

## **Automated Monitoring of the Rhizosphere using a Microbial Sensor**

### **A. Identification / Significance of the Problem and Technical Approach**

Measurement of electrical potential has been identified as an important parameter in plant/microbial/soil investigations.<sup>1,2</sup> With an emphasis on the rhizosphere, the difficulty of measuring soil redox potential (Eh) has limited certain soil investigations and particularly the measurements of Eh in aerobic soils. A review of the use of soil potential indicated there were two main problems identified: 1) reliability of the equipment including leaks and polarization of the electrodes, 2) sampling error due to the high spatial and temporal variability of the soils. It was hypothesized in the previously cited article “that plants physiologically function within a specific Eh-pH range and that, along with microorganisms, they alter the Eh and pH in the rhizosphere to ensure the homeostasis at the cell level”.<sup>1</sup> The primary goal of this scope of work is provide a tool to generate data to support this “self-regulation hypothesis.”

Current monitoring technologies include oxidation-reduction potential (ORP) electrodes (platinum electrode with a reference cell (typically Ag/AgCl electrode). The review articles acknowledge the usefulness of electrical potential measurement while noting the difficulty in acquiring reliable data in many soil types. The available technologies are simply inadequate for measuring soil types with low moisture and/or oxidizing soils.

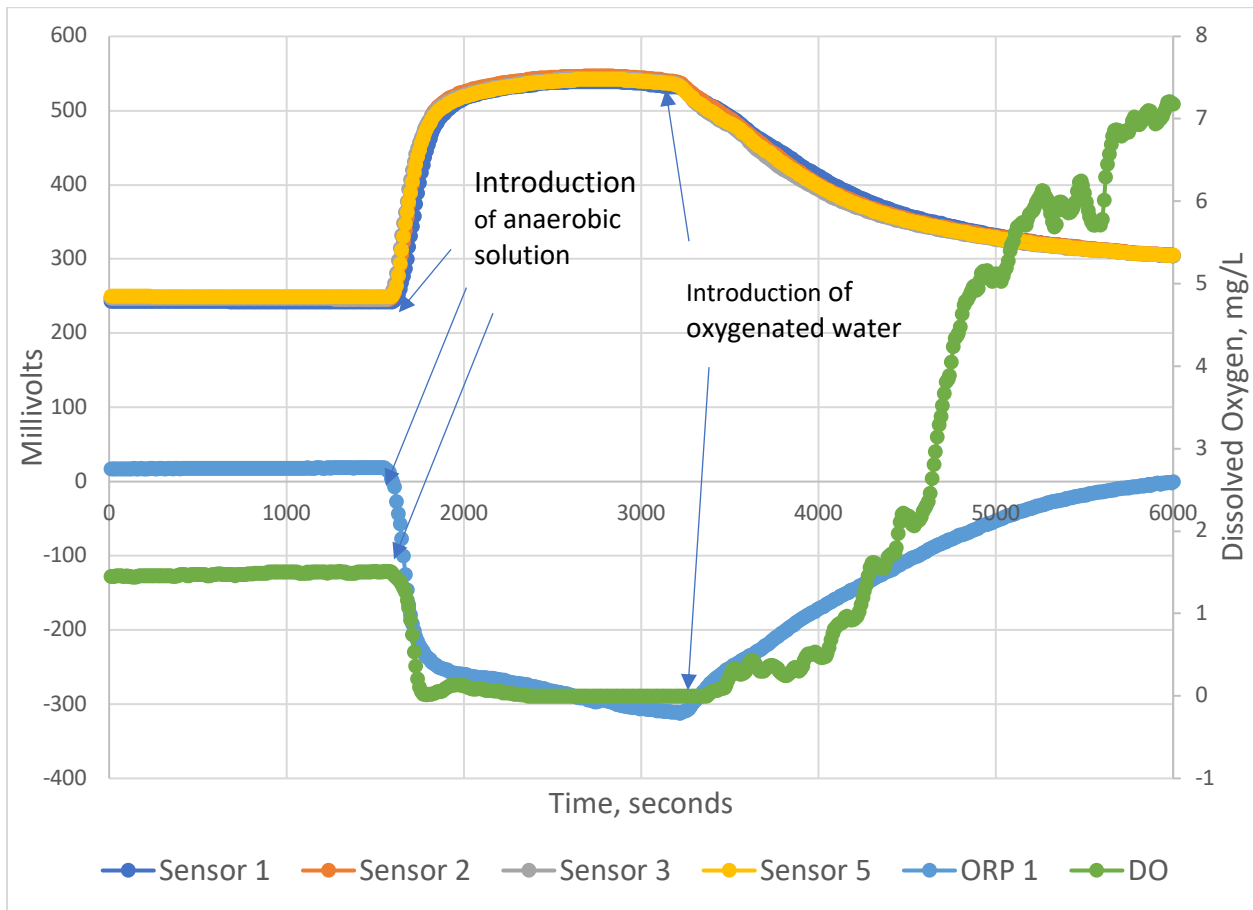
The proposed microbial sensor is an electrochemical analytical system functionally related to microbial fuel technology. The microbial sensor is composed three primary components: 1) a graphite anode, 2) a carbon cloth cathode exposed to atmospheric oxygen and 3) measurement circuitry. The anode, when exposed to the environment, becomes populated with bacteria forming a biofilm. The biofilm metabolizes the substrates in the environment and transfers the electrons to the anode (intermediate electron acceptor). The electrons flow through the measurement circuitry to the oxygen (the ultimate electron acceptor) surrounding the cathode. The microbial sensor is capable of three modes of operation: potentiometric, amperometric, and kinetic.

The measurement circuitry is designed and programmed to perform all three modes of operation. The three modes of operation provide different and complementary information on the environment being studied. The potentiometric signal provides information whether the system being studied is aerobic or anaerobic (the signal can be correlated to ORP and dissolved oxygen (DO) measurements), while the kinetic signal can be used to determine the substrate concentration of the environment. This proposal will focus on the potentiometric and kinetic signal generated by the sensor system.

A microbial sensing system (three anodes and a common cathode), ORP and optical DO probes were placed into a stirred chamber containing aerobic natural pond water. The correlation between sensor signals for aerobic and anaerobic solutions is illustrated on Figure 1. The experiment was performed for a total of 6000 seconds. A flow (150 ml/min) of an anaerobic solution (anaerobic water) was initiated at 1500 seconds for 240 seconds. A flow (100 ml/min) of oxygenated water was initiated at 3000 seconds until the end of the experiment. The experiment illustrates that the microbial sensor signal (potentiometric mode of operation) is related to the ORP and DO signals

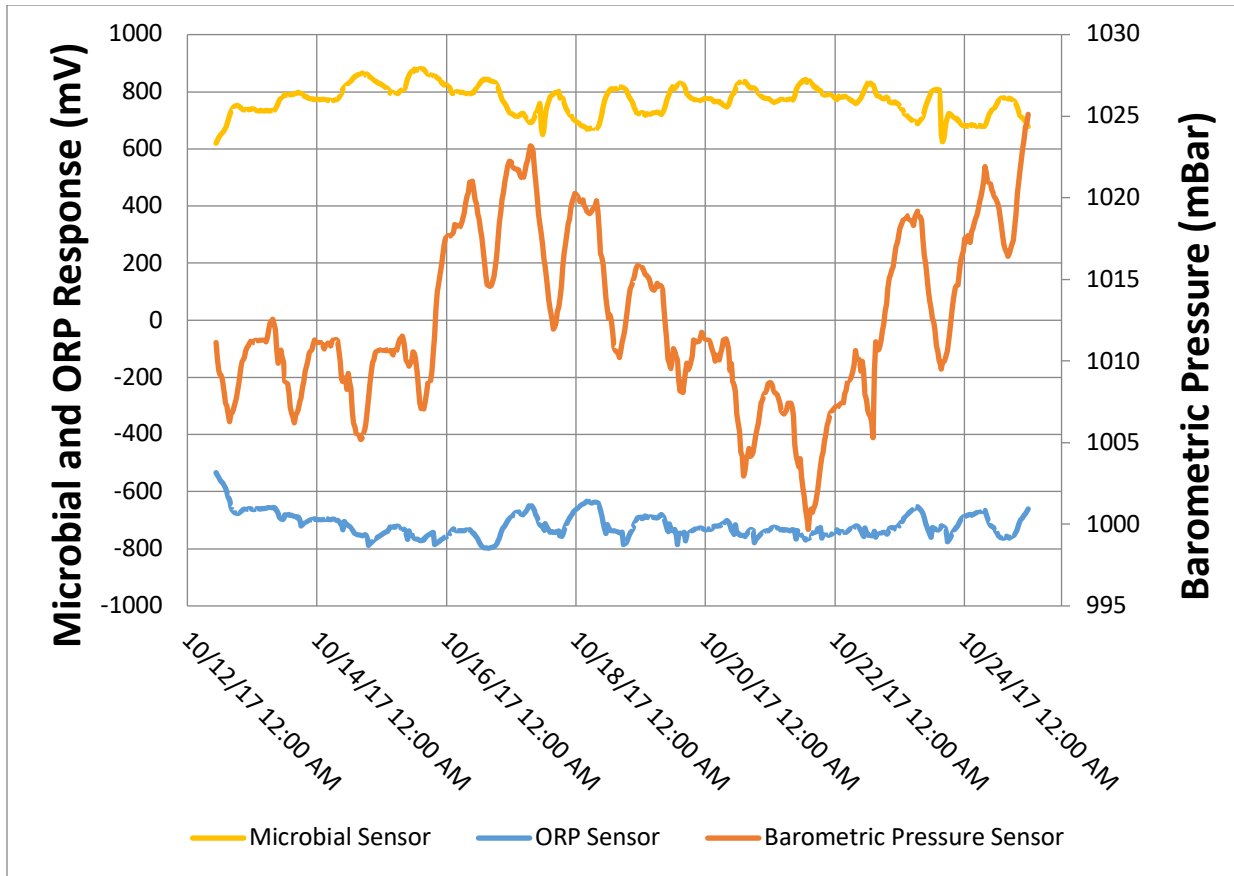
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in aqueous or saturated environments. This proposal will extend the investigation of the microbial sensor into the unsaturated zone including well-drained or dry soils. The ability to be deployed in unsaturated soils will make this an ideal sensor system for investigations of the rhizosphere.



**Figure 1: Relationship of Microbial, ORP and DO sensors in aqueous environment**

The automated monitoring system was deployed in an aquifer in costal Texas to determine the relationship between ORP and microbial signals over time. The results of the investigation indicated a daily signal fluctuation of the two sensor systems. We deployed additional sensors in the field including water level and barometric pressure to determine the origin of the fluctuations. It was determined that barometric pressure was causing the fluctuation. We have hypothesized that the barometric pressure is releasing the daily mass of metabolic gas (methane, carbon dioxide) production of the aquifer. The signals of both the ORP and microbial sensors fluctuate between aerobic and anaerobic conditions based on the release of the metabolic gases by the pressure differentials. This indicates the utility of this monitoring system and its ability to determine very small differences in redox conditions in natural environments.



**Figure 2: Data generated from a field deployment (coastal Texas) of the monitoring system in an aquifer illustrating the relationship between microbial sensors, ORP probe and barometric pressure**

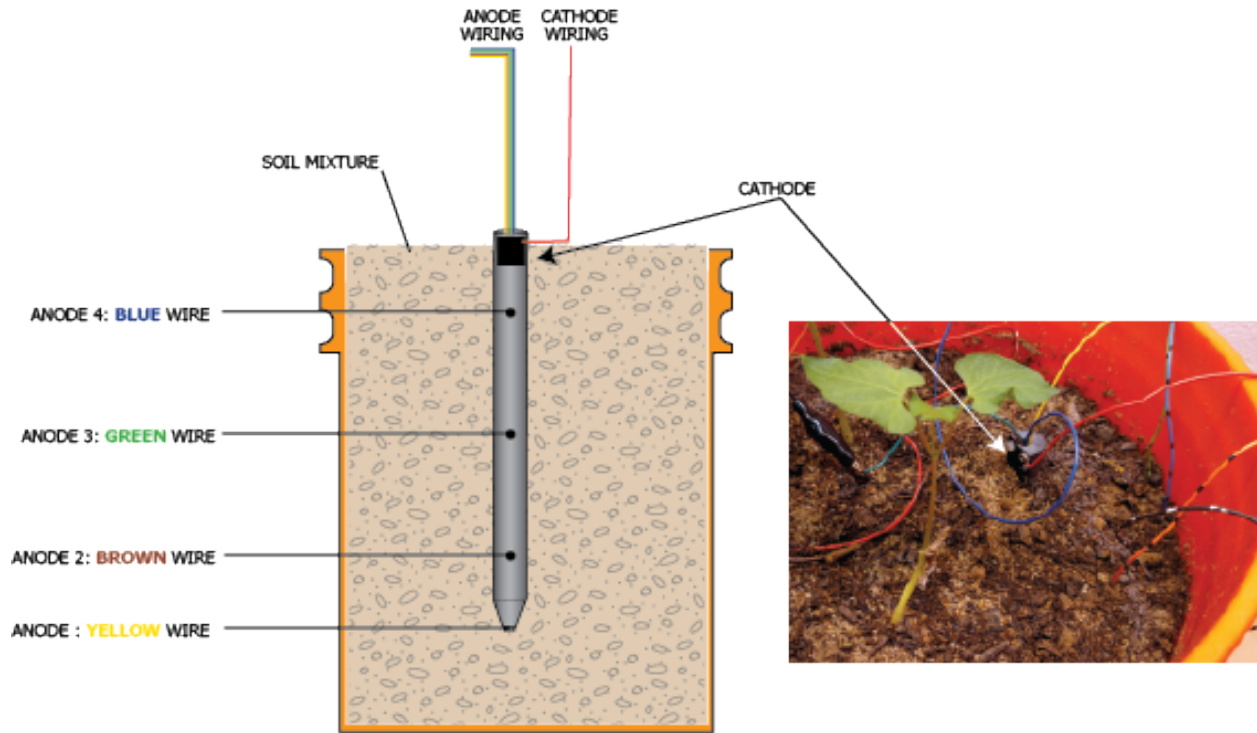
One of the problems associated with baseline technologies for the measurement of soil potentials in actual soils are related to the reference cells (silver/silver chloride or calomel). The ORP is a platinum wire typically coupled to a silver/silver chloride electrode reference cell. The reference cell is composed of the actual reference cell (silver wire in a solution of AgCl) and a salt bridge (solution of KCl). The reference cell is ideal for aqueous solutions but of limited value when measuring dry soils. Therefore, many soil investigations collect the soils and place the soils in solutions to make the measurement. An investigation requiring the collection of soils samples invites the problems associated with sampling error. An actual field deployment of the ORP using dry soils requires the contact of the inlet port of the salt bridge with dry soils resulting in loss of moisture from within the reference cells leading to inconsistent potential values. In contrast, the microbial system uses a cathode fabricated from a platinum-coated carbon fabric exposed to the atmosphere. The microbial sensor system does not require the presence of solutions at either the anode or cathode which is a major advantage.

The proposed sensor system is unique because multiple anodes can be referenced using one cathode and the graphite anodes can be miniaturized (less than 0.2 mm in diameter). This allows a monitoring system with multiple anodes (>10) being deployed throughout the soil profile. The

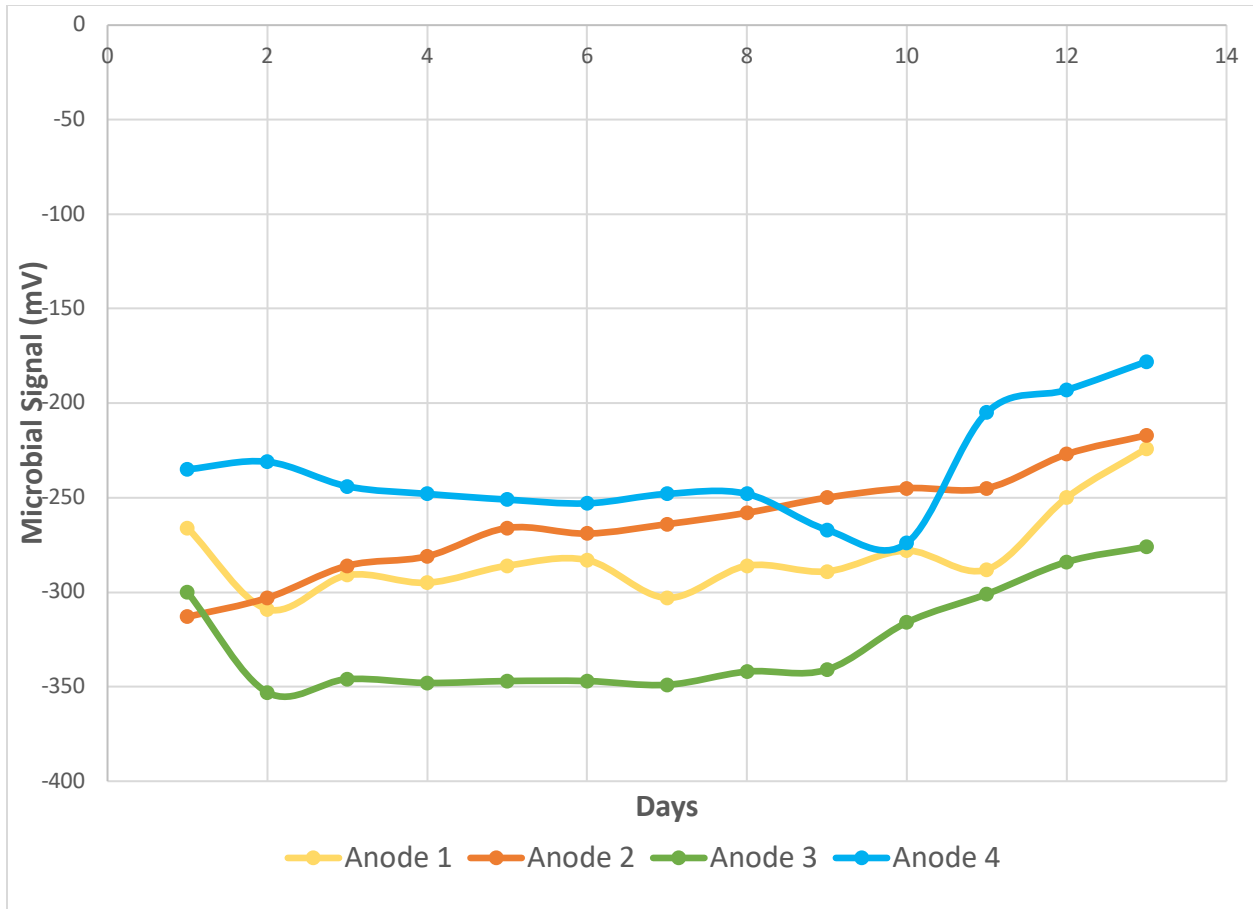
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multiple sampling points addresses the spatial variability of the soils while the automated collection of data from the multiple anodes addresses the temporal variability.

A preliminary experiment using the microbial sensing technology for monitoring the unsaturated zone is presented on Figure 3. A sensor array with four anodes and a single cathode were fabricated into a probe that can be pushed into the soil. Measurement circuitry used high impedance (950 M $\Omega$ ) potentiometers to determine the potential difference between each of the anodes and the common cathode. The preliminary data is presented on Figure 3.



**Figure 3: Preliminary Investigation of microbial sensor deployment in moist to dry soils**



**Figure 4: Soil potentials of moist/dry soil**

Please note: the microbial sensor registers negative values in the oxygenated environments (see Figure 1 and 3) which is opposite of ORP measurements.

The preliminary investigation demonstrated several attributes of the sensors deployed into an unsaturated soil including:

- The sensor system operates in dry and well oxygenated soils
- The cathode can be directly exposed to dry soil and atmosphere and serve as a reference cell for the sensor system
- Multiple anodes may be monitored using a common cathode (allowing definition of the vertical and horizontal distributions of soil potential using one reference)
- Soil potentials attained values of over -200 mV

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The proposed microbial sensor has several advantages in the measurements of soil potentials in the investigation of the rhizosphere including:

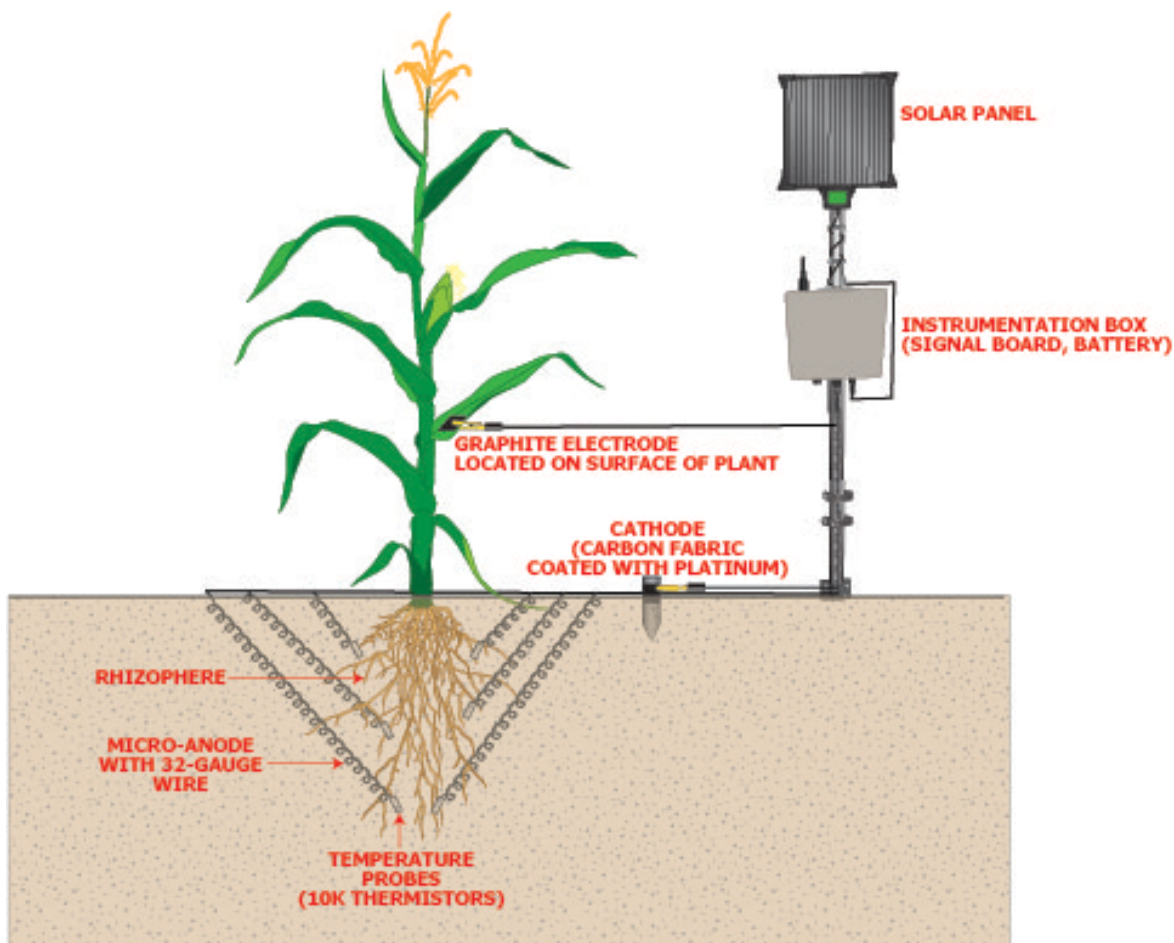
- The cathode and anodes can be located in and operated in well-drained to dry soils
- Anodes can be miniaturized
- One cathode can be used as a reference for multiple anodes
- Surface of the anode does not require maintenance because the self-replicating biofilm on the surface is resilient to poisoning and/or oxidation
- System performs multiple analyses (e.g., potentiometric, kinetic)
- Simple, robust and capable of field deployment
- System is easily automated to provide for real-time long-term monitoring

#### Proposed Scope of Work

This proposal will develop the technology from characterizing saturated (natural waters and submerged sediments) zones to characterizing unsaturated zones. This will result in the development of an important tool in understanding the rhizosphere.

The Phase I scope of work will consist of design, fabrication and testing (laboratory and field) of the microbial system (Figure 4). If Phase II funding becomes a reality, the system will be further developed for deployment of long-term monitoring of various agricultural areas and vegetative habitats in the United States.

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**Figure 5: Schematic of the proposed microbial sensor system configuration**

Burge Environmental has over 25 years of experience in sensor design and the deployment of sensors in unattended remote locations. The reasons the microbial system was selected for this scope of work included:

- Capable of operation of in a variety of environments
- Low instrumentation cost
- Low power requirements
- Very low maintenance (no support reagent)
- Applications in other fields (environmental remediation of soils, agricultural investigations)

## **Technical Approach**

### **Background & Expertise**

Burge Environmental was successful in obtaining Phase I and Phase II STTRs for the development of field-deployable Cr(VI), TCE, <sup>90</sup>Sr, <sup>99</sup>Tc, <sup>129</sup>I, tritium and uranium monitoring systems. In addition, the instruments supported additional sensors (e.g., water level, conductivity, pH) to aid in the characterization of the system under investigation.

The instruments are capable of off-grid operation, remote telecommunications, and self-calibration. The monitoring systems were developed to aid researchers and remediation experts in evaluation of the fate and transport of those radionuclides in the subsurface environment.

### **B. Anticipated Public Benefits**

The funding opportunity to develop a fully automated, field-deployable microbial sensor system for the unsaturated zones, including most rhizospheres, would yield several anticipated benefits. The primary benefit would be the development and deployment of a monitoring system capable of assisting DOE and its contractors in understanding the movement of the substrates (fate and transport) and/or microbial activity in the rhizosphere. The system will produce real-time spatial and temporal data at lower cost than the current baseline technologies.

#### **Additional Benefits**

- **Agricultural Investigation**

The microbial sensor technology can determine the response of microbes in dry and/or oxidizing soils supporting investigations into how fertilizers, pesticides and other chemicals impact the soils and rhizosphere. Additionally, the system may provide real-time monitoring of other parameters causing stress in agricultural crops.

- **Long-term Habitat Monitoring**

The microbial sensing system may allow remote monitoring of many natural habitats including tundra, forests and grasslands to determine parameters that impact the health of these habitats. An important aspect of the monitoring is the impact of the rhizosphere on the health and water production from important watersheds such as the Colorado River basin. The ability of the system to be deployed in unsaturated zones with little maintenance may allow for long-term monitoring and aid in the calibration of satellite investigations and modelling of these areas.

- One related aspect of rhizosphere management concerns the expanding science of phytoremediation that uses plant systems to be harnessed for a range of environmental management tasks. The scope of this spans the use of tree root systems to exert hydraulic control of contaminated groundwater to the activity of the root-microbial matrix that is capable of metabolizing contaminants.

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### Baseline Technology

Previously cited papers have indicated the problems associated with the baseline methods and the inability of the methods to correctly characterize many soil conditions. The current baseline technologies include ORP electrodes.

### Proposed Technology

It is anticipated a sensor system (several sensors in system) deployed in the field will continuously operate for several years with minimal if any maintenance.

## C. TECHNICAL OBJECTIVES OF PHASE I

The three technical objectives of this Phase I include:

1. Determine microbial sensor response in several types of soils at various moisture levels
2. Determine if the miniaturized sensor design becomes entangled and expands into the soil profile during the growth of the plant
3. Determine relationship of sensor signals between the rhizosphere and the surrounding soils

## D. PHASE I WORK PLAN AND WORK SCHEDULE

The Phase I will be divided into six tasks for accomplishing the technical objectives.

Task 1: Design a miniature, multiple anode microbial system with temperature probes

Task 2: Design and fabricate a laboratory measurement system


Task 3: Laboratory evaluation of the sensor system

Task 4: Design and fabricate a field-deployable measurement/communication system

Task 5: Laboratory (greenhouse) test the complete system within the rhizosphere of plants in dry and oxidizing soils to rhizospheres in saturated, acidic soils

Task 6: One limited field demonstration of the complete system

### **Task 1: Design a miniature, multiple anode microbial system with temperature probes**

Investigators: 

Duration: 12 weeks

Two types of microbial sensors will be designed: 1) a system for determining the background conditions, and 2) a miniature system designed to become entangled within the growing roots. The entanglement will allow the sensors to be located within the rhizosphere. The measurement system will allow the collection of data from ten (10) anodes and temperature probes. The team will design a unified graphite sensor system for deployment in oxidizing and dry soils (Figure 5). One of the primary elements of this task will be the design and fabrication of miniature anodes. The first anodes will be fabricated from small diameter graphite rods. The anodes will be electrically connected to very small diameter wires (<30 gauge). It is anticipated that the small

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diameter anode will require very high impedance potentiometers (>100 giga ohms) to insure adequate measurement of the microbial signal. Therefore, special electronic boards will be designed to allow the measurement of ten anodes with high impedance inputs. A programmable microprocessor-based electronic board will have the ability to measure current, temperature, and control all operational functions for investigations lasting several weeks to several months.

**Task 2: Design and fabricate a laboratory measurement system**

Investigators: [REDACTED]

Duration: 12 weeks

A laboratory system will be designed and fabricated to acquire real-time data from ten microbial and temperature sensors in the rhizosphere. A signal acquisition board will be capable of collecting MPS and MKS for each of the ten microbial sensors.

**Task 3: Laboratory Evaluation of the Sensor System**

Investigator: [REDACTED]

Duration: 12 weeks

A test chamber will be designed and fabricated to allow monitoring of soils with and without plants. The chambers will allow the control and measurement of the moisture content of the soils. Additionally, the chamber be designed to allow the introduction of gases (methane, carbon dioxide and air) into the subsurface of the soils within the chamber. The sensor systems will be deployed within chambers containing plants and chambers containing soils. The conditions within the two populations of chambers will be varied (moisture, subsurface gases) and the responses of the sensors recorded over several days to several weeks.

Evaluation 1: The sensors will be allowed to condition in the chambers for approximately five to seven days. The conditioning time is required for the surface of the anode to populate with a biofilm. After the conditioning period, the soil moisture of the test moisture will be increased. Gases (carbon dioxide, air) will be periodically flushed with the pore volume to determine if the sensors respond to soil moisture or the composition of the gases in the subsurface. The flushing of the pore volumes with methane or nitrogen should produce anaerobic conditions within the soils while flushing with air should produce aerobic conditions.

Evaluation 2: The test chamber will be fitted with several ports to allow the introduction of substrates. The second evaluation will introduce a substrate (acetate) into the test chamber to determine the effect of the substrate concentration on the biofilms populating the anodes. The sensor system will be operated in both the potentiometric and kinetic modes of operation. The test methodology will allow include an evaluation of the effects of each of the electrodes present in the test chamber.

**Task 4: Design and fabricate a field-deployable measurement/communication system**

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Investigator: [REDACTED]  
Duration: 12 weeks

Based on the laboratory evaluation, a field-deployable sensor system with measurement/communication system will be designed and fabricated. The system will be designed to allow for long-term monitoring of soils and/or the rhizosphere in a variety of locations including agricultural fields and natural environments. The sensor system will be designed to support at least 10 anodes with the ability to expand the number of sensors by the addition of an auxiliary board. All the signals will be capable of being processed and data transmitted by one system. The system will operate using a 12-volt power supply and exhibit low power consumption. The design will allow a solar panel with battery to operate the system during an entire year.

**Task 5: Laboratory test the complete system with rhizosphere of plants in dry and oxidizing soils to rhizospheres in saturated, acidic soils**

Investigator: [REDACTED]  
Duration: 12 weeks

Laboratory testing will be conducted in the laboratory and a greenhouse (Arizona State University). The testing will include data collected from the roots of mature plants and the developing roots during the germination and growth of plants. The primary experiments will focus of the impacts of soil moisture and conductivity of the soils in test chambers with and without plants.

The system will be deployed to an agricultural field and/or greenhouse. A field deployment unit will collect data for 30 to 60 days to determine if the design of the field deployment system is adequate for long-term deployments for monitoring agricultural and natural habitats

**Task 6: One limited field demonstration of the complete system**

Investigator: [REDACTED]  
Duration: 8 weeks

The complete system is illustrated on Figure 5. The design will include the following attributes:

- Measurement/communication module capable of measuring: MKS, MPS
- Measurement of temperature
- Control/communication module capable sending the data through cellular communications to a web site
- Measurement/communication module powered by solar cells

The performance schedule for the six tasks presented in the work plan schedule is presented on **Table 1**.

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**Table 1: Task Schedule for Phase I**

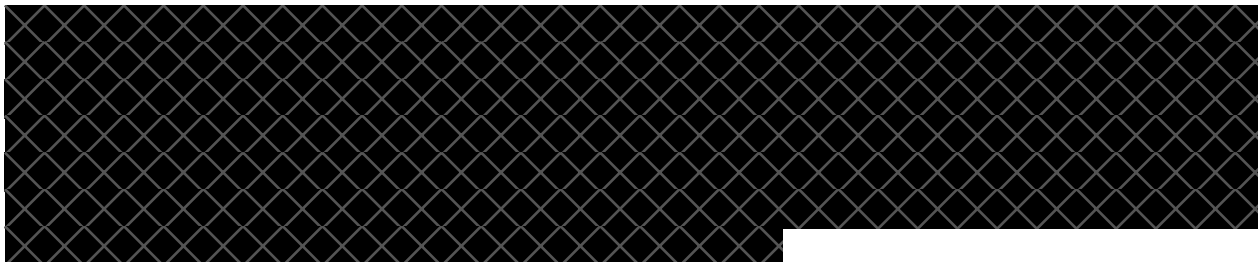
Month:	1	2	3	4	5	6	7	8	9
<b>Task 1:</b> Design a miniature, multiple anode microbial system with temperature probes									
<b>Task 2:</b> Design and fabricate a laboratory measurement system									
<b>Task 3:</b> Laboratory evaluation of the Sensor System									
<b>Task 4:</b> Design and fabricate a field-deployable measurement/communication system									
<b>Task 5:</b> Laboratory Evaluation of the Monitoring System									
<b>Task 6:</b> Field Evaluation of the Monitoring System									

**E. 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. The research is the subject of fifteen published articles and more than 30 papers delivered at conferences. The research and development has been divided between the deployment of sensors in the field and the development of simple sensors for the detection of environmental contaminants at the ppb level in ground water and surface waters.



**F. PRINCIPAL INVESTIGATOR & OTHER KEY PERSONNEL**

**Burge Environmental**





## G. FACILITIES / EQUIPMENT

The proposed work will be performed at the Burge Environmental, Inc. facility located in   
 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 containing the target compounds of the sensors used in the sampling/analytical 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. It is not anticipated that any equipment will be purchased for this scope of work.

## H. CONSULTANTS & SUBCONTRACTORS



## REFERENCES CITED

- (1) Olivier Husson. *Plant Soil* **2013**, 362, 389.
- (2) Rabenhorst, M. C.; Hively, W. D.; James, B. R. *Soil Scii. Soc. Am. J.*. **2009**, 73 (2), 668.