatility, as demonstrated by acceptable performance under a variety of conditions.

Two performance levels were established with regard to the data that the biosensors might be used to collect:

Level 1: Semiquantitative screening concentration data
Moderate accuracy
Moderate quantitation limit
Moderate specificity and selectivity

Level 2: Quantitative concentration data
High accuracy
Low quantitation limit
High specificity and selectivity

The interference of parameters affecting the pH of the groundwater being measured impacted the biosensor's performance against several performance criteria, including accuracy, precision, sensitivity, and range. The biosensor measures small pH changes produced by the reaction of an enzyme with 1,2-DCA, and techniques are required to distinguish these pH changes from pH changes due to other processes. For vial measurements, this interference can be significantly reduced by proper calibration. However, for flow-through cell and down-hole measurements, calibration procedures have not been developed to reduce the pH interference. Because the biosensor measures small pH changes produced by the reaction of an enzyme with 1,2-DCA, methods are required to distinguish these pH changes from pH changes due to other processes. This can readily be accomplished by adding an optical fiber (bundled with the biosensor) and a second measurement channel to the hardware, thus providing optical pH measurement for correction of the pH changes. At the present level of development, the biosensors would most appropriately be used to provide semiquantitative data regarding 1,2-DCA concentrations in groundwater.

The biosensors can be used to collect Level 2 quantitative data when used in the vial measurement mode; however, further investigation into development and testing of the biosensors is required for them to be reliable field instruments for all the applications originally intended.

2.0 TECHNOLOGY DESCRIPTION

2.1 TECHNOLOGY DEVELOPMENT AND APPLICATION

The CSU biosensor is a two-layer detection element immobilized on the tip of an optical fiber (Figures 1 and 2). The outer layer of the detection element contains bacteria with an enzyme that catalyzes a reaction with the analyte resulting in protons being released. The inner detection layer contains a pH-sensitive fluorescent dye (fluoresceinamine). Thus, the presence of the contaminant leads to a pH change on the fiber tip, which can be measured as a change in fluorescence intensity (Figure 3). Since the change in fluorescence depends on the contaminant concentration, these optical, enzymatic biosensors provide quantitative output.

Many enzymes catalyze reactions that result in a pH change. CSU researchers have worked primarily with the class of enzymes known as hydrolytic dehalogenases, which catalyze the introduction of water into a halogenated organic compound with the production of a hydrohalide (e.g., hydrochloric acid [HCl]) (Figure 4). However, a biosensor based on organophosphorous hydrolase has also been developed to detect members of the organophosphorous family (which includes many nerve agents).

One of the advantages of fiber-optic sensors is their small size, typically about 1 millimeter (mm) in diameter (Figure 5). These optical sensors can be used at much longer distances than electronic sensors because signal loss in optical fibers is extremely low. In the field, the fiber-optic biosensors can be lowered into a small well (e.g., Geoprobe well) for measurement.

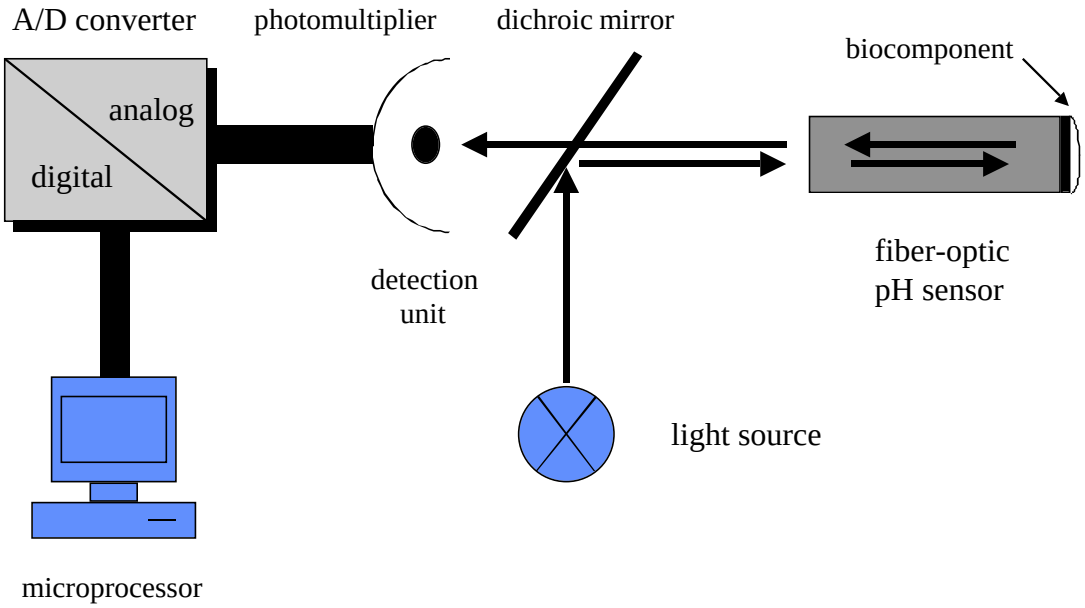

Figure 1. Schematic of the Fiber-Optic Biosensor System.

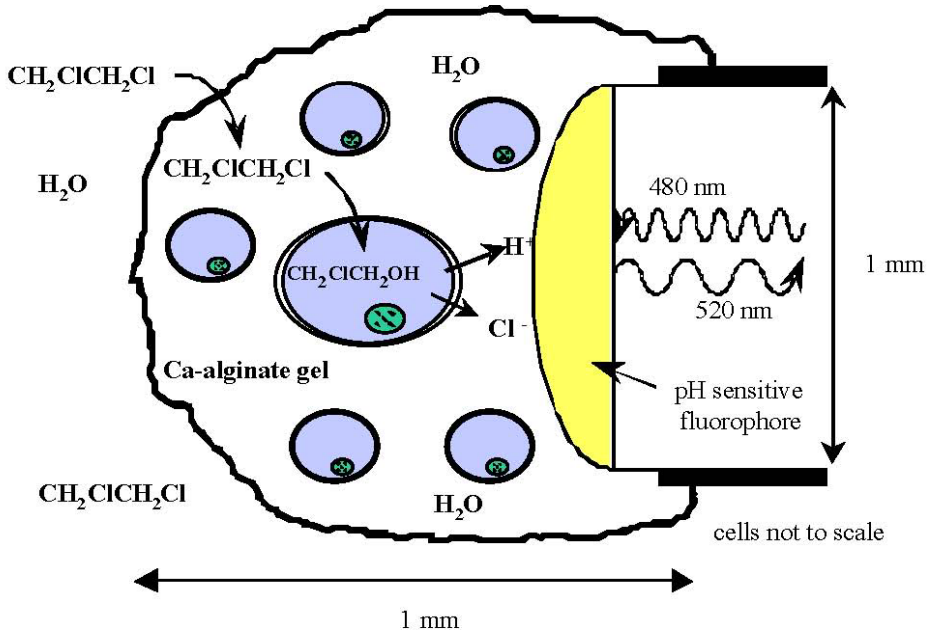

Figure 2. Schematic of the Two-Layer Detection Element of the CSU Biosensor, Illustrated for the Ethylene Dibromide Biosensor. (The pH-sensitive fluorophore is excited with 480-nanometer (nm) light and emits fluorescence at 520 nm, which is transmitted along the optical fiber to a photomultiplier.)

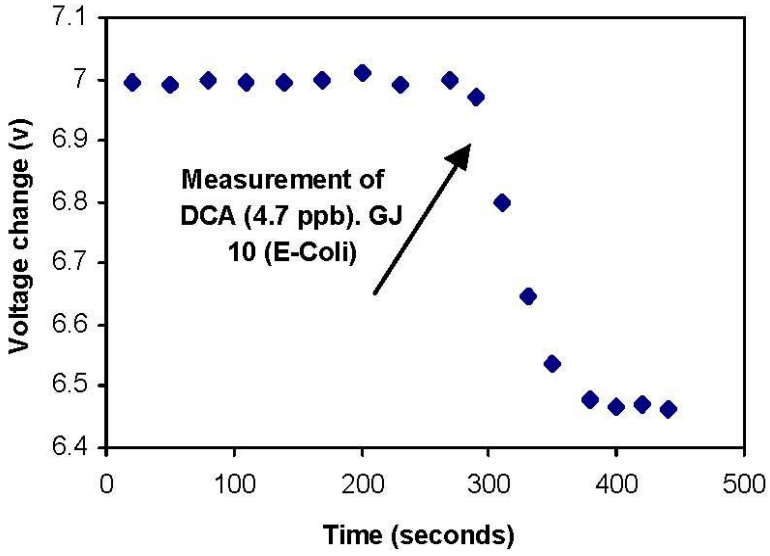

Figure 3. Biosensor Response as Photomultiplier Voltage Change Following a Change in Analyte Concentration.

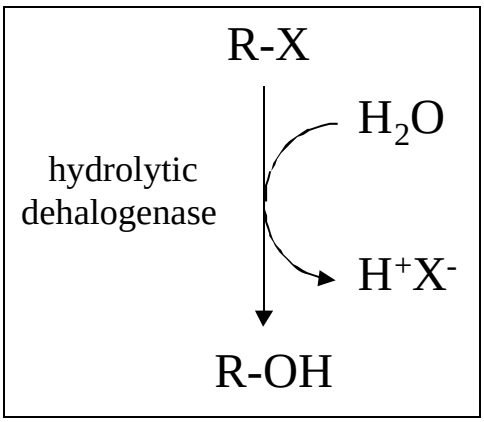

Figure 4. Reactions Catalyzed by Hydrolytic Dehalogenases Produce Protons, Which Change the pH of the Environment Near the Enzyme.

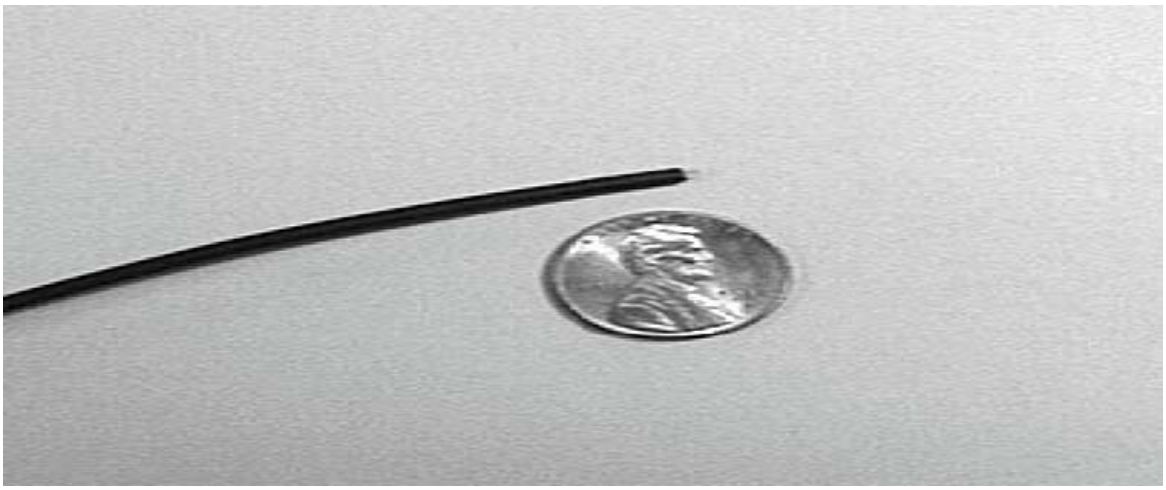

Figure 5. Comparison of Fiber-Optic Sensor to a Penny to Demonstrate Small Size.

2.2 PROCESS DESCRIPTION

Biosensor Construction Protocols

Biosensors consist of a layer of calcium-alginate-entrapped cells or purified enzymes in direct contact with a layer of a pH-sensitive fluorophore immobilized on one end of an optical fiber (Figure 6). Optical fibers coated only with fluorophore are termed pH optodes. To prepare these pH optodes, the cladding of fibers was removed from 1 mm of the distal end of the optical fiber, then polished with very fine grit paper. A pH-sensitive fluorophore was affixed to the distal end of the fiber-optic cable. The fluorophore, fluoresceinamine, was first coupled to polyvinyl alcohol (PVA) using cyanuric chloride, and the resulting product was cross-linked with glutaraldehyde in presence of HCl to form a hydrogel that was applied subsequently to the

polished optical fiber tip by using a micropipette. After polymerization, the resulting pH optode was stored in 0.1 M Na_2HPO_4 at room temperature.

Figure 6. Fiber-Optic Biosensor. (One end of the optical fiber is coated by a pH-sensitive fluorophore, which in turn is covered by cells or enzymes entrapped in Ca-alginate.)

Whole cell biosensors were created by entrapping a small amount of concentrated resting cells in a calcium alginate hydrogel on the fluorophore end of a pH optode. Previously cultured cells were combined with a 4% sodium alginate solution to give a mixture at a desired ratio. Five microliters of this gel mixture were deposited on the end of a pH optode. The resulting biosensor was immediately immersed in ice-cold 0.47 molar (M) $CaCl_2$ for 30 min, placed into a buffered measurement solution (BMS) (1 millimolar (mM) $C_6H_{13}NO_4S$+ 25 mM $CaCl_2$ + 150 mM NaCl) and stored at 4°C.

Enzyme biosensors were created by entrapping pure enzymes in a calcium alginate hydrogel on the fluorophore end of a pH optode. Enzymes were previously isolated from cells by a special procedure consisting of disrupting fresh cells by sonication to get a cell-free extract that is purified on a Ni-nitrilotriacetic acid Sepharose column HR 16/10. The pure enzymes were combined with 4% sodium alginate solution to give a mixture at a desired ratio. The subsequent steps to create an enzyme biosensor are similar to those for preparation of whole cell biosensors.

Biosensors were treated with the cross-linking agent glutaraldehyde to improve their physical stability. Biosensors were suspended in 6 M glutaraldehyde for 30 min at room temperature with stirring. The treated biosensors were washed with deionized water and stored at 4°C in measurement solution until used.

The treatment improved the stability of biosensors but lowered the diffusion of substrate and product in and out of the gel matrix, resulting in a slight decrease in the sensitivity of the biosensors.

2.3 PREVIOUS TESTING OF THE TECHNOLOGY

No significant field testing of the biosensors was performed prior to the Environmental Security Technology Certification Program (ESTCP) demonstrations.

2.4 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

In situ measurements by fiber-optic biosensors could be used to reduce costs at DoD sites in at least four scenarios. First, biosensors could be used to monitor groundwater contaminant concentrations in existing plumes, either by permanent installation of wells for monitoring over

time or by analyses of wells at discrete time points. Second, biosensors could be placed in sentinel wells between a plume and a receptor to detect offsite contaminant migration. Third, biosensors could be used to continuously monitor treatment system effluent to determine treatment efficiency and provide evidence as to whether regulatory limits for discharge are met. Finally, they could be used for site characterization—as soon as a Geoprobe or well is placed, a biosensor could determine the contaminant concentration and the results could direct the placement of subsequent Geoprobes or wells.

Relative to traditional, discrete sampling approaches, biosensors have the following advantages:

- The capability of providing low-cost, simultaneous measurements at different depths in a well (i.e., spatial resolution). Currently, average values over a screened interval are obtained because discrete interval monitoring, although more informative, is too expensive and complicated.

- The capability of providing low-cost, continuous monitoring (i.e., temporal resolution). Current methods rely on single periodic measurements that may not be representative.

To achieve the full potential of this biosensor technology, it will be necessary to develop biosensors that are stable over long (> 2 months) periods. For many applications, such as on-site vial sampling, the requirement is that the rate of sensitivity loss be low enough to allow recalibration to occur only once per day, as would be typical of any sensor. For down-hole monitoring, the rate of sensitivity loss should be lower; if this cannot be achieved, then the down-hole monitoring mode will be limited to qualitative rather than quantitative measurements.

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3.0 DEMONSTRATION DESIGN

3.1 PERFORMANCE OBJECTIVES

Performance of the biosensors was compared to the GC/MS method for groundwater analysis. Performance was evaluated based on the following objectives identified in the Demonstration Plan:

- Accuracy, as demonstrated by a one-to-one correlation between the two analytical techniques
- Range, as demonstrated by a response from less than 5 µg/L to greater than 500 µg/L 1,2-DCA
- Precision, as demonstrated by a low relative percent deviation between duplicate analyses
- Sample throughput, as demonstrated by low analysis time in the field
- Mechanical reliability, as demonstrated by a low incidence of failure
- Versatility, as demonstrated by acceptable performance under various conditions.

Table 1 presents a summary of the performance objectives and indicates which objectives were met during the demonstration.

Table 1. Performance Objectives.

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance Objective Met?
Qualitative	Sample processing rate	>6 samples/day	Yes
	Mechanical reliability	Low breakdown incidence	Yes
	Versatility	Applicability to all conditions	No
	Ease of use	Typical operator training and labor required	No
Quantitative	Accuracy	Relative percent difference (RPD) <25% (RPD for GC/MS method); correlation coefficient (r^2) >0.9	No
	Precision	RPD for biosensor equal to or less than 25% (RPD for GC/MS method)	No
	Sensitivity	<5 µg/L	Yes (if no interference)
	Range	> 500 µg/L	Yes (if no interference)

This section describes the criteria used to select a demonstration site. These criteria included:

- Presence of a contaminant detectable by biosensors
- Existence of an ongoing groundwater monitoring program with which data can be coordinated and shared
- Preference for many monitoring points and monitoring wells with long screen intervals to facilitate discrete depth measurements
- Preference for nonhomogeneous aquifer concentrations to demonstrate the importance of discrete depth monitoring.

The demonstration site selected was SUBASE Bangor since it met all the above criteria, including having a groundwater plume with 1,2-DCA as a major component. 1,2-DCA is one of the compounds for which a biosensor had already been developed and lab-tested.

3.2 TEST SITE/FACILITY HISTORY/CHARACTERISTICS

SUBASE Bangor

The study area is OU8 in the Public Works Industrial Area (PWIA) of SUBASE Bangor, which is located near the town of Silverdale, Washington. An on-site underground storage tank (UST) is believed to be the source of a release of unleaded gasoline into the surrounding media between 1982 and 1986. In 1986, soil vapor extraction/air system and product recovery were implemented to clean up the site. To date, liquid petroleum hydrocarbons remain in several monitoring wells at the PWIA. Chlorinated volatile organic compounds (VOC) are also present in site groundwater. EA Engineering, Science, and Technology (EA) conducted an investigation to assess natural attenuation processes at OU8.

OU8 geological conditions have been highly characterized by drilling and monitoring well installation. The area consists of four stratigraphic units: construction fill, Vashon till (Qvt), Vashon advance outwash (Qva), and Lawton clay. The construction fill can be found 2 to 3 feet below ground surface (bgs) and consists of a sandy material. Underlying the construction fill and ranging to a depth of about 45 ft bgs is the Qvt, which consists of silt, sand, gravel, and cobbles. This unit is 20 to 40 ft thick. The Qva (location of the shallow aquifer) is beneath the Qvt and consists of sand, silt, and gravel. The thickness of the Qva is about 100 to 130 ft. Beneath the Qva is the Lawton clay aquitard. A silty transition zone in the bottom of the Qva separates the shallow aquifer from the lower aquitard.

There are approximately 100 monitoring wells at OU8. The wells were installed at three different depth intervals: shallow, intermediate, and deep. The depth to groundwater is about 20 ft bgs, and the general flow direction is southeast. The Qva lies beneath the Qvt at OU8 and is the location of the shallow unconfined aquifer. The shallow aquifer contained in the Qva is about 125 ft thick. The shallow wells are screened within 30 ft of the water table; intermediate wells are screened within the middle 40 ft of the aquifer thickness; and the deeper wells are screened within 30 ft of the Lawton clay aquitard. The plume contains dissolved petroleum contaminants (including benzene) and dichloroethane (DCA). Most of the contaminants are in the shallow and intermediate zones of the Qva. Site characterization data for SUBASE Bangor can be found in Appendix A of the Final Report (Olsen and Reardon, 2005).

3.3 PHYSICAL SETUP OPERATION

All equipment and supplies necessary for measurements were mobilized to and around the demonstration site in a van. No site utilities were required. Power was obtained from either a vehicle battery or a portable generator. Biosensors were transported to the site from CSU on ice. The tips were stored in a 0.01 M buffer solution at pH 7.0 with no contaminant present and were maintained in that solution on ice until shortly before their use.

3.4 SAMPLING/MONITORING PROCEDURES

The fiber-optic biosensor demonstrations used the following sampling methods.

- **VOA Vial Measurements.** A biosensor was inserted into a vial containing a sample of the groundwater from a monitoring well. A split sample was sent to an off-site laboratory for analysis by GC/MS.
- **Flow-Through Cell Measurements.** A biosensor was inserted into an aboveground, flow-through cell (with continuous flow of groundwater from the monitoring well) in conjunction with recording routine measurements of the field parameters pH, dissolved oxygen (DO), oxidation/reduction potential (ORP), temperature, and specific conductance.
- **Down-Well Measurements.** A biosensor was lowered down-hole in an unpumped monitoring well. Measurements were taken at several depth intervals to define contaminant gradients.
- **Sampling of "Sentinel" Wells.** A biosensor was installed down-hole in a selected monitoring well. The fiber-optic cable and analyte probe were left in the hole and monitored on a routine basis over the period of a day. Results from this type of sampling provided a basis to determine if the biosensors could be left in a well for longer periods and what calibration needs are necessary for such sampling.

The above procedures allowed for comparison of biosensor readings with analytical results from GC/MS laboratory analysis. The results were also used to compare sampling methods and concentration profiles with depth. The results and details concerning measurement methods used at each monitoring well are provided in Section 4.

Field QC Samples

The following types of quality control (QC) samples were collected and analyzed:

- **Duplicate samples**. Two of the VOA vials filled with groundwater were analyzed on site using the bsiosensor. The second sample was analyzed immediately after the first sample and was identified as a duplicate sample. A third sample was retained for potential later analysis.
- **Colocated samples**. As previously described, concentrations of 1,2-DCA were measured down-hole in selected wells. At one of these locations, the biosensor was removed and cleaned. The down-hole analyses were then repeated at the same depths in the same well.
- **Additional QC samples**. Additional quality assurance/quality control (QA/QC) samples are discussed in the following paragraphs that would typically be used only in an off-site

laboratory. However, because the biosensor is being evaluated for use as a replacement for off-site analyses, additional samples were analyzed.

3.5 ANALYTICAL PROCEDURES

Groundwater samples collected from monitoring wells during the demonstrations at SUBASE Bangor were analyzed for VOCs using Environmental Protection Agency (EPA) Method 8260B for GC/MS.

4.0 PERFORMANCE ASSESSMENT

4.1 PERFORMANCE DATA

Volatile Organic Analyte (VOA) Vial Analysis. The objective of this type of analysis was to compare the biosensor readings to off-site laboratory results (GC/MS) for 1,2-DCA. After field parameters indicated that stable groundwater conditions had been reached during pumping, two VOA vials were filled. Three additional VOA vials were filled for analysis by the biosensors. Filling alternated between vials for biosensor and off-site analyses. The cap of one of the biosensor vials was removed briefly and immediately replaced with a cap fitted with a biosensor. A biosensor reading was recorded after sufficient time had elapsed to obtain a stable reading. This procedure was then repeated for the second vial (duplicate).

Flow-Through Cell Analysis. While groundwater from the sample pump was being measured for field parameters (pH, DO, etc.) in a flow-through cell, a second flow-through cell (connected in series and attached to the effluent port of the first cell) was utilized to take biosensor readings of the groundwater flowing from the well. Before placement in the cell, a biosensor was inserted into a field standard of 1,2-DCA at a concentration that was similar to the anticipated concentration from the well being sampled (based on the last lab results for that well). This helped to minimize the time needed for a stable reading when the biosensor was put into the flow-through cell. Readings were recorded from the biosensor at regular intervals until field parameter readings stabilized. In addition to individual biosensor readings, notes were taken as to the range in fluctuation of readings and the approximate average reading.

Down-Well Measurements. A biosensor was lowered down-hole in an unpumped monitoring well. Measurements were taken at several depth intervals to define contaminant gradients.

Sampling of "Sentinel" Wells. A biosensor was installed down-hole in a selected monitoring well. The fiber-optic cable and analyte probe were left in the hole and monitored on a routine basis over the period of a day. Results from this type of sampling provided a basis to determine if the biosensors could be left in a well for longer periods and what calibration needs are necessary for such sampling.

4.2 PERFORMANCE CRITERIA

The performance of the biosensor was assessed at two levels: Level 1 is the ability to provide qualitative, screening data with definitive compound identification. Level 2 is the ability to provide definitive compound identification and quantitative concentrations.

Level 1: Semiquantitative screening concentration data
Moderate accuracy
Moderate quantitation limit
Moderate specificity and selectivity

Level 2: Quantitative concentration data
High accuracy
Low quantitation limit
High specificity and selectivity

Table 2 provides the data quality objectives and evaluation criteria. Evaluation criteria for 1,2-DCA were selected to be consistent with those standard procedures used by the off-site laboratory (GC/MS methods equivalent to EPA method 8260B).

Table 2. Expected Performance and Performance Confirmation Methods.

Performance Criteria	Expected Performance Metric (pre-demonstration)	Performance Confirmation Method	Actual (post-demonstration)
PRIMARY CRITERIA (Performance Objectives) (Qualitative)			
Sample throughput	> 6 samples per day	Experience from demonstration operation	For vial measurements, >6 samples per day
Mechanical reliability	Low breakdown incidence	Experience from demonstration operation	Further development needed to improve mechanical reliability of biosensor tips. Hardware reliability was high.
Versatility	Applicability to all conditions tested	Comparison of results from different wells and laboratory testing	Further development needed for the biosensors to address interference of pH on measurements.
Ease of use	Operator training and labor required similar to other field equipment	Comparison to operator requirements for other commonly used field instruments	Ease of operation similar to other field instruments, although calibration could be simplified.
PRIMARY CRITERIA (Performance Objectives) (Quantitative)			
Accuracy	RPD <25% (the RPD for EPA Method 8060B) r2 >0.9	Correlation with GC/MS reference method	Accuracy was dependent on ability to correct for non-analyte-related pH changes. For vial measurements, r2 = 0.934 and average RPD = 45.6%
Precision	RPD for biosensor equal to or less than RPD for reference method	RPD between replicates, taking into account best RPD attained with the GC/MS reference method	Average RPD for vial measurements = 45.6% Overall, RPDs higher than reference method.
Sensitivity	Detection limit for 1,2-DCA <5 µg/L	Detection of 1,2-DCA concentrations less than 5 µg/L as determined by GC/MS reference method	Detection limit for 1,2-DCA <5 µg/L
Range	> 500 µg/L 1,2-DCA	Ability to quantify 1,2-DCA concentrations greater than 500 µg/L as determined by GC/MS reference method	> 500 µg/L 1,2-DCA
SECONDARY PERFORMANCE CRITERIA (Qualitative)			
Hazardous materials	No hazardous materials produced	Evaluate materials needed for operation	No hazardous materials produced
Process waste	No process waste produced	Observation	No process waste produced

Table 2. Expected Performance and Performance Confirmation Methods (continued).

Performance Criteria	Expected Performance Metric (pre-demonstration)	Performance Confirmation Method	Actual (post-demonstration)
Factors affecting performance • Throughput groundwater quality	• Analysis rate >6 samples/day • No interferences under typical groundwater conditions	• Time/sample analysis Performance not affected by groundwater characteristics	• Analysis rate >6 samples/day • In some cases, pH changes interfered with biosensor analysis • Biosensor tips need to be stabilized for long-term immersion
Maintenance	Maintenance requirements similar to other field instruments	Comparison of field records to operator requirements for other commonly used field instruments	Biosensor tips need refrigeration and have a finite shelf life. Durability of tip could be improved. Hardware maintenance not dissimilar to other field instruments.
Scale-up constraints	No commercialization constraints	Investigate ability to easily produce commercially	Likely no commercialization constraints; however, depends on further development results.

4.3 DATA ASSESSMENT

This section presents the results for the various types of biosensor measurements taken during the second demonstration at SUBASE Bangor. The first demonstration was ineffective due to damage to the biosensor hardware during shipping to the site. The hardware was repaired on site; however, few usable measurements were collected. Valuable experience was obtained during the first demonstration (logistics, sampling methods, field calibration, etc.) The following sections describe results from the second demonstration.

4.3.1 Vial Measurements

Results of the biosensor and the off-site laboratory measurements (GC/MS) are summarized in Tables 3 and 4. The biosensor results for these measurements are plotted against results of the laboratory method (GC/MS) in Figures 7a and 7b. The correlation coefficient (r2 value) for the two methods was 0.934. This indicates good agreement between biosensor readings and the laboratory results under the conditions of the vial measurements.

Figure 7b shows the biosensor results for vial measurements plotted against the laboratory GC/MS results. The one-to-one correlation line is shown as the dashed line. The 50% and 100% error lines represent the areas of the graph where points must fall to be within 50 and 100% of the one-to-one correlation.

Table 3 also presents the RPD values for the biosensor and laboratory analyses. The average RPD for nine vial samples was 45.6 %, with a range of 16.2 to 80.0%. This is greater than the RPD for the reference method (EPA Method 8260B for GC/MS) of 25%.

Table 3. Comparison of Biosensor and Laboratory (GC/MS) Measurements of 1,2-DCA Concentrations Along with Laboratory Data on Co-Contaminants in Each Well.

Well	Laboratory DCA (µg/L)	Biosensor DCA (µg/L)	RPD (%)	Aromatic VOCs (µg/L)	Chlorinated VOCs (µg/L)
8MW35	17	10	51.8	ND	18.4
8MW33	18	38	71.4	ND	55
8MW33	19	15	23.5	78	51
8MW33	18	36	66.7	77	52
MW-05	900	475	61.8	14,090	1,520
8MW49	730	610	17.9	40,460	730
8MW06	990	842	16.2	4,548	1,031
8MW25	<1	<27*	--	ND	ND
8MW03	6	14	80.0	ND	6
8MW47	600	483	21.6	38,100	600
8MW08	<20	<107*	--	9,780	58
Average			45.6		

* Unreliable delta V/pH (pho)

Table 4. Comparison of Biosensor and Laboratory (GC/MS) Measurements of 1,2-DCA Concentrations Along with Field Parameter Results for Sampled Groundwater.

Well	Laboratory DCA (µg/L)	Biosensor DCA (µg/L)	pH (S.U.)	ORP (mV)	DO (mg/L)	Specific Conductance (µS/cm)
8MW35	17	10	6.7	132	0.07	128
8MW33	18,19, 18	38, 15, 36	7.1	92	0.11	346
MW-05	900	475	6.5	-46	0.13	457
8MW25	<1	<27	6.6	22	1.11	131
8MW03	6	14	6.7	46	1.63	140
8MW47	600	483	6.7	-59	0.09	704
8MW08	<20	13	6.6	6	1.18	764

Table 3 data show that samples with high 1,2-DCA concentrations had high concentrations of aromatic VOCs (e.g., benzene). No correlation between aromatic VOC concentrations or chlorinated VOC concentrations and the RPD of laboratory and biosensor measurements was observed. This indicates that the biosensors were not affected by the presence of relatively high concentrations of these co-contaminants.

The vial measurement results indicate that at their current state of development, the biosensors would be appropriately used as a Level 1 instrument, providing semiquantitative screening concentration data.

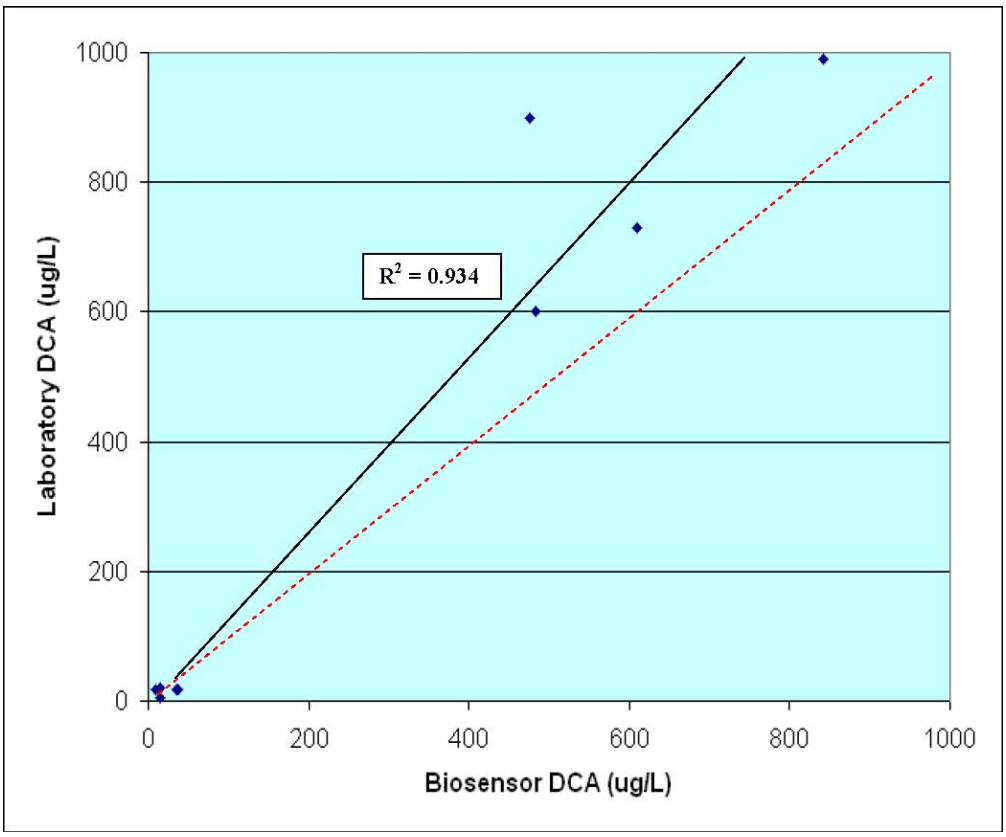

Figure 7a. Correlation Between Biosensor and Laboratory Results.
(Dashed line is the one-to-one correlation line.)

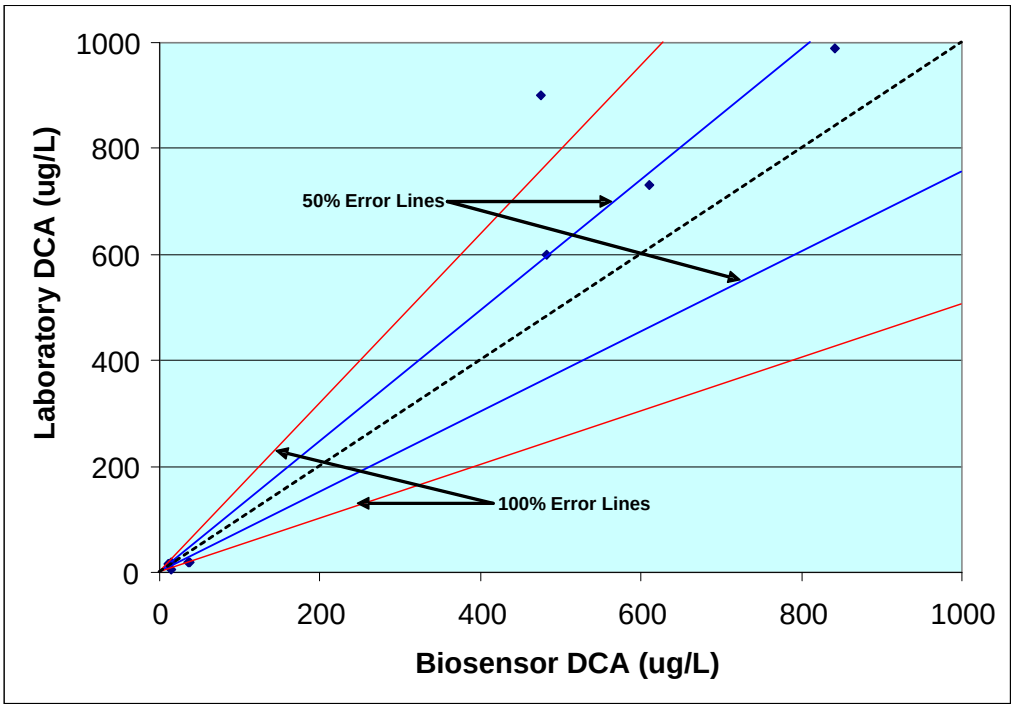

Figure 7b. Biosensor and Laboratory Results for Vial Samples Shown with Percent Error Lines.

4.3.2 Flow-Through Cell Measurements

Flow-through cell measurements were taken with the biosensors at two monitoring wells—8MW47 and 8MW33. Figure 8 shows the setup for taking biosensor readings in a flow-through cell. As biosensor readings were taken in the flow-through cell, measurements of pH, specific conductivity, temperature, ORP, and DO were also recorded.

8MW47

Figure 9 shows the flow-through cell setup at 8MW47. Figures 10, 11, and 12 show the flow-through cell biosensor readings plotted along with pH, ORP, and DO readings, respectively. Since flow-through cell measurements are frequently used to indicate when a well has been pumped sufficiently to allow for sampling of groundwater, it is of interest to note that the biosensor measurements were steady before ORP and at about the same time as DO and pH. The data in these three figures do not indicate a strong correlation between ORP or DO with the biosensor response (and none is expected). Since the biosensor signal is composed of a response to the analyte concentration as well as a response to the environmental pH, some correlation of the biosensor response with pH signal might be expected. This was not the case in the first 15 min of the test, suggesting that changes in analyte concentration were dominant during this period (recall that lower biosensor signal indicates increased analyte concentration). These two effects could be resolved by including a second optical fiber on the instrument for measurement of pH.

Figure 8. Flow-Through Cell Setup.

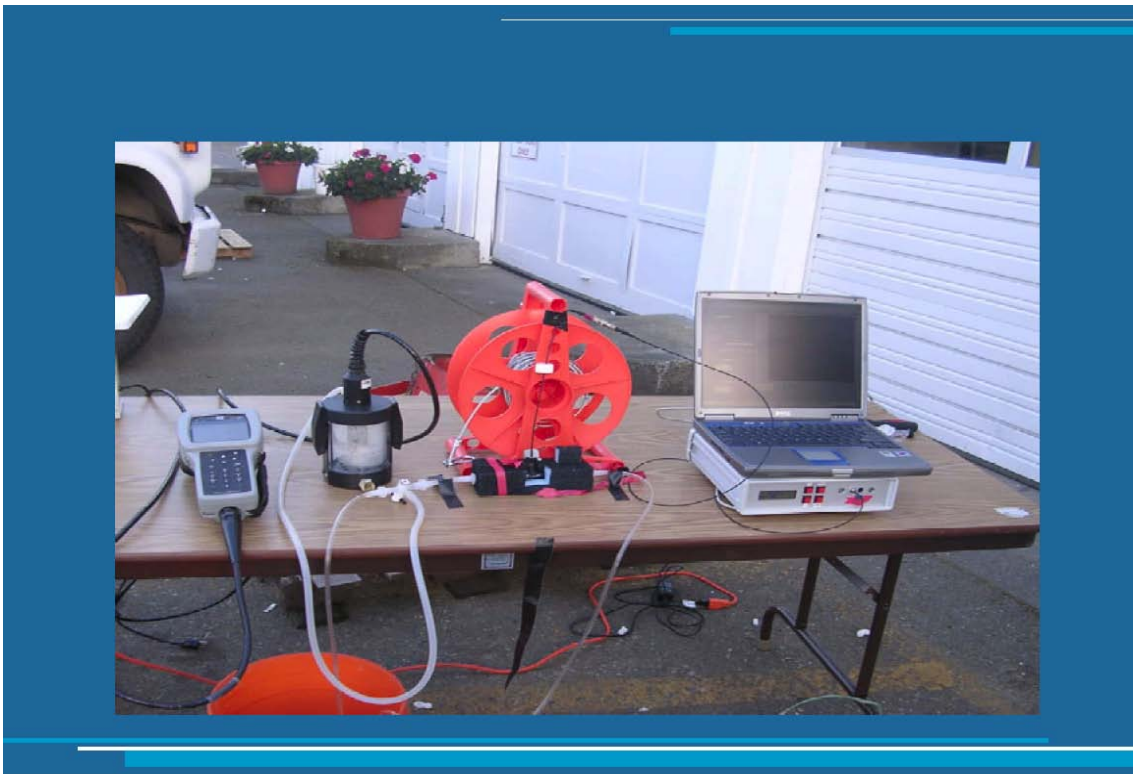

Figure 9. Flow-Through Cell Setup at 8MW47.

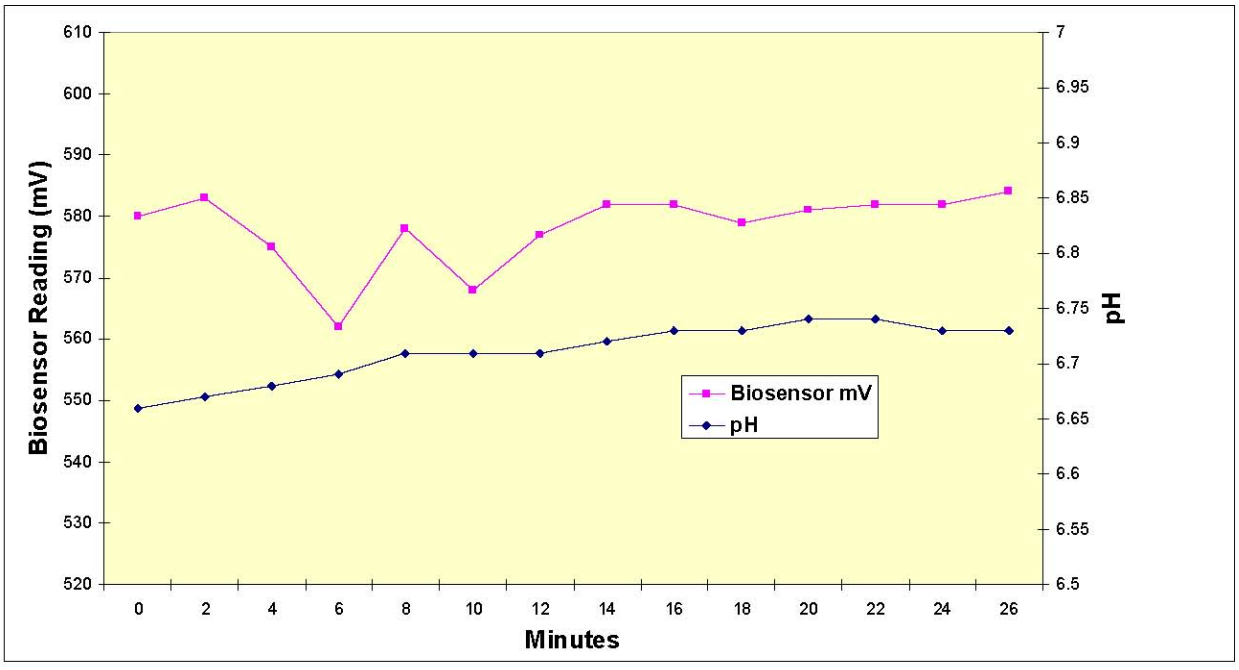

Figure 10. Flow-Through Cell Results at 8MW47—pH Versus Biosensor Readings.

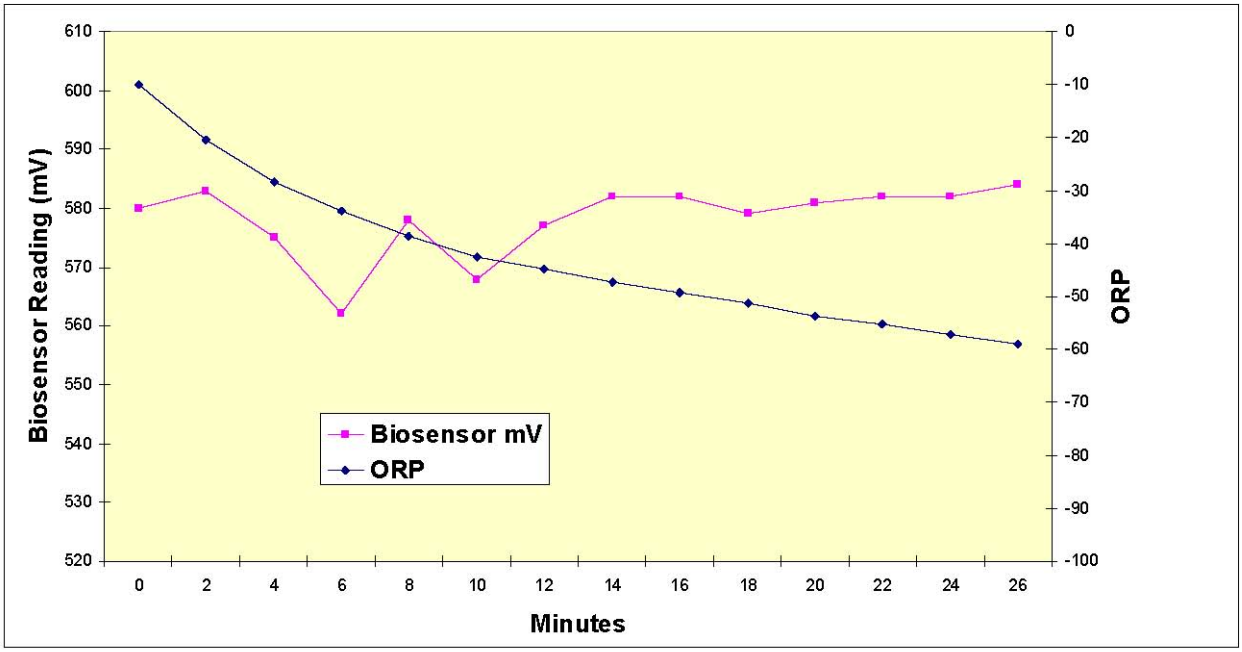

Figure 11. Flow-Through Cell Results at 8MW47—ORP Versus Biosensor Readings.

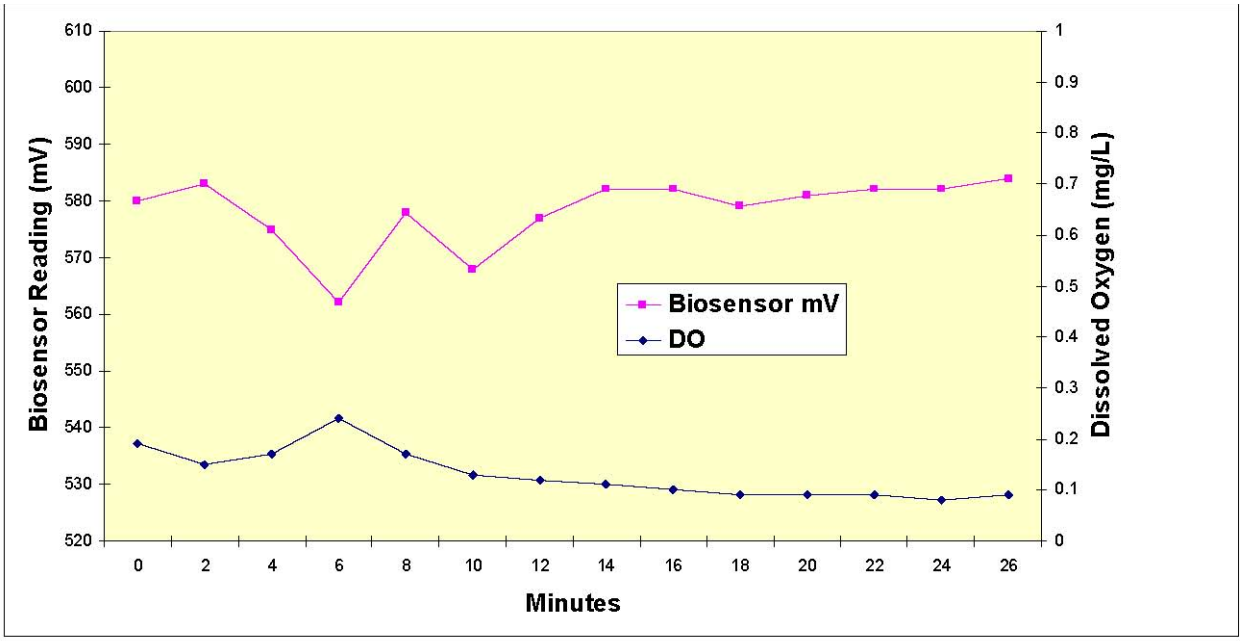

Figure 12. Flow-Through Cell Results at 8MW47—DO Versus Biosensor Readings.

8MW33

Figures 13 and 14 show the flow-through cell setup at 8MW33. Figures 15, 16, and 17 show the flow-through cell biosensor readings plotted along with pH, ORP, and DO readings, respectively. The results were similar to those obtained from 8MW47 in that the biosensor measurements did not correlate with ORP or DO. The influence of pH on the biosensor measurements can be noted when the two data sets are parallel (after approximately 15 min of pumping). However, in the initial phase of the experiment, the biosensor and pH measurements change at different rates, indicating that the biosensor measurements reached a steady value earlier. Inclusion of an optical pH measurement as a second channel on the biosensor instrument would allow analyte measurements to be separated from these environmental pH changes (not related directly to the analyte).

Overall, the results indicate that the biosensors can be used to determine when water quality during pumping and sampling has reached stable conditions. At these wells (at least 8MW33), the water could have been sampled earlier based on the stable biosensor readings. The results are classified as Level 1.

4.3.3 Down-Hole Profiling

A biosensor was placed in a protective sheath (Figure 18) to take measurements down hole for the purpose of defining the 1,2-DCA vertical profile within a monitoring well. This setup was lowered into well 8MW47 and readings were recorded at 2-ft intervals from the water table to the bottom of the screened zone. Measurements were also made at the same 2-ft intervals as the biosensor was raised from the bottom of the screen zone. The results were identical to those observed as the biosensor was lowered into the well. The results are shown in Figure 19, which shows measurements in millivolts because calibration procedures for a flow-through setup have not yet been developed to effectively translate millivolts readings to 1,2-DCA concentrations. Although a firm assessment of 1,2-DCA concentrations cannot be made without having an optical pH measurement at the same location as the biosensor, a preliminary evaluation of the data in Figure 19 suggests that the concentration of 1,2-DCA was highest at the surface, decreased over the next 5 ft until a layer of higher concentration was reached, then decreased again (recall from Figure 3 that higher 1,2-DCA concentrations lead to lower fluorescence measurements). Small amounts of light non-aqueous phase liquid (LNAPL) were encountered in this well, and thus it is possible that the high surface concentrations were caused by 1,2-DCA that was partitioned into the LNAPL. However, multidepth groundwater sampling would be needed to confirm these conclusions (i.e., determine the extent to which an increase in biosensor response was due to an increase in DCA concentration).

These biosensor readings may be among the first near-real-time readings to allow detection of varying low 1,2-DCA concentrations in groundwater with varying hydraulic conductivity in a vertical profile. The results clearly show that stratification within the screened interval occurs. Therefore, the typical pumped samples will depend on placement of the pump and the mixing of stratification that occurs. Development of a tool to measure stratification in situ is a significant advancement. The results are classified as Level 1 (no quantitative data were obtained). However, estimates of the changes in concentrations between the depths were made and the changes were significant.

Figure 13. Flow-Through Setup at 8MW33.

Figure 14. Flow-Through Cell Readout Setup.

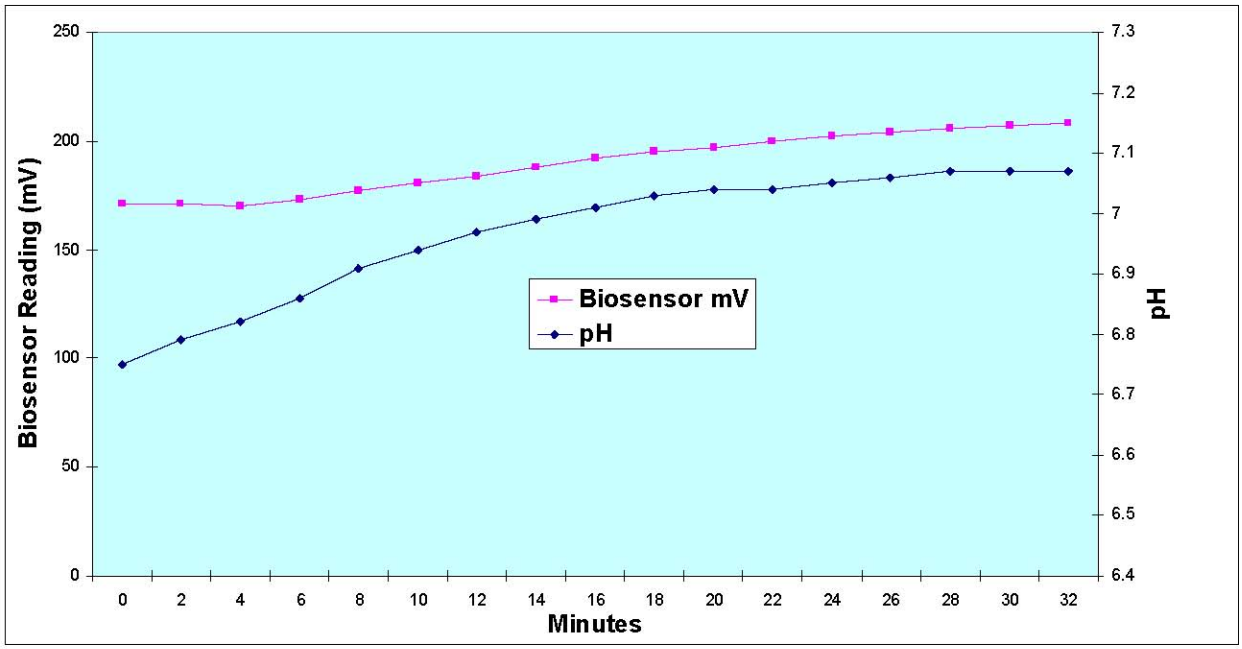

Figure 15. Flow-Through Cell Results at 8MW33—pH Versus Biosensor Readings.

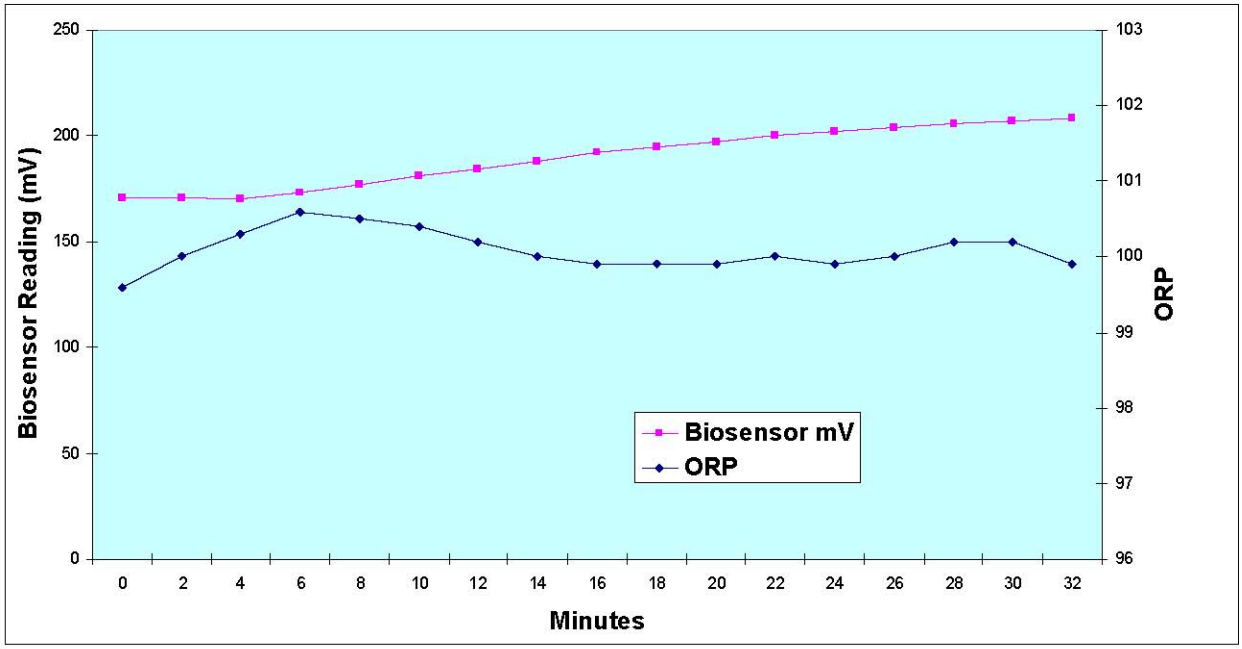

Figure 16. Flow-Through Cell Results at 8MW33—ORP Versus Biosensor Readings.

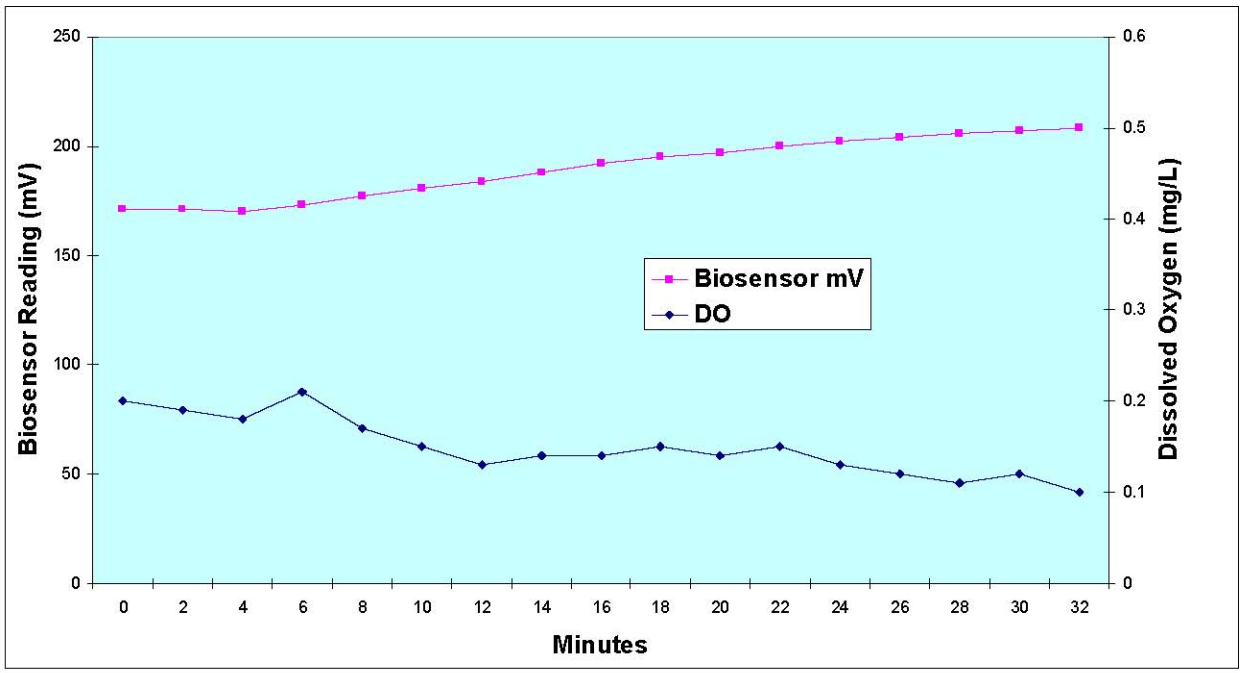

Figure 17. Flow-Through Cell Results at 8MW33—DO Versus Biosensor Readings.

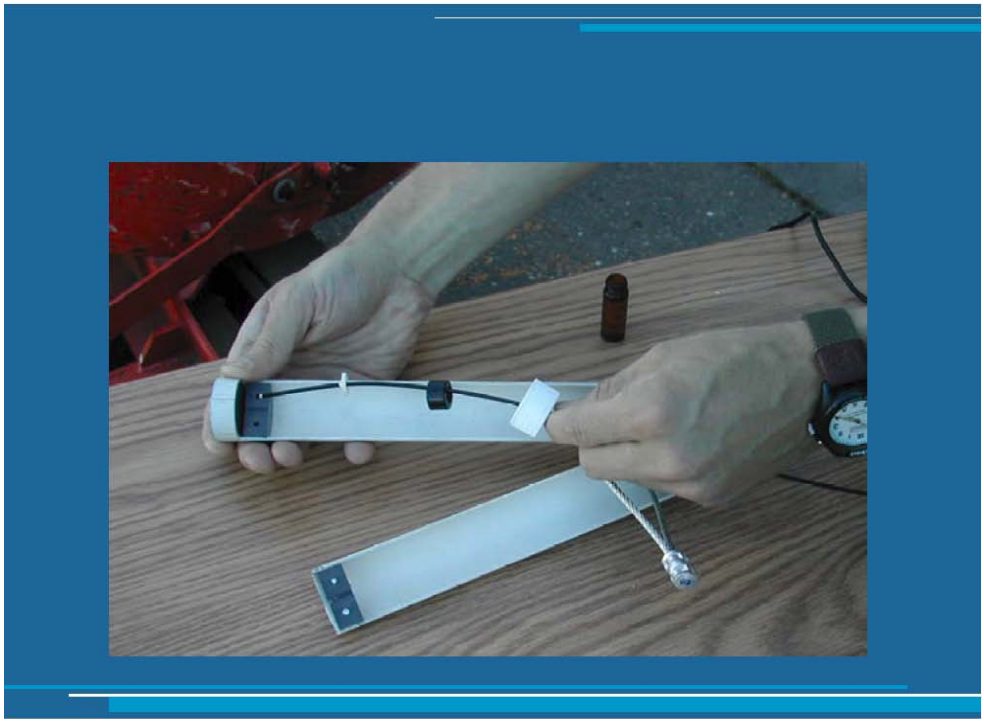

Figure 18. Down-Hole Profiling Setup.

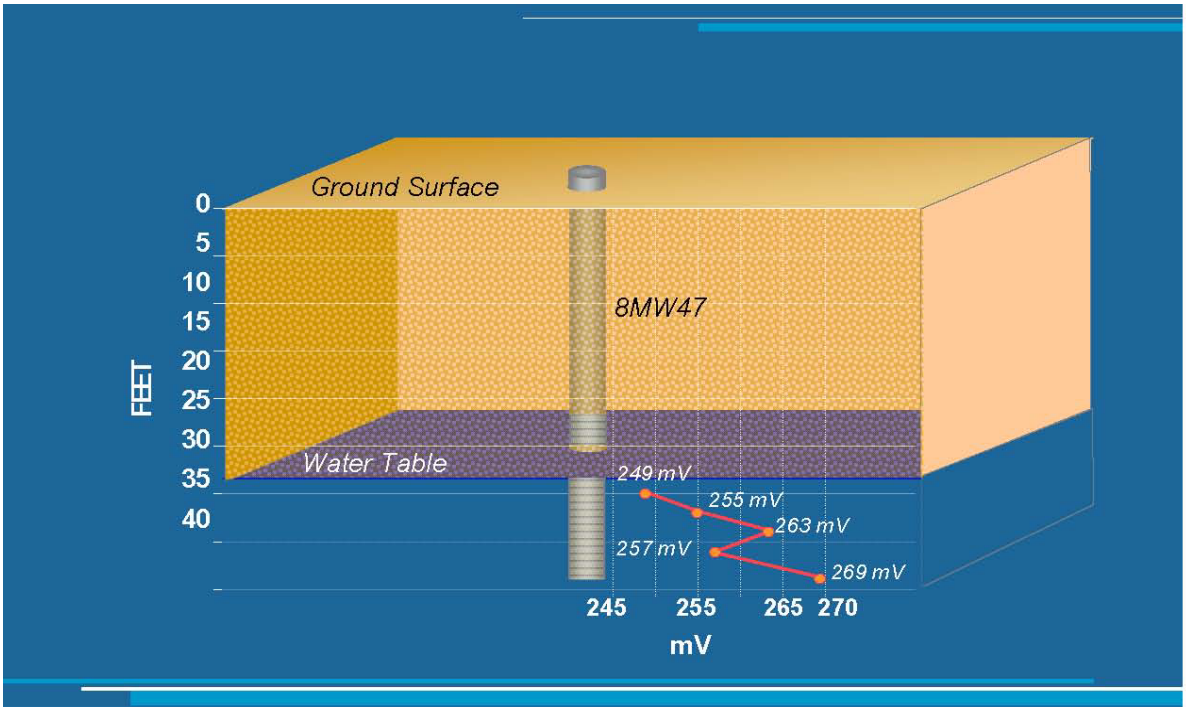

Figure 19. Down-Hole Profiling Results (biosensor readings versus depth).

4.3.4 Sentinel Well Measurements

A biosensor was placed down hole in the protective sheath used for down-hole profiling in monitoring well 8MW47 and was left in place for 24 hours. Periodically, readings were recorded by connecting the hardware to the biosensor. The results are shown in Figure 20. The biosensor signal decreased about 20% over the first 18 hours, and the signal was essentially constant from 15 to 18 hours. However, the biosensor output then dropped another 65% in the next 6 hours. Since the biosensors were shown to have significantly longer lifetimes in laboratory studies, the observed decline was expected to be caused by a factor other than loss of enzyme activity. Visual inspection of the tip of the biosensor after 24 hours down hole indicated the alginate layer containing the bacteria (and enzyme) had become detached from the tip. If the biosensors are to be used in a down-hole mode, then the biosensor tips need to be stabilized to allow for long-term immersion in groundwater. This can be accomplished by cross-linking the alginate polymer or by choosing a different immobilization matrix.

4.4 TECHNOLOGY COMPARISON

In general, the biosensors functioned as Level 1 measurement devices and provided measurements that were not impacted by the presence of other groundwater contaminants. When used in flow-through cells and for vertical profiling, the biosensors produced significant data that were not readily available by other means. Three factors that limit the performance and utility of this measurement technology must be addressed:

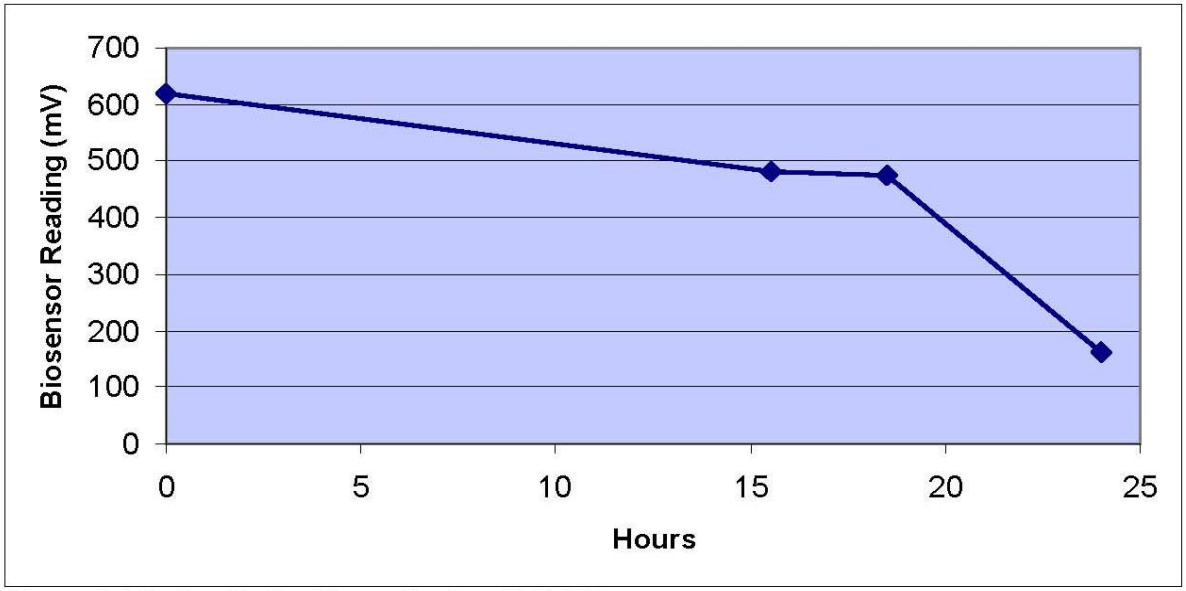

Figure 20. Sentinel Well Results for 8MW47.

1. **The influence of pH on the biosensor measurement**. Because the biosensor measures small pH changes produced by the reaction of an enzyme with 1,2-DCA, methods are required to distinguish these pH changes from pH changes due to other processes. This can readily be accomplished by adding an optical fiber (bundled with the biosensor) and a second measurement channel to the hardware, thus providing optical pH measurement for correction of the pH changes.
2. **Calibration procedures**. An adequate calibration procedure has been developed for vial measurements; however, calibration procedures must still be developed for flow-through cell and down-hole measurements.
3. **Robustness**. The biosensor tips should be designed to be more durable. Methods to do this (e.g., cross-linking the alginate layer) have been tested in the laboratory and appear to be feasible.

The biosensors can be used to collect Level 2 data when used in the vial measurement mode; however, further investigation into development and testing of the biosensors is required for them to be reliable field instruments for all the applications originally intended.

5.0 COST ASSESSMENT

5.1 COST REPORTING

Given the developmental requirements of the biosensors before they can be commercialized and being at ESTCP's direction, no costs for their use have been developed at this time.

After further development, the potential benefits of using biosensors in groundwater monitoring can be assessed by comparing costs associated with biosensor use with conventional monitoring methods (i.e., laboratory methods similar to EPA Method 8260B) on a per well basis as well as on a sampling event basis.

The primary cost driver for the biosensor technology is the capital cost of the optical-electronic system that includes the light source and detection units. Although the cost of this unit is currently approximately $5,000, the figure is for custom construction. If manufactured commercially, the price would be substantially lower.

One cost issue with biosensors is the length of time a biosensor tip will last during regular use. To date, biosensor tips have been prepared with very good activity retention over 10 days, and further improvements are anticipated. However, the biosensor tips themselves are inexpensive to prepare and thus should not be costly to purchase. Installation of new tips and disposal of old ones is not labor-intensive. Recalibration must be done periodically, regardless of whether a new tip has been installed or an old tip is being used in a new location.

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6.0 IMPLEMENTATION ISSUES

6.1 COST OBSERVATIONS

As noted above, no costs for the biosensors' use have been developed at this time.

6.2 PERFORMANCE OBSERVATIONS

These demonstrations showed that, while the biosensors are not yet ready for commercialization, with further development they can be valuable tools for providing accurate field analyses.

6.3 SCALE-UP

Scale-up is not an issue for the biosensors.

6.4 OTHER SIGNIFICANT OBSERVATIONS

There are no other significant observations regarding the biosensors at this time.

6.5 LESSONS LEARNED

This demonstration showed that, while the biosensors are not yet ready for commercialization, with further development they can be a valuable tool for providing accurate field analyses of several groundwater contaminants. This further development needs to focus on:

- Improving calibration methods to increase accuracy and precision
- Improving field usability
- Adding multichannel capability to hardware to facilitate calibration and analyze multiple compounds.

6.6 END-USER ISSUES

Potential end-user issues that exist for the use of biosensors for groundwater monitoring include:

- Is the instrument easy to use?
- Is calibration an easy process?
- Are the results accurate and repeatable for conditions at the site?
- What is the detection limit and does it change with changing conditions?
- Can biosensors detect other and/or multiple compounds?

The demonstration was designed to address each of these issues. Ease of use and calibration procedures were documented. The evaluation criteria that have been presented for comparing biosensor and conventional laboratory method results address accuracy, interference, and detection limit issues.

After the required additional development, procurement of the biosensor technology is expected to be straightforward. Although CSU is pursuing patent protection for this technology being done

for the purpose of providing incentive for an equipment manufacturer that would require intellectual property protection to commercialize the device (Patent application, Reardon, and Das, 2001). The goal is to license the patent to such a company, which would then manufacture the biosensors commercially with no restrictions; i.e., the biosensors would be available to DoD and remediation professionals similar to oxygen and pH sensors.

Also, the long-term performance of this sensor technology is an important factor for its commercialization. Although this performance characteristic was not within the scope of this demonstration, we have evidence from laboratory tests that storage lifetimes of at least 50 days are possible with less than 10% loss in sensitivity. If sensitivity loss is limited to the same low rate when the biosensors are in frequent or continual use, this would mean that recalibration would need to occur only weekly in the vial or depth profiling measurement modes. For down-hole monitoring, that rate of sensitivity loss would mean that the biosensors would need to be recalibrated every 50 days to retain accuracy within 10%. However, if only semiquantitative or presence/absence signals are required, recalibration could occur much less frequently. Future research could target this aspect of the biosensor performance. Once the causes of sensitivity loss (e.g., enzyme leakage from the biosensor tip, enzyme degradation, fluorophore bleaching) are evaluated, the appropriate redesign could take place.

6.7 APPROACH TO REGULATORY COMPLIANCE AND ACCEPTANCE

Comparison of the biosensor results to conventional results will be necessary to obtain regulatory approval of biosensor use. With respect to execution of the demonstration, minimal regulatory involvement was needed since this was a demonstration of analytical technology and not of a remediation technology.

7.0 REFERENCES

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