Automated Monitoring of Subsurface Microbial Metabolism using Graphite Electrodes

Small Business Innovative Research (SBIR)

Phase IIB Proposal

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A. IDENTIFICATION/SIGNIFICANCE OF THE PROBLEM AND TECHNICAL APPROACH

The Phase I/Phase II awards focused on the use of a microbial sensing system to determine the transport and fate of organic carbon in natural subsurface environments. Seven automated, field-deployable systems with cellular communications were designed, fabricated and tested at three sites to determine the fate and transport at natural and contaminated aquifers in Texas, New Mexico and California (Figure 1). The field deployments were successful with several new chemical/physical observations recorder previously unreported in the literature (Figure __). During the field deployment task, several additional market opportunities were identified to monitor agricultural, wastewater treatment and fish farming facilities. Microbial monitoring systems were deployed at four (wastewater treatment (2), algae and aquaculture) facilities (Figure 1). The deployments performed at the wastewater treatment facilities exceeded our and the facility operator's expectations. At the request of one facility operator, we have deployed five systems to monitor five different processes within the wastewater treatment facility. The market opportunities of the technology were greatly expanded based on these additional deployments. It is interesting to note that most processes at any given facility are not monitored. The microbial sensing system is potentially a "disruptive technology" capable of redefining the way wastewater treatment facilities are operated. The U.S. has over 17,000 municipal wastewater treatment facilities and the microbial sensor can be employed at almost all the facilities as process monitoring to increase the efficiency of the operations.

Microbial sensor technology is described as "disruptive" because the microbial monitoring system can largely replace ORP and dissolved oxygen (DO) probes in wastewater treatment, industrial and bioreactor processes. Microbial sensor technologies have two primary advantages over ORP and DO probes: 1) ORP and DO become covered with biofilms reducing the response of the sensors, thus requiring frequent maintenance and/or replacement, while for microbial sensors the biofilm is the sensing surface, and 2) ORP and DO are surrogate methods that attempt to correlate their measurements with biological activity, while the microbial sensor is a direct measurement of microbial response to changing conditions. The total market for these ORP and DO sensors is approximately ___year with __% devoted to our target markets.

Electrical power is the largest operating cost at a wastewater treatment facility. Over 50% of the operating cost of wastewater treatment is from electricity usage <evan has this reference>. The microbial sensor has the potential of optimizing wastewater processes through real-time process feedback (reduced energy usage overall) or increased treatment capacity (reduced energy intensity per gallon), saving billions of dollars per year throughout the U.S. Increased treatment capacity could enable forestalling the 7-8 figure capital costs of planned infrastructure expansions that municipalities face every day. The expansion of the microbial sensor technology into these significantly larger markets will insure a return on investment provided by DOE.

Lab investigation revealed potential applications in unsaturated soils and root zone (rhizosphere). The microbial sensor technology provides significant and reproducible signals in moist to dry oxiziding soils. There are no commercially-available products that can be used for long-term monitoring of these types of soils. This capability may have significant applications in the precision agricultural market, including the ability to determine plant stressors and optimal amendment applications.

The Phase IIB scope of work will focus on the transition of the microbial sensing system from monitoring relatively quiescent anaerobic environments (sediments, aquifers) to rapidly changing environments (wastewater treatment) that can vary from anaerobic to aerobic in a matter of minutes. The original models for the microbial biofilms populating the surface of the anodes in anaerobic environments were based on the investigations performed with microbial fuel cells in anaerobic conditions. The use of microbial sensors in very oxidizing conditions (trout farm) was not anticipated during the Phase II scope of work. The transition of the technology to the rapidly changing conditions of wastewater treatment facilities requires a reevaluation of assumptions concerning the composition of the biofilm made during the original Phase II scope of work. A better understanding of the biofilm composition and the origin of the potentiometric signal is required for full market acceptance.

Figure 1: Photographs of field deployments: Goleta wastewater treatment and Grants Superfund Site

B. ANTICIPATED PUBLIC BENEFITS

The development of a fully automated, field-deployable monitoring system to characterize organic carbon in both the natural environments and process control applications will result in several benefits.

Natural Environments

- Remedial Actions: The in-situ monitoring system may be used for performance monitoring of active remedial actions. The ability to perform continuous monitoring will facilitate optimization of the remedial action decreasing the time to complete the remediation. This results in reduction of the total cost of the project.
- Tool for achieving monitored natural attenuation (MNA): Most MNA programs are projected to last ten years or longer. Significant cost reductions could be realized with the implementation of an automated long-term monitoring program.
- Sentinel Monitoring: Oxidizable carbon from a variety of sources (oil spills, fracking, leaking underground storage tanks, and landfills) released into an aquifer can rapidly change the aquifer to very anaerobic conditions. A continuous monitor can quickly and cost-effectively detect these environmental impacts reducing the long-tern costs of the release.
- Tool for Determining the fate and transport of organic carbon in Subsurface Environments.

Process Control

- Wastewater treatment: The development of a sensor capable of providing long-term, maintenance-free, reproducible data for both aerobic and anaerobic conditions will allow for optimization of the numerous processes performed within a treatment facility
- Bioreactors: A sensor system based on microbial activity will allow for better understanding and control of bioreactors (e.g., waste-to-energy/methane, pharmaceutical, and algal systems).
- Aquaculture: The system can be applied to optimizing growth conditions for both plants and animals in controlled environments (fish farms, hydroponics)

Baseline Technology:

Natural Environments

The current baseline technology requires the collection of sediment and/or water samples followed by incubation and/or laboratory analysis. Previously cited papers have described problems associated with these methods including the inability to correctly characterize many anaerobic systems. The cost, in terms of labor and laboratory fees, makes detailed studies of many anaerobic systems prohibitive where frequent data is required.

Process Control

The baseline sensors include ORP and DO probes that are subject to biofouling and poisoning of the analytical surfaces. Both types of probes are surrogate measurements (Redox potential or dissolved oxygen concentrations) collected to correlate the measurements with the activity and health of microbial communities. Conversely, the microbial sensor signal is a direct measurement of the response of the microbial biofilm populating the surface of the anode. Neither ORP nor DO probes can function in all microbial process environments, microbial sensors do not appear to be so limited.

Baseline Methods

Natural Environments

Costs

- Labor Intensive (sampling and data management).
- Expense (laboratory analysis and data reduction) limits the number of sampling episodes capable of being performed to once a quarter.

Disadvantages

- Not cost competitive for frequent sampling intervals (monthly, weekly, etc.).
- Trend analysis for the fate and transport of organic carbon is difficult in changing environments with infrequently collected data (quarterly, monthly). Parameters such as precipitation, temperature and even seasonal carbon inputs such as with falling leaves can all perturb system and complicate trend analysis.
- Data collected once every quarter does not allow optimization of the parameters for remedial actions.
- Sentinel monitoring (leak detection) requires frequent collection and analysis of data and is not cost-effective using baseline technologies.

Process Control

Costs

- Automated sensors (ORP, DO) sensors costs: 1) initial, and 2) replacement and maintenance

Disadvantages

- Automated probes (DO, pH and ORP) constant maintenance to clean and remove biofilms
- Automated probes (ORP, pH) periodic replaced because of failure of the reference cell
- Laboratory Analysis: All other types of analysis: MLSS, organic carbon performed by laboratory analysis

Cost of Proposed Technology

The microbial monitoring system is unique because it cannot be used in the same manner as most sensor systems. Typical sensor probes (pH, ORP, DO, etc.) allow the operator to perform multiple analyses on different samples by inserting the probes into each of the different solutions and/or calibration solutions and recording the results. This methodology will not be successful with the microbial system, because the microbial biofilm populating the surface of the anode is intimately associated with the environment it developed. The microbial monitoring system appears ideal for the long-term or in-situ monitoring of the environment and/or treatment process (wastewater treatment facility, etc.) where typical sensor probes are ill-suited. Therefore, the microbial monitoring system is designed and programmed for long-term deployments at remote sites and periodically collect the microbial signals (for example every hour) transmit real-time data to a remote user. The existing microbial monitoring system transmits the data to a web-based analytics platform (Groundswell Technologies) allowing the user to access and review the data or be alerted to significant changes.

These characteristics present an opportunity for the real-time microbial sensor as the core of a Device-as-a-Service business model. This simplifies and integrates typical device cost, service contracts, and data software subscriptions into a single competitive product offering. Current market research indicates that most (i.e. 9 MGD or larger) wastewater treatment plants spend over $100,000 per year on sensor probes, service contracts, and data subscriptions despite the limitation of existing technologies. Monitoring well sensor probes, equipment, and sampling can be tens of thousands to hundreds of thousands of dollars per well. The resiliency of this microbial sensor technology enables an economically viable method of monitoring these industrial processes or natural environments. This model forecasts a $2500 capital cost and $250 per month per probe data subscription.

C. DEGREE TO WHICH TECHNICAL FEASIBILITY HAS BEEN DEMONSTRATED

The Phase II scope of work presented nine technical objectives. This section documents the results of the Phase II scope of work.

Phase II Technical Objectives

- Design and fabrication of a ten (10) microbial probe “plug and play” monitoring system
- Integration of the microbial probes with an extended monitoring system
- Testing of the combined (microbial amperometric and microbial potentiometric) probe system with calibration/sampling module in the laboratory
- Determine which chemical/biological parameter (ORP, dissolved oxygen, etc.) are measured by MPS
- Development of the (microbial kinetic sensor) into an analytical technique
- Development of the microbial metabolic cell into a stand-alone method for measuring microbial activity
- Design and fabrication of an outdoor test facility
- Field deployment at a natural environment to determine fate and transport of organic carbon
- Field deployment at a contaminated site to determine fate and transport of organic carbon

The final work product of the Phase II was monitoring system capable of the following attributes:

- Electrical connections for ten (10) independent microbial probes
- Capable of performing both MPS and MAS modes of operation

- Capable of performing MKS (after the development of the technique)
- Capable of collecting calibration data (CO_2) measurements for the probes
- Interface with the extended sampling and analytical system
- System capable of collecting soil-gas and/or water samples for independent testing
- Capable of 180 days of monitoring without servicing
- Capable of wireless communication and remote web based geospatial data visualization and alerting
- Integration of the microbial probes with the extended monitoring system

All the technical objectives were fulfilled except of the fabrication and testing of a test facility. It was determined to bypass this objective and use the resources for additional field deployments. Eleven microbial monitoring systems were deployed at six sites (Table __). Three monitoring systems were deployed at one site for over 9 months. Papers and/or posters were presented at five conferences. Two papers of publication were prepared and will submitted with the next 30 days.

Several parties have indicated an interest in the technology resulting in an additional four posters presented at several conferences.

Nine provisional patents and one patent application (15/237,230) were filed covering the novel inventions developed during the PhaseI/Phase II scope of work.

Instrumentation

Two types of instrumentation (laboratory and field) were developed and tested during the Phase II scope of work:

Laboratory Instrumentation

The microbial monitoring system is composed of three primary components: anode, cathode and measurement circuitry (Figure 2). The anode provides a conducting surface for the establishment of a biofilm. The biofilms populating the surface of the anode oxidize hydrocarbons and use the anode as the electron acceptor. The anode assembly consists of a graphite rod bonded within polymer housings with a signal cable connecting the assembly to the measurement circuitry.

The cathode assembly allows the introduction of atmospheric oxygen (the ultimate electron acceptor of the system) to the cathode when the assembly is deployed in aqueous environments. The cathode assembly is composed of a polymer tube (cathode body) allowing free exchange of gases between the atmosphere and the cathode via the hollow interior (“snorkel”) of the tube. The cathode and accompanying permeable membrane are located at the bottom of the cathode body and are exposed to the environment to be characterized The upper terminal opening of the cathode body is exposed to the atmosphere. The permeable membrane provides a water-proof seal between the cathode (immersed in the aqueous environment) and the snorkel of the cathode body. The membrane performs two functions: 1) prevents the introduction of water into snorkel, and 2) allows atmospheric oxygen to diffuse from the snorkel through the membrane to the cathode. An electrical cable connects the cathode to the measurement circuitry.

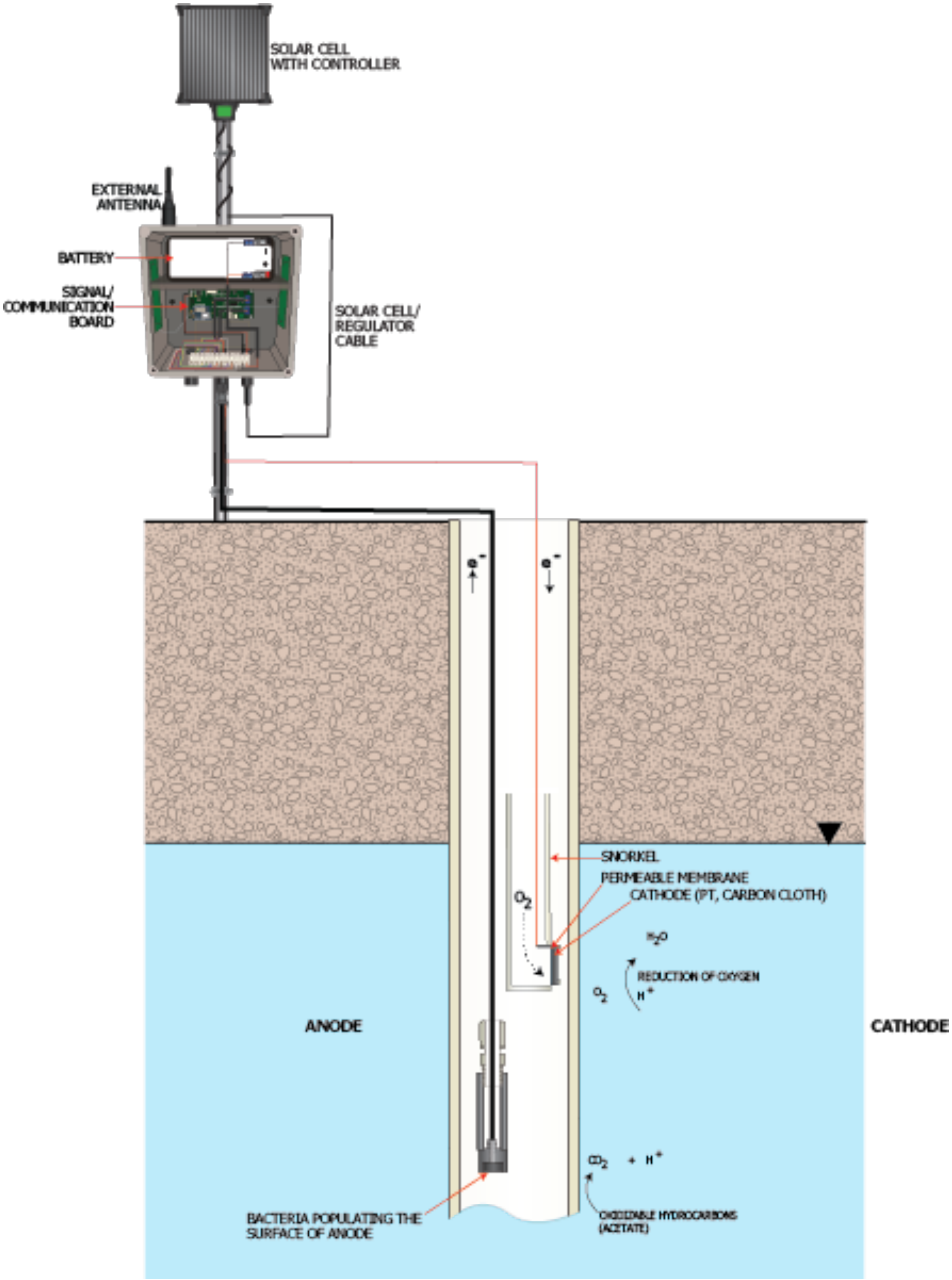


Figure 2: Microbial Sensor System

An electrical potential develops between the biofilms on the anode(s) and the cathode. The measurement of open-circuit potential (no current flowing) allows the use of multiple anodes referenced against a common cathode exposed to atmospheric oxygen.

The measurement circuitry includes two types of electronic boards (motherboard and signal). Each of the signal boards are designed and programmed to collect data from one microbial sensor and one temperature probe. The motherboard accommodates up to seven signal boards allowing serial communications between the signal boards and a laptop computer. The boards are programmed to collect three types of electrical measurements from the microbial sensors. The electrical measurements, ranges and the uses of the analytical measurements are listed on Table 1.

Name	Electrical Measurement	Units/range	Use of the measurement
Microbial Potentiometric Sensor (MPS)	Open-circuit voltage	Volts/0-1.3 volts	Determination of redox conditions
Microbial Kinetic Sensor (MKS)	Recovery voltage after shunting anode with the cathode	Volts (0-1.3 volts)	Substrate concentrations
Microbial Amperometric Sensor (MAS)	Electrical current	uA	Substrate concentrations

Field Instrumentation

The field instrumentation includes anode and cathode assemblies similar in designs of with the laboratory instrumentation except the assemblies are connected to cables for deployment in wells. A board was designed, fabricated and tested for field applications. The board performs two primary functions: 1) signal acquisition/processing of the sensor signals, and 2) cellular data communications. The signal acquisition capabilities included collection of data from the following sensors:

- Three (3) microbial sensors with temperature probes
- ORP probe
- pH probe
- water level probe
- barometric sensor

The signal data is transmitted using cellular (3G or 4G) data modules. The board is programmable allowing the user to select the data collection/transmission frequency (typically every 30 minutes). The system is powered by a solar cell and 12-volt battery (Figure 2).

Microbial Sensor Results

Laboratory Results

Relationship of microbial sensor (MPS) versus ORP and Dissolved oxygen (DO) measurements

A 2-liter, flow-through chamber with stirring bar was used to evaluate four microbial sensors versus ORP and optical dissolved oxygen (DO) probes (Figure 3). The testing procedure allowed the three sensor systems (microbial, ORP, DO) to attain constant signals in aerobic conditions. After 10 to 20 minutes elapsed, a volume (200-ml) of an anaerobic solution was introduced into the chamber and the signals of the sensors were again allowed to equilibrate. After equilibration, an aerobic solution was introduced into the chamber for the remaining time of the experiment. The microbial signal (MPS) versus DO and ORP measurements are presented on Figure 4.

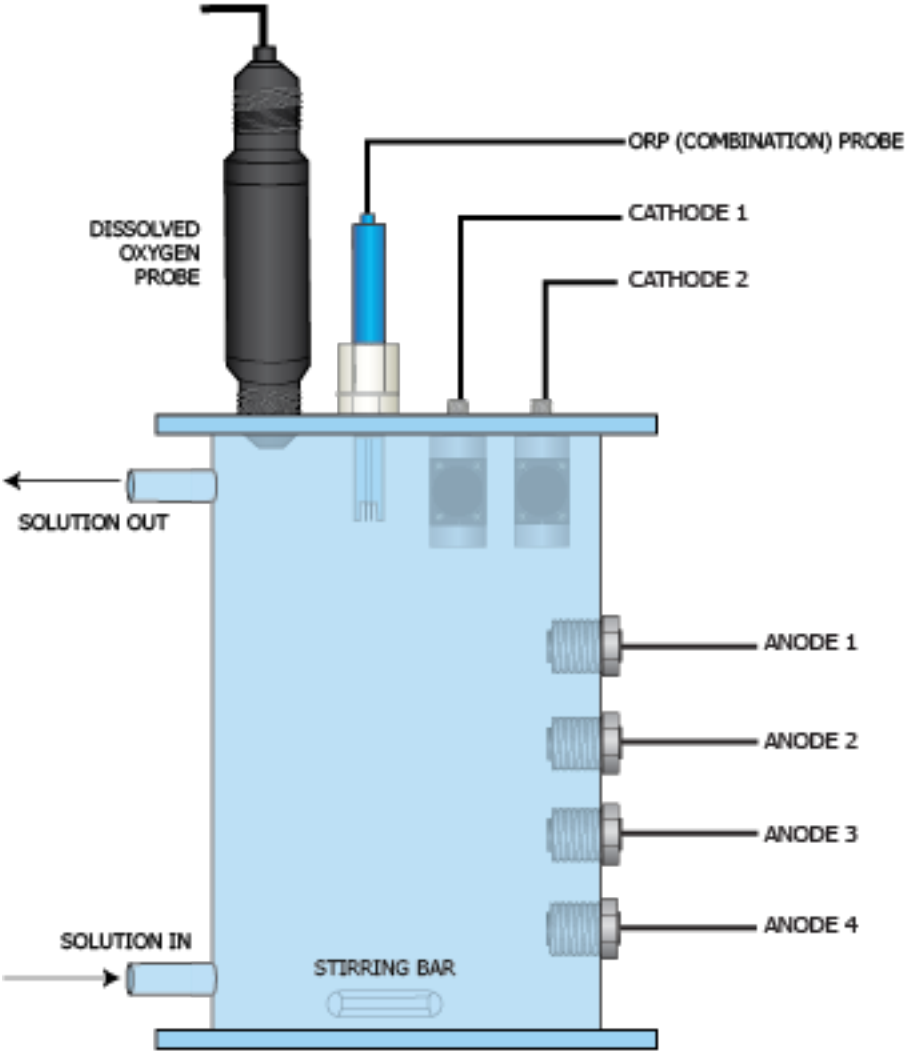


Figure 3: Microbial Sensor, ORP, DO probes versus time

Longevity of the Analytical System

The microbial sensor (MPS) signals versus time (days) plots for three microbial probes located within a sealed test chamber (18 liters) filled with an anaerobic sediment/water mixture are illustrated on Figure 5. The anodes were located at three different depths within the chamber. The gas composition of the headspace (atmosphere) of the test chamber was alternated between nitrogen or air. The presence of atmospheric oxygen (creation of aerobic conditions) depressed the signal of the probes and the introduction of nitrogen increased the signal (creation of anaerobic conditions). The shallowest probe (pink trace) displays the greatest signal response while the deepest probe (blue trace) displays the least signal in response to the changes in gas composition. The results presented on Figure 5 illustrate how MPS can be used to determine the environmental conditions surrounding a given probe.

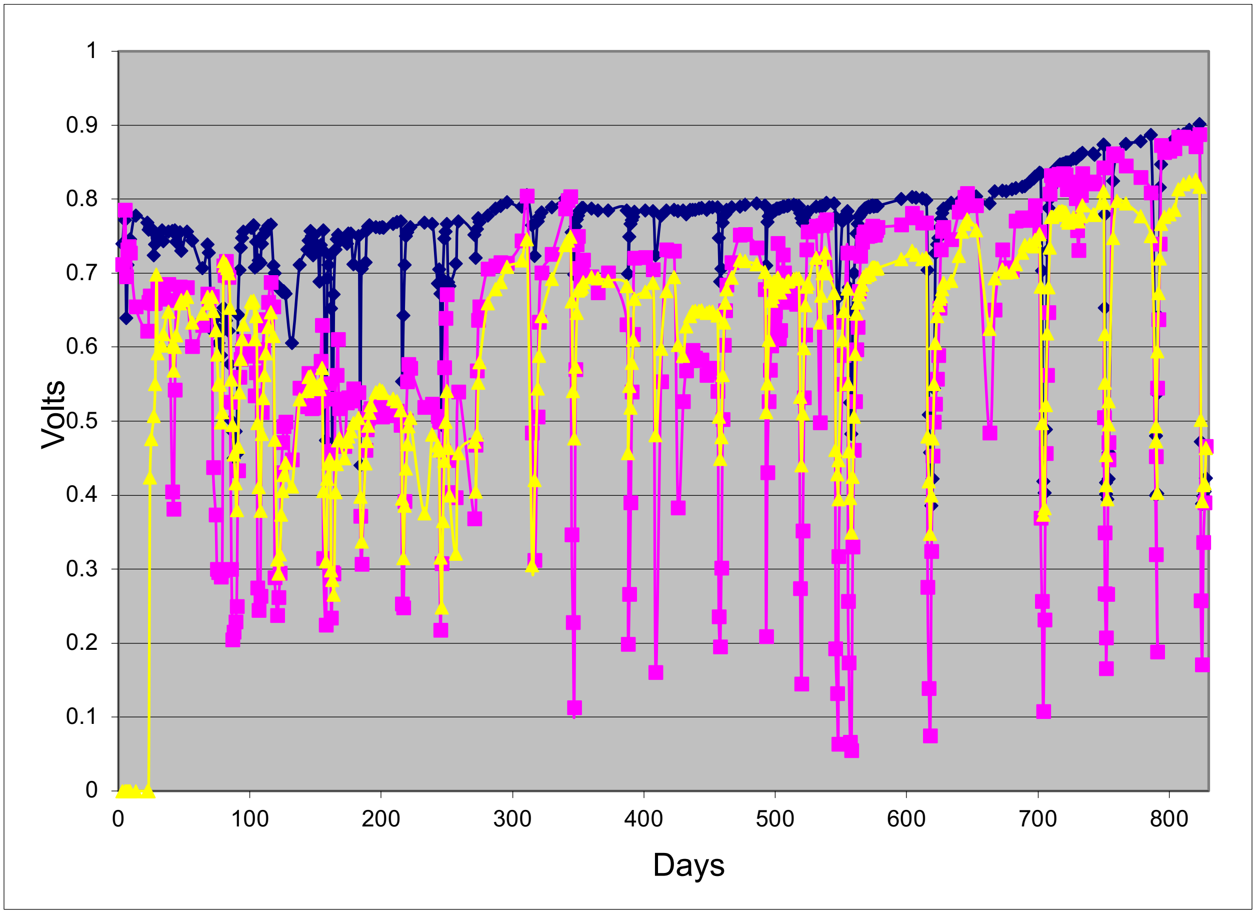


Figure 4: Microbial signals versus time

The experiment was performed for over 900 days with no significant signal degradation observed for the three sensors located in a sediment over time.

Field Results

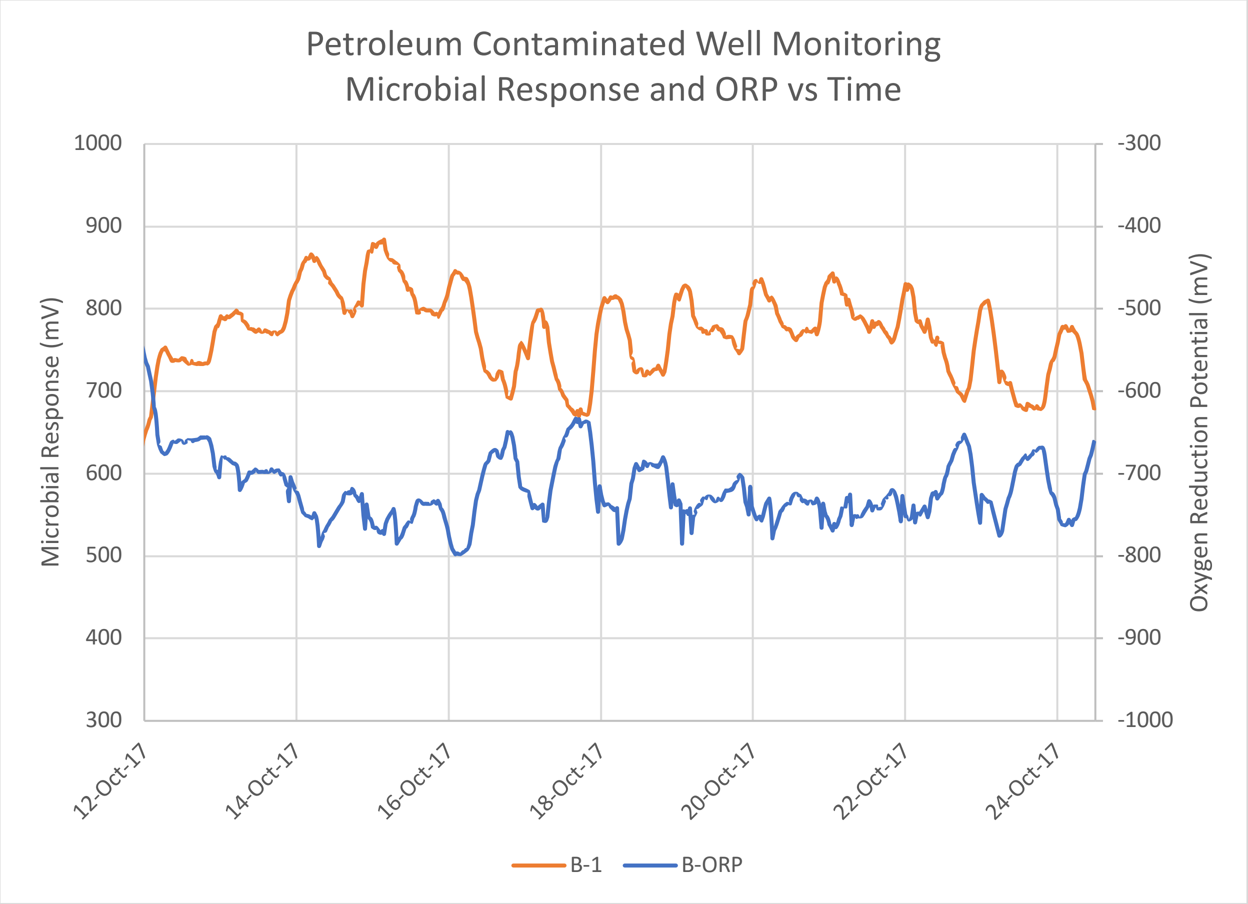
The system was deployed to several locations for a variety of applications,

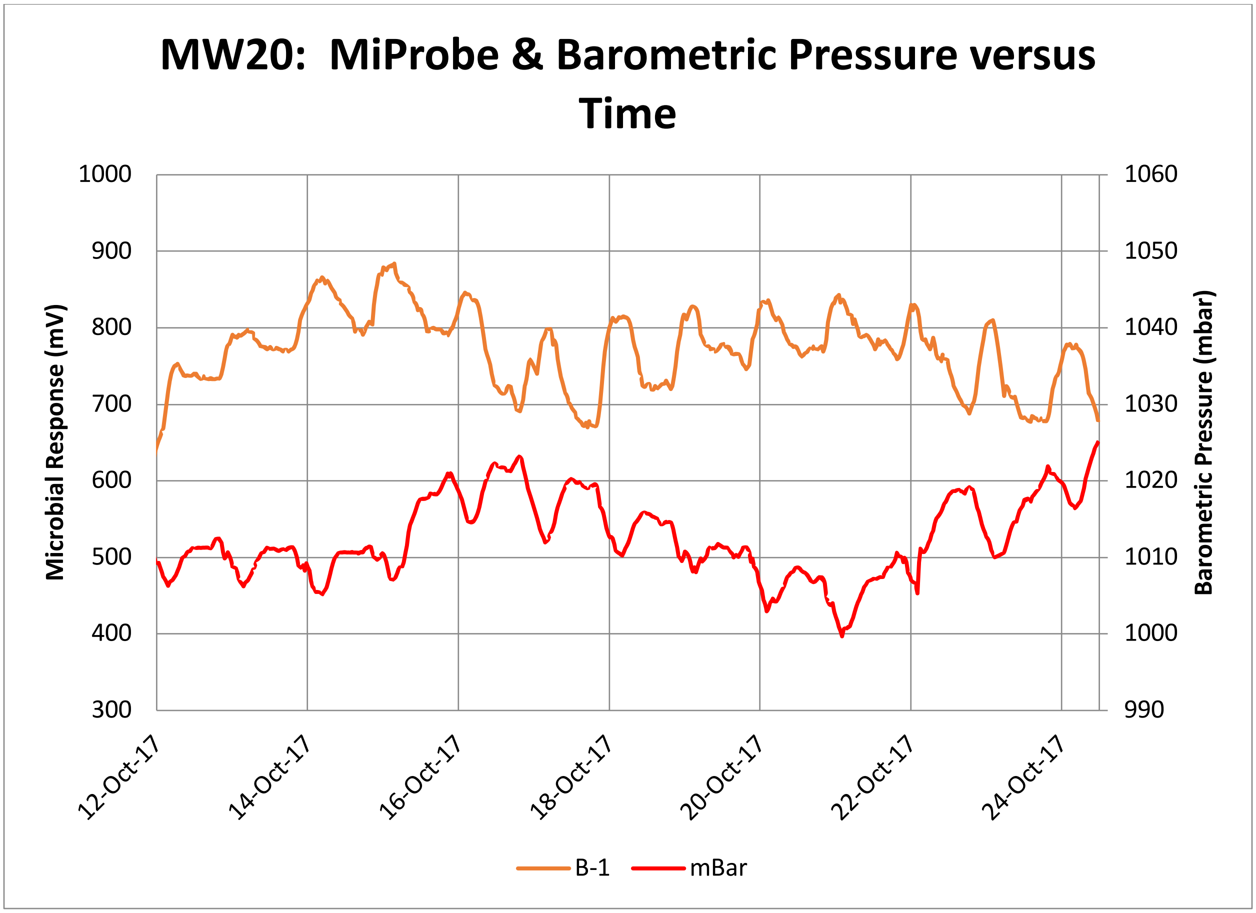
Location	Site Characteristics	Number of Systems Deployed	Sensors Deployed	Deployment Dates
Grants NM	Superfund Site (PCE)	2	Microbial Temperature Water Level ORP	10/2017 to present
[redacted] Coronado, CA	Contaminated Site Hydrocarbons/TCE	2	Microbial Temperature Water Level ORP	11/2017 to present
Texas	Contaminated Site Petroleum	3	Microbial Temperature Water Level ORP Barometric Pressure	3/2017 to 12/2017
Goleta Ca	Wastewater Treatment	2	Microbial ORP	11/2017 to present
Mesa AZ	Wastewater Treatment	1	Microbial	9/2017 to present
Arizona State University	Algae Testing Facility	1	Microbial	10/2017 to present

A few case studies for the above sites include:

Texas

Several shallow groundwater wells were monitored to adjacent to a petroleum spill. During the investigation, a repeating pattern was observed in the microbial data. The monitoring system was fitted with additional sensors including water level probes, temperature (water and air), barometric pressure and ORP. The ORP was added to the monitoring system as a check of the microbial sensor results. The sensor data for one of the microbial sensors, ORP and barometric sensor in one of the monitoring wells is illustrated on Figure __.


Petroleum Contaminated Well Monitoring
Microbial Response and ORP vs Time
Microbial Response (mV)
1000
900
800
700
600
500
400
300
Oxygen Reduction Potential (mV)
-300
-400
-500
-600
-700
-800
-900
-1000
12-Oct-17
14-Oct-17
16-Oct-17
18-Oct-17
20-Oct-17
22-Oct-17
24-Oct-17
B-1
B-ORP


MW20: MiProbe & Barometric Pressure versus Time
Microbial Response (mV)
1000
900
800
700
600
500
400
300
Barometric Pressure (mbar)
1060
1050
1040
1030
1020
1010
1000
990
12-Oct-17
14-Oct-17
16-Oct-17
18-Oct-17
20-Oct-17
22-Oct-17
24-Oct-17
B-1
mBar

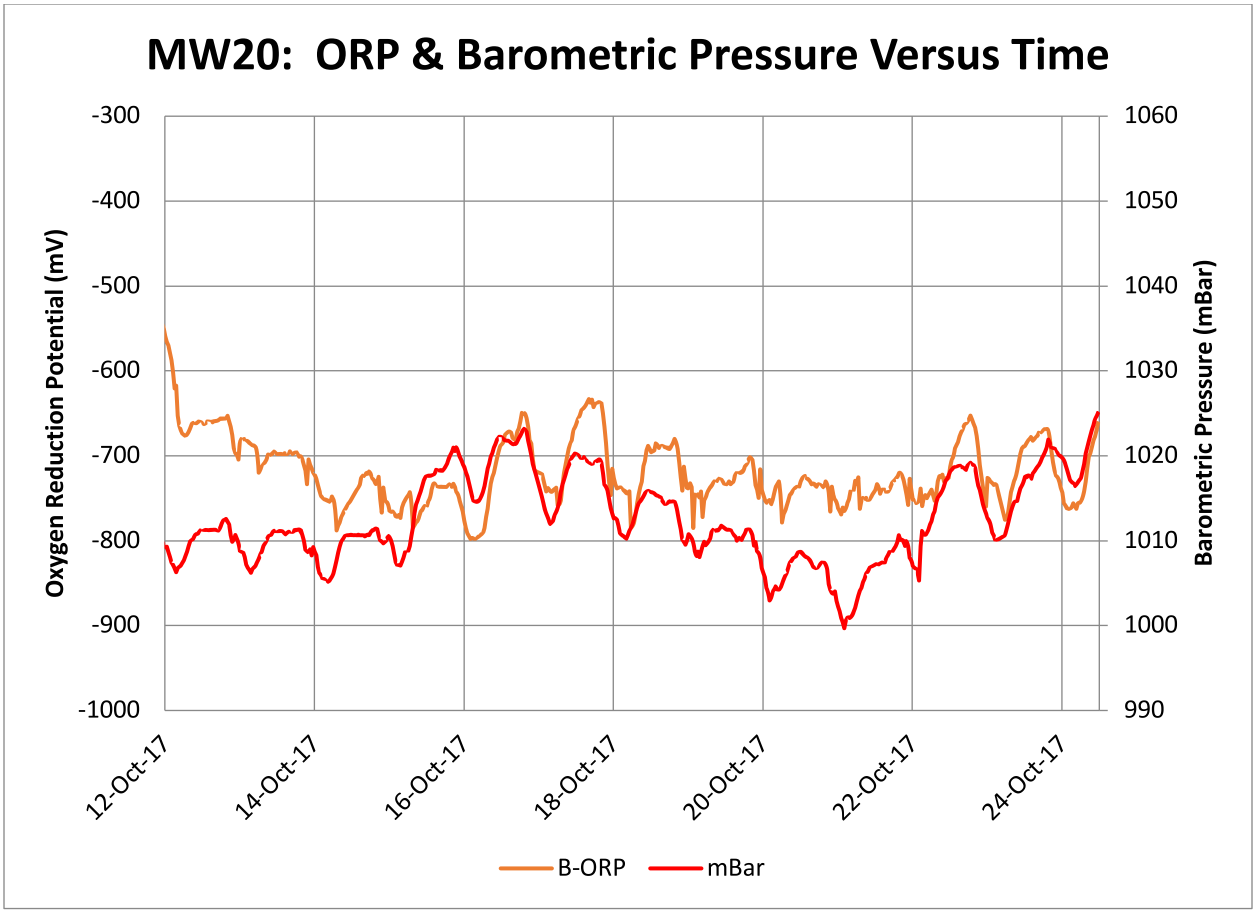
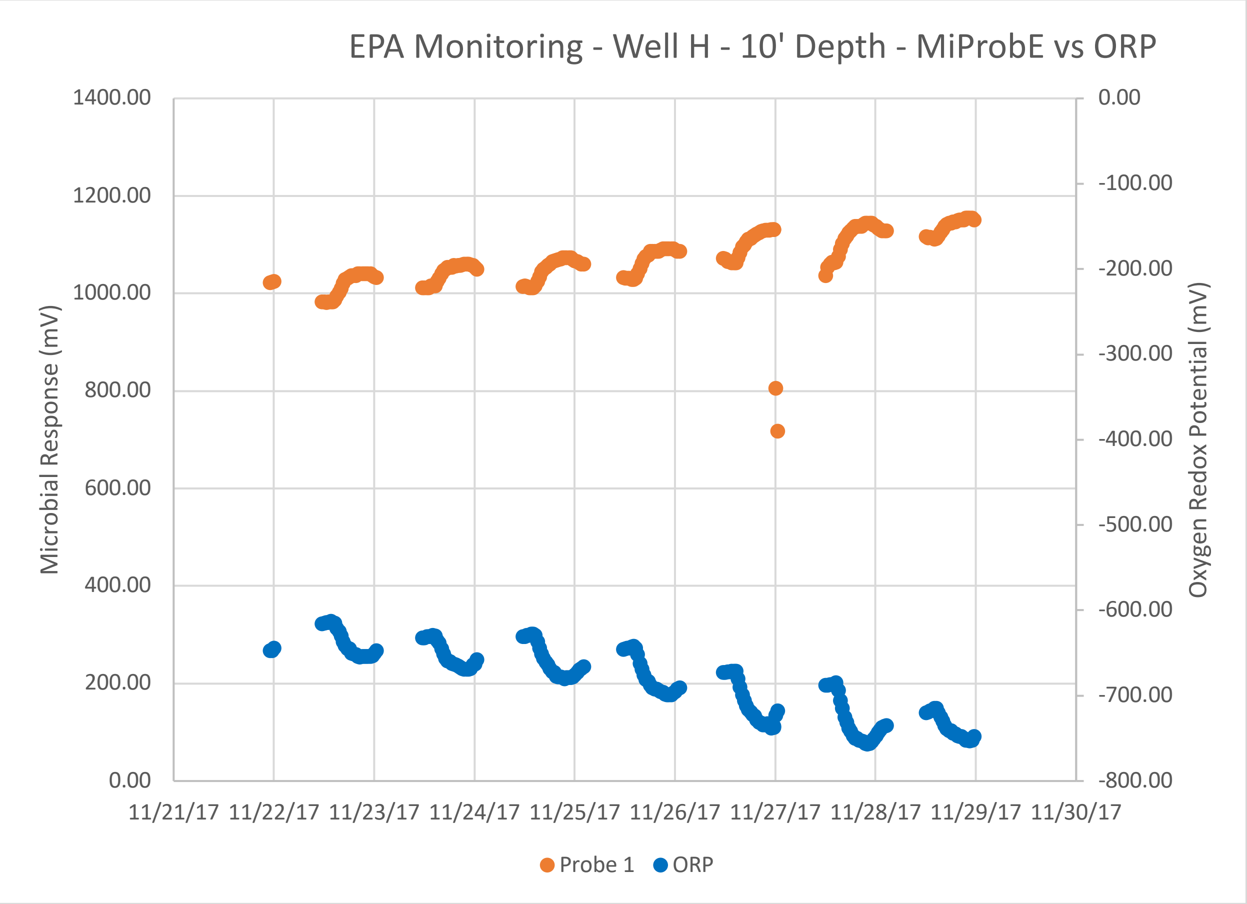


Figure 5: Texas Data

The results indicate that both the microbial sensor and ORP sensor fluctuations correlated with change in barometric pressure.

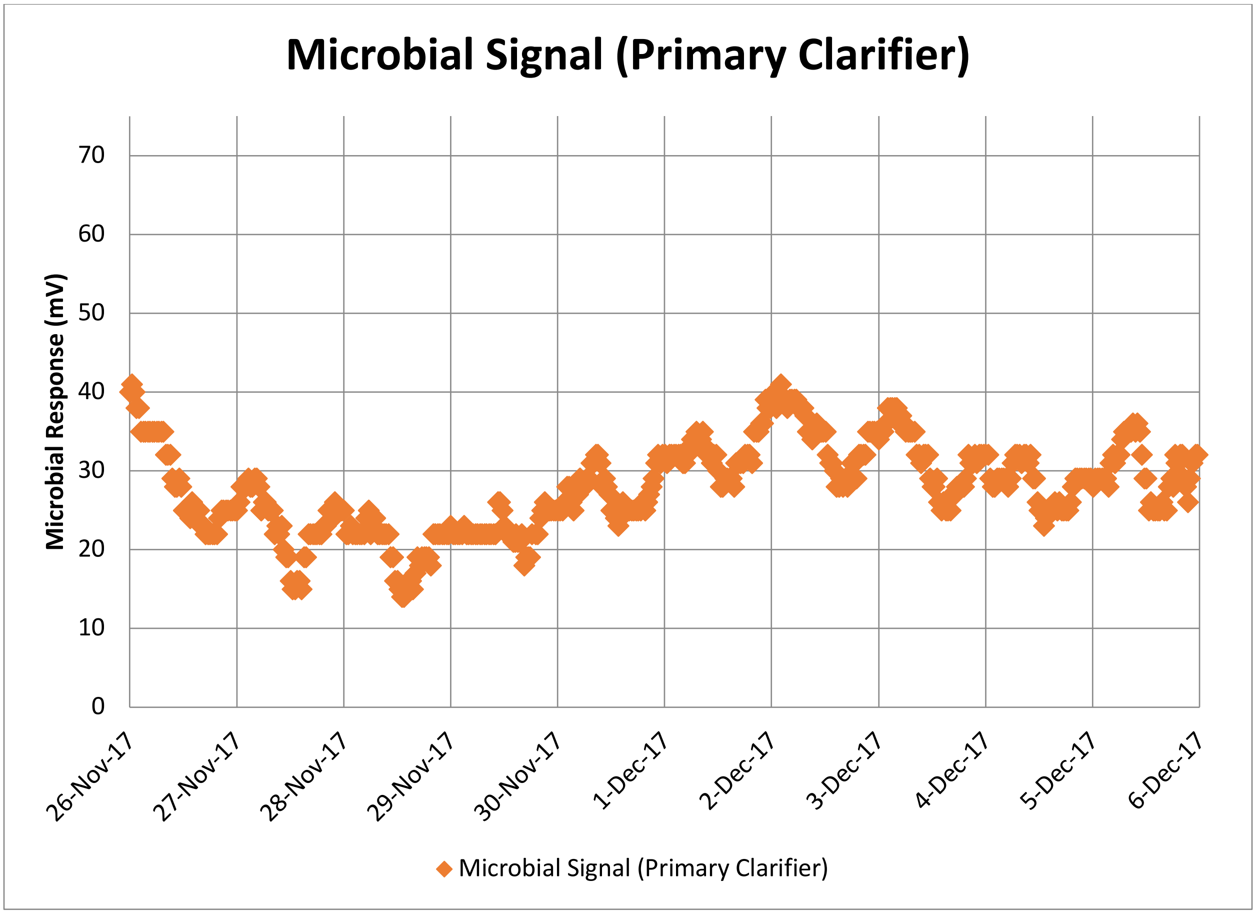
Grants NM

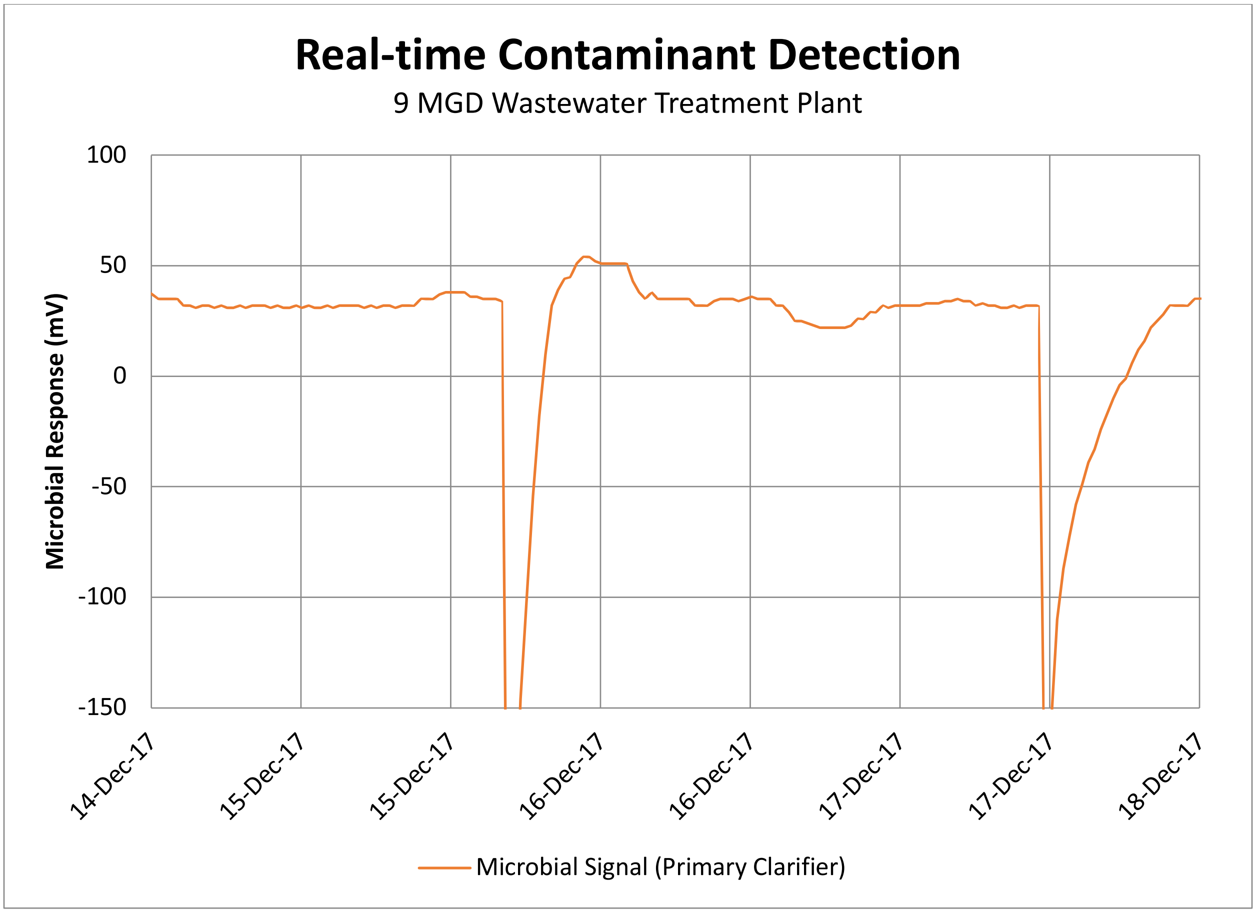
The results of the two sensors indicate daily fluctuations similar with the daily fluctuation observed at the Texas Site.



Goleta CA

The signals of a microbial sensor and dissolved oxygen probe monitoring the activated sludge process of the wastewater treatment facility is presented on Figure 6. The results of the two sensors indicate a significant correlation. (Note: the daily fluctuations are due to the time when the greatest volume of biomass is discharged to the treatment facility-after the work day).


Microbial Signal (Primary Clarifier)
Microbial Response (mV)
70
60
50
40
30
20
10
0
26-Nov-17
27-Nov-17
28-Nov-17
29-Nov-17
30-Nov-17
1-Dec-17
2-Dec-17
3-Dec-17
4-Dec-17
5-Dec-17
6-Dec-17
◆ Microbial Signal (Primary Clarifier)


Real-time Contaminant Detection
9 MGD Wastewater Treatment Plant
100
50
0
-50
-100
-150
Microbial Response (mV)
14-Dec-17
15-Dec-17
15-Dec-17
16-Dec-17
16-Dec-17
17-Dec-17
17-Dec-17
18-Dec-17
Microbial Signal (Primary Clarifier)

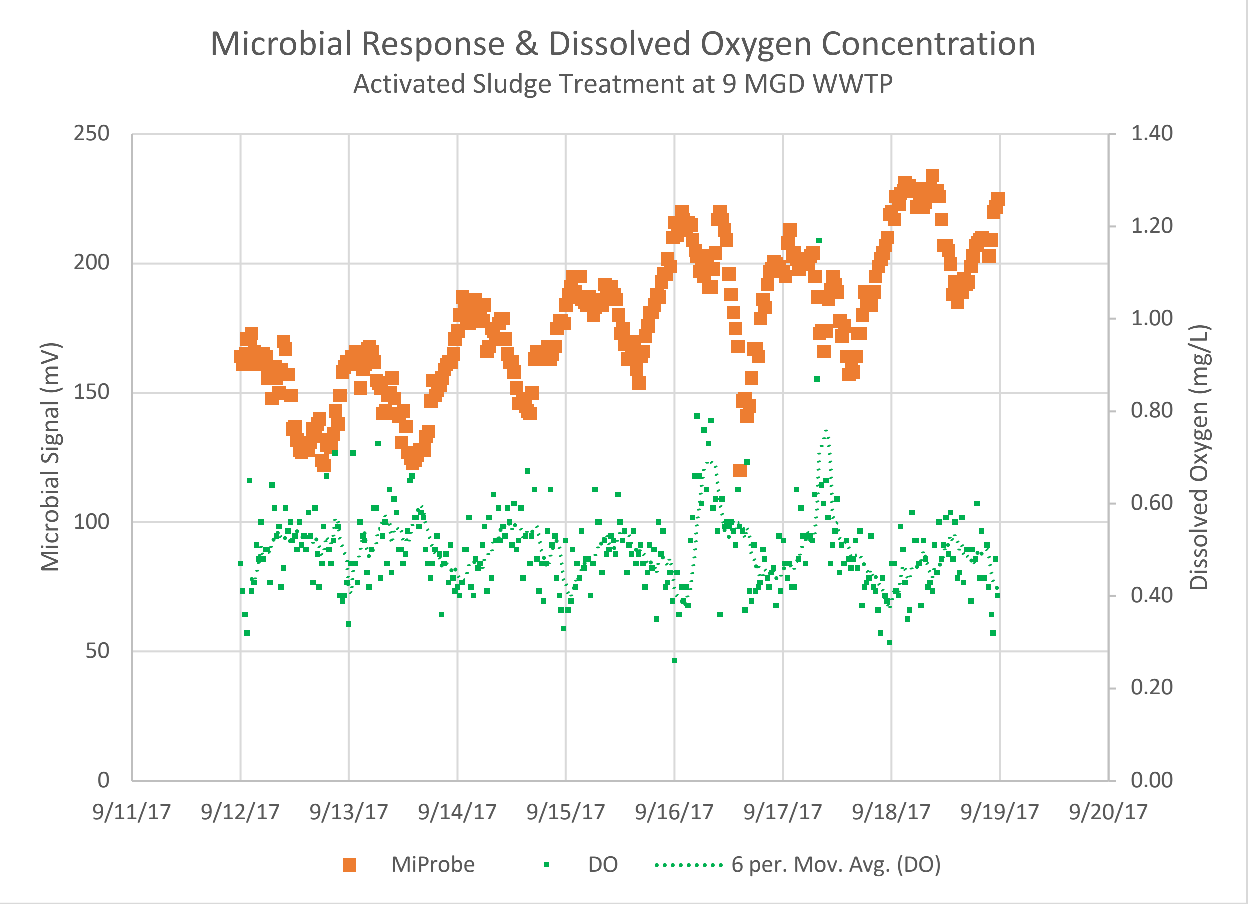


Figure 6: Goleta: Microbial sensor versus DO probe

The field deployment revealed the complexity of the microbial signal. Although the microbial signals appear to be a direct replacement of the signals generated by ORP (redox potential) or dissolved oxygen (DO) probes (Figures __ and ___) in relatively simple environments. In environments where substrate concentrations are changing, the microbial signal appears to be considerably more complex (Figure __), functioning as a composite signal of both DO and substrate concentrations. The figure indicates microbial signal correlates with the daily fluctuations of the dissolved oxygen concentrations and a longer periodic fluctuation of MLSS. MLSS is a measurement of organic carbon in the system. This very significant because the treatment operators use a combination of DO and MLSS to determine the efficiency of the treatment operation. The microbial sensor may allow the complete evaluation of the treatment operation using one sensor due to this composite signal characteristic. This the reason that the technology may be disruptive, not because it is a direct replacement of ORP or DO probes with lower maintenance cost, but because it allows real-time measurement to determine the efficiency of the operation. MLSS testing is typically done only daily as it is an expensive and time intensive lab technique limiting its value to process optimization and control.

Figure 7: Goleta: Microbial sensor and biocide

A second unexpected attribute of the microbial sensor was its response of a biocide. Occasionally, the biocide bronopol, used in septic systems of commercial planes and "port-a-pottys", is discharged to the facility from the Santa Barbara Airport and other unknown locations. The discharges can have extremely detrimental impacts to the operation of the treatment facility impacting treatment cost (counter-acting agents) and man-hours. The microbial sensor detected these discharges of the biocide to the treatment facility in real-time (Figure 7). The sensor showed both a drop in microbial activity upon the bronopol contamination at the beginning of the treatment process (primary clarifier, Figure 7) and an increase in non-degraded material at the last stage (activated sludge, Figure 8) of the process 6-8 hours later, consistent with residence time of each process and step of the treatment plant.

D. TECHNICAL OBJECTIVES OF PHASE IIB

The technical objectives of Phase IIB include:

- Better understanding of the microbial potentiometric composite signal
- Determine the composition of the microbial communities populating the surface of the anode for various processes (wastewater treatment plants, bioreactors) in anaerobic and aerobic conditions
- Determine the microbes populating with the cathode
- Determine performance of the microbial monitoring system in the unsaturated zone
- Design, fabricate and test methods of calibration of in domestic and industrial wastewaters
- Determine the extent of the microbial sensors to determine the biocide concentrations in wastewater.
- Design, fabricate and test microbial monitoring system for wastewater treatment facilities and bioreactors
- Deploy at multiple sites in several regions in the U.S.

The final work product of this work plan will be a fully operational field-deployable system tested at several sites.

E. PHASE II WORK PLAN AND WORK SCHEDULE

The Phase IIB will be divided into twelve tasks for accomplishing the eight technical objectives for a two-year period.

Task 1: Determine the composition of the microbial biofilms located on the surface of the anodes in process control processes

Task 2: Determine the microbes associated with the cathode in aqueous environments

Task 3: Determine the nature of the microbial signal (potentiometric) in aqueous environments

Task 4: Design and fabrication of a method of calibration of the domestic and industrial waste water
Task 5: Determination of the microbes associated with the anode and cathode in unsaturated environments
Task 6: Determine the impacts of biocides to the operation of the microbial sensor
Task 7: Design and fabrication of a unified microbial monitoring system
Task 8: Unified microbial monitoring system at natural subsurface environments
Task 9: Unified microbial monitoring system at contaminated sites
Task 10: Unified microbial monitoring system at municipal treatment facilities
Task 11: Unified microbial monitoring system at industrial treatment facilities
Task 12: Unified microbial monitoring system deployed for determining microbial activity in the unsaturated zone

Task 1: Determination of the composition of microbial biofilms in natural and process control processes

Investigators: [redacted] Duration: 16 weeks

The microbial composition of the biofilms populating the surface of the anodes in aquifers and sediments was assumed to be relatively constant over the timeframe of any monitoring program because the anaerobic environments being measured were assumed to be relatively constant (temperature, substrate concentrations). This assumption was reinforced by the laboratory chamber investigations performed during the Phase I and Phase II scope of work. This assumption became tenuous during the field investigations performed at wastewater treatment facilities. Assumed compositions of anaerobic biofilms (Geobacter, ____, etc.) of subsurface, anaerobic environments may be not valid for aerated municipal wastewater. An understanding of the composition of the microbial biofilms are important in understanding the exact nature of the microbial signal generated in aerobic wastewater treatment facilities and bioreactors.

The biofilms will be investigated by collecting samples of the biofilm populating the anodes followed by genome screening to ascertain the microbial composition. The experimental procedure includes laboratory testing where the biofilms in anaerobic and aerobic conditions to determine the changes in the microbial composition occurs during the transition between the redox conditions. The biofilms will be sampled in actual field conditions at least three wastewater treatment facilities at the various treatment operations (headworks, activated sludge, etc.) to determine the microbial composition.

Task 2: Determine the microbes associated with the cathode

Investigators: [redacted] Duration: 16 weeks

The original designs of the cathode used a platinum-coated carbon fabric exposed to atmospheric oxygen. The material was an adaptation of the cathode material commonly used with microbial fuel cells. The platinum is used as a catalyst to aid in the reduction of the atmospheric oxygen during the production of power. The material was used because it provided reproducible signals, however, it was soon discovered that platinum was not required at the cathode to provide reproducible signals. It appears the cathode is a biocathode. Therefore, it is probably microbes associated (biofilm) or adjacent to the cathode that are responsible for providing the stable cathodic signal when measurements are performed in the open-circuit mode of operation (no current flowing). The exact nature how oxygen was reduced, platinum catalyst or microbial, was not investigated because in quiescent, anaerobic environments of natural aquifers the cathodes were well behaved and other parameters required investigation. However, in the rapidly changing environments of wastewater treatment facilities the exact nature of the cathode is becoming a factor in determining the nature of the microbial signal.

Task 3: The nature of the microbial signal (potentiometric)
Investigators: [redacted] Duration: 16 weeks

The deployment of potentiometric microbial sensors at wastewater treatment facilities revealed the complexity of the microbial signal (Figure __). Based on the observations of wastewater treatment operators, the microbial signal may be a metric for the total efficiency of the treatment operation.

The interpretation and calibration of the microbial signal cannot be successfully performed until the exact nature of the microbial signal is unraveled. The wastewater investigations revealed that the signal appears to be composite signal containing information of both DO and MLSS measurements. This task will focus of designing and testing chambers where DO and MLSS levels can be changed independently to determine the nature of the signal.

The task will attempt to design and fabricate test chambers to flow various nutrient solutions through the test chamber while attempting to hold the DO concentrations (or ORP reading constant). This will be a challenging task because the presence of bacteria will in the test chambers will be altering the both the nutrient and oxygen concentrations.

Task 4: Design and fabrication of a method of calibration of the domestic and industrial waste water
Investigator: [redacted] Duration: 12 weeks

A method of calibration for subsurface application was proposed during the Phase I scope of work. The method was to capture the metabolic gases (CO_2, methane) below the static water level using a microbial metabolic cell. The gases captured in the cell are volumetrically measured. The method of calibration was adequate in aquifers and sediment where gas evolution is restricted to the microbial activity in anaerobic environments. However, in municipal wastewater and industrial processes air is commonly injected to aid in the conversion of oxidizable carbon to carbon dioxide. The presence of air bubbles precludes the use of microbial metabolic cells. One line of investigation places microbial sensors in-situ calibration chambers within the wastewater treatment operations. A "calibration" solution, such as water equilibrated with the atmosphere and at a specific substrate (i.e. acetate) concentration, could be periodically flowed through the chamber to determine the response of the sensor to water.

Task 5: Determine the impacts of biocides to the operation of the microbial sensor
Investigator: [redacted] Duration: 24 weeks

The monitoring of a primary settling basin revealed that the microbial sensor could determine a biocide in very highly diluted solution. This ability to determine chemical agents impacting the operation (decreasing the microbial population in the wastewater) of a treatment facility would allow its deployment at locations (lift stations, etc.) prior to the introduction to the treatment facility for: 1) locating the offending discharger and/or 2) mitigate the potential impact to the wastewater treatment operation by diluting the discharge before reaching the facility. The microbial sensor is unique because the active surface of the sensor is a biofilm and any chemical with the toxicity and sufficiently high concentrations impacting the bacteria in the treatment process can impact the bacteria biofilm changing its electrical signature. After the chemical passes through the system the microbial sensor will simply regenerate the biofilm similar with the way the bacteria in the treatment operation recover after impacts of toxic chemicals. It is recognized that the sensor may not be capable to determining a specific toxic chemical, however it may be capable of recognizing a variety of chemicals in an effort of determining when a chemical capable of impacting a treatment facility is occurring.

The task will identify chemicals that are associated with industrial or commercial discharges known to impact wastewater treatment operations and determine impacts to the microbial sensor and if impacts are determined at what concentrations do impacts first occur. A flow-through chamber will be designed and fabricated allowing the installation of multiple sensors (microbial, ORP and DO). Various concentrations of biocides and other chemicals known to impact the operation of treatment facilities will be passed through the chambers. The investigation will determine if any other attributes of the microbial signal (potentiometric and/or kinetic) can be used to assess the type of biocide passed through the chamber.

Task 6: Design and fabrication of a unified microbial monitoring system
Investigator: [redacted] Duration: 24 weeks

The present microbial monitoring system was designed and fabricated for monitoring subsurface environments including aquifers and sediments. The eleven field systems have been deployed in the field for a total of 48 months and many problems were identified in the field including temperature, moisture and cellular coverage. The instrumentation was modified to address almost all the identified problems and allow the monitoring of treatment facilities, however during the field deployment several important parameters were identified including: 1) higher impedance inputs, 2) additional probes, 4) moisture-resistant electronic board coatings and 3) incorporation of calibration system for long-term monitoring. The unified system will allow deployment in both saturated and unsaturated environments. The system will be designed for easier fabrication and maintenance than the current systems that were placed in to the field.

Task 7: Unified microbial monitoring system at unsaturated environments
Investigator: [redacted] Duration: 24 weeks

The microbial monitoring system will be modified to allow for the deployment of 10 by 10 by 10 sensor arrays with accompanying very high impedance measurement system. The system will be designed to allow for a 3-D data collection and data visualization of the rhizosphere using the Groundswell Technology web-based programs

Task 8: Unified microbial monitoring system at contaminated sites
Investigator: [redacted] Duration: 24 weeks

Task 9: Unified microbial monitoring system deployed municipal treatment facilities
Investigator: [redacted] Duration: 24 weeks

Four wastewater treatment facilities will be selected to deploy multiple systems at various locations within each of the facilities: headworks, primary clarifier, activated sludge, disinfectant. The automated systems will transmit data to the Groundswell Technology website and the data will processed to allow a completer understanding of the entire process. This will allow optimization of the facility with the capability of increasing throughput with less energy consumption.

Task 10: Unified microbial monitoring system at industrial treatment facilities
Investigator: [redacted] Duration: 24 weeks

The performance schedule for the ten tasks (by quarters) presented in the work plan schedule is presented on *Table 2.*

Table 2: Task Schedule for Phase II

Month:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Task 1	■	■						
Task 2	■	■	■					
Task 3			■	■				
Task 4			■	■				
Task 5	■	■	■	■				
Task 6					■	■		
Task 7						■		
Task 8						■	■	■
Task 9						■	■	■
Task 10							■	■

RELATED RESEARCH & DEVELOPMENT

Research and development by Burge Environmental, Inc. has been focused on problems associated with automated, remote, long-term monitoring of environmental contaminants in the field. This research began in 1989 and is the subject of twenty published articles and more than 30 papers delivered at conferences.

PRINCIPAL INVESTIGATOR & OTHER KEY PERSONNEL

[redacted]

[redacted]

[redacted]

F. FACILITIES / EQUIPMENT

The proposed work will be performed at the Burge Environmental, Inc. facility located in [redacted]. The Burge Environmental, Inc. facility has approximately 3,000 square feet of laboratory and office space. The laboratory contains several types of analytical instrumentation and test equipment. The facility has the instrumentation to independently analyze samples from the monitoring system. The facility is fully equipped with computers, tools, and electronic instrumentation necessary to perform most of the operations outlined in the scope of work

G. CONSULTANTS & SUBCONTRACTORS

[redacted]

[redacted]

REFERENCES CITED