



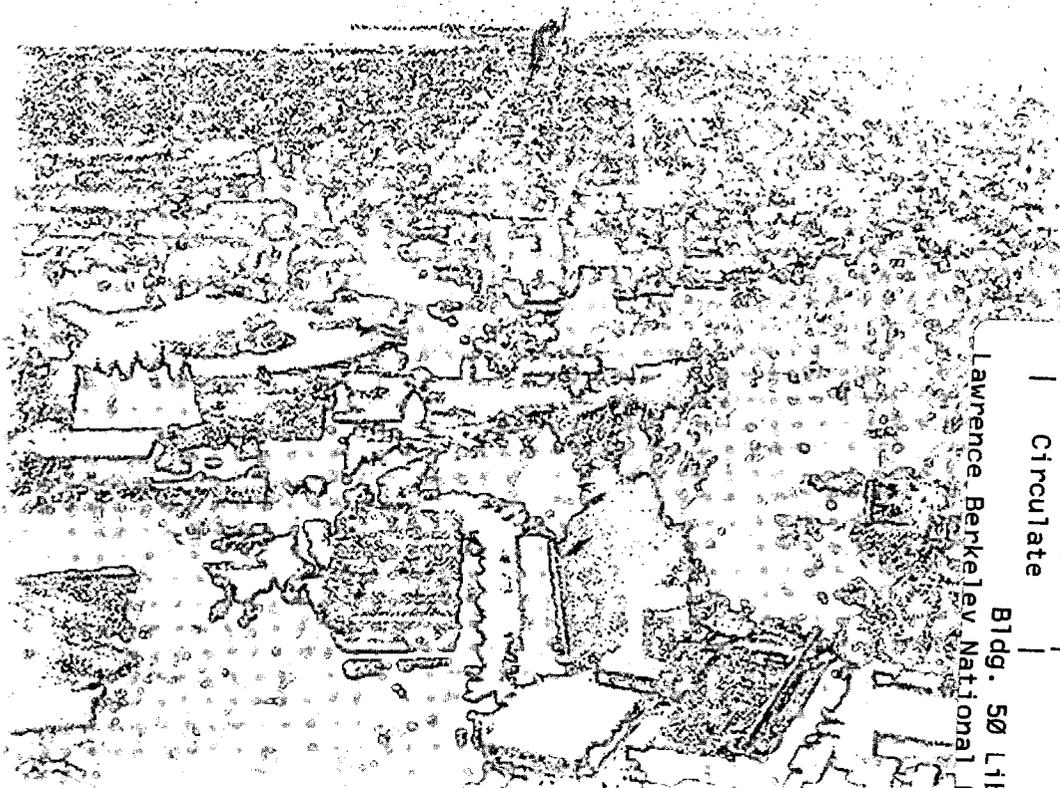
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Application of Electrical Methods to Measure Microbial Activity in Soils: Preliminary Microcosm Results

B.L. Cox, A. Sweet, and E. Majer

Earth Sciences Division

December 1997



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B.L. Cox, A. Sweet, and E. Majer

Earth Sciences Division
Ernest Orlando Lawrence Berkeley National Laboratory
University of California
Berkeley, California 94720

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Lawrence Berkeley National Laboratory, 1 Cyclotron Rd., Berkeley, CA 94720 USA

ABSTRACT

The application of the geophysical technique known as self-potential to the measurement of microbial activity was tested on laboratory microcosms containing ferric iron and iron-reducing bacteria *Shewanella alga* BrY. Measurements of the electrical response of silver-coated copper electrodes distributed along a teflon probe inserted into sterile and inoculated layers containing either ferric chloride, ferric citrate, or ferric oxide rich soil were recorded over hours or days. Strong electrical signals reached values more negative than -400 mV for all types of inoculated ferric iron layers. Electrical signals in sterile control layers, by contrast, rarely reached values more negative than -150 mV. These preliminary experiments indicate that it may be possible to apply the self-potential geophysical method to monitor bioremediation in the field.

Introduction

In situ bioremediation is an important strategy for the environmental restoration of contaminated subsurface sites. Bioremediation strategies must be designed around the particular characteristics of the site of interest. However, one general problem common to all bioremediation scenarios is that of monitoring the microbial activity. Whether the bioremediation employs intrinsic microorganisms and stimulates them by feeding (nutrients and water), or the strategy involves the injection of bacteria designed to react with the contaminant, there is a need to monitor the progress of the microbial reactions. The purpose of this work was to perform some initial scoping studies to examine the application of the geophysical method called self-potential to the measurement of microbial activity in the subsurface. If self-potential could be used as an indicator, or better yet, as a direct measurement of the location of microbial activity, then the monitoring of bioremediation could be greatly advanced.

Self-potential, also known as spontaneous potential or SP, has been used for mineral exploration since 1830 (8, referenced 18). Spontaneous ground potentials are attributed to either background potentials or mineralization potentials.

Background potentials show anomalies less than 100 mV and result from such processes as fluid streaming, bioelectric activity in trees, and electrolytic concentration gradients in groundwater(19). Mineralization potentials, associated with minerals such as magnetite, pyrite, chalcopyrite, pyrrhotite, sphaalerite, galena, and graphite, may exhibit electrical anomalies up to 800 mV(19).

Field equipment consists of a pair of electrodes connected by wire to a millivoltmeter. Electrodes are made of metal immersed in a saturated solution of the metal (such as Cu in CuSO_4) contained in a porous pot. The millivoltmeter should have an input impedance large enough that negligible current is drawn from the ground during the measurement (19). Measurements are made either along the surface of the ground, in order to define some hidden electrical anomaly, or vertically through a drill-hole, as a means of delineating strata of differing chemical and physical properties. A typical self-potential well log is shown in Figure 1.

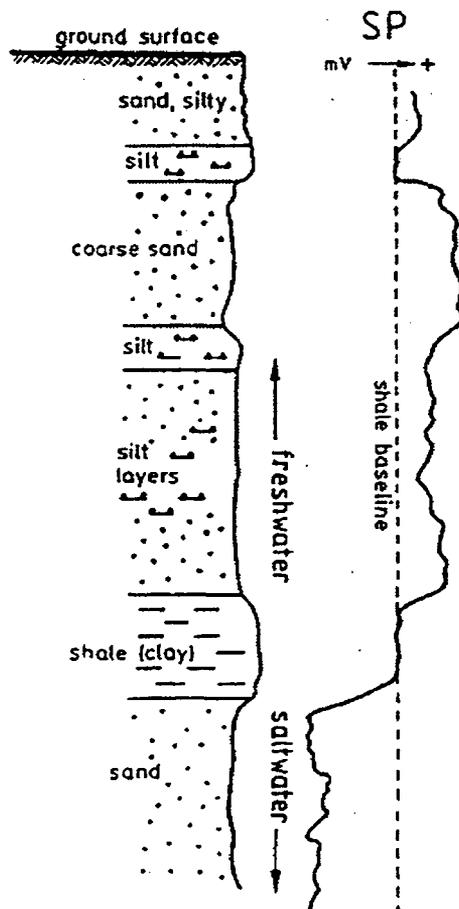


Figure 1. Typical self-potential well-log (from reference 21).

We tested the idea that a down-hole self-potential probe might be used to monitor the microbial activity in an iron-rich layer. As a preliminary test of the possible range in electrical signals we might detect in the field, we made laboratory microcosms containing a ferric iron rich layer and iron-reducing bacteria *Shewanella alga* BrY. The microbial activity was measured with a voltmeter connected to a vertical probe containing 10 equidistant electrodes. By monitoring the difference in electrical potential between a control electrode and the other electrodes over time, we could obtain a range of signals resulting from different initial conditions.

Recent studies have demonstrated the use of metal reducing bacteria (*Shewanella* and *Geobacter* genus) to degrade organic contaminants such as benzene and toluene under anaerobic conditions (11,5). Polycyclic aromatic hydrocarbons (PAHs) and alkanes can be degraded anaerobically by sulfate reducing bacteria(6). Microbial degradation of hydrochlorofluorocarbons (HCFCs) and chlorofluorocarbons (CFCs) has been measured in anoxic sediments (10,15) and has been linked to a methanogenic archaea *Methanosarcina barkeri* (9).

Shewanella and *Geobacter* bacteria are able to couple the oxidation of the organic molecules with the reduction of different metals, including iron, manganese, chromium, and uranium (12,14). These bacteria have potential for bioremediation of waters and soils contaminated with heavy metals (chromium) and actinides (uranium) because reduced chromium (Cr(III)) and uranium (U (III)) are precipitated out of solution. Iron and manganese oxide minerals become soluble and mobile when reduced. Ferric iron is ubiquitous in subsurface sediments, and layers of iron oxides are important indicators of redox processes. Observations of a landfill leachate pollution plume in Vejen, Denmark indicated that organic degradation occurred primarily in the iron-rich zones (13).

Shewanella alga BrY was isolated from the Great Bay estuary, New Hampshire (2,17). *Shewanella putrefaciens* and *Shewanella alga* are similar but distinct species, based on numerical analysis of whole cell protein profiling, ribotyping patterns, 16S rRNA gene sequencing, and phenotypic characterization (20). *S. alga* species are mesophilic and halotolerant with 52 to 55 mol% G+C. *S. putrefaciens* are more heterogeneous, typically psychrotolerant, with 43 to 47% G+C (20). Kinetic experiments have shown that *S. alga* BrY can dissolve iron oxide minerals such as hematite, goethite and ferrihydrite (4, 7,16). *S. alga* BrY respond to carbon and nitrogen starvation by decreasing the mean cell volume by more than 50% which may provide a means of delivering these bacteria to contaminated subsurface environments (3).

We hypothesized that the growth and respiration of metal-reducing bacteria in ferric iron rich layers could produce an electrical signal strong enough to be detected and monitored by a voltmeter. We developed a probe to measure the electrical potential anomaly resulting from laboratory microcosm experiments. Nutrient-rich ferric iron layers were established and inoculated with the iron-reducing bacteria

Shewanella alga BrY , and sealed with agar gel. Electrical activity was measured in layers composed of ferric iron as either ferric chloride, as ferric citrate or as iron oxides in soils. This allowed us to measure systems with soluble ferric iron (ferric chloride and ferric citrate) as well as lattice-bound ferric iron (iron oxide minerals in soils). Results were compared with those obtained from sterile controls.

Our experimental objective was to design and demonstrate a miniature geophysical probe to measure the electrical signal produced by microbial activity in ferric iron solutions and in soils. Based on the results of preliminary experiments, we discuss possibilities for scaling up to a field experiment.

Materials and Methods

Experimental Setup

Experiments were performed either in 50 ml Pyrex screw-cap test tubes (15.24 cm in length) or in 600 ml Mason canning jars (Figure 2). Teflon rod (0.635 cm diameter) was used as the support for the wire electrodes. Teflon can withstand autoclave temperatures ($>121^{\circ}\text{C}$) necessary for sterilization. For the test-tube experiments the rod length was 12.7 cm, and the electrodes were spaced 1 cm apart along the length of the rod. For the Mason jar experiments the rod length was 20.32 cm. Five electrodes were spaced one centimeter apart starting one centimeter from the bottom of the rod; four more electrodes were spaced two centimeters apart.

All but one of the experiments employed a 10 gauge (0.254 cm diameter) silver plated copper wire insulated with a Kynar (polyvinylidene fluoride) coating. For one experiment, a 0.2032 mm diameter 99.9% gold wire was used. The gold wire was insulated with "Insul-Spray" manufactured by Rotanium, a clear high dielectric strength sealer for electronic components. Four coats were applied to each wire. Once the rod was completely strung with wire, 1 centimeter of insulation was stripped off the end of each wire to enable electrical measurements of the wire by the voltmeter. A distinct possibility is that anomalies we observe in this experiment would be due to electrode effects rather than microbial activity.

A size 1 rubber stopper was placed snugly at the mouth of the test tubes. The Mason jar lid had two components, a top with a seal around the rim of the jar and a screw cap for around the perimeter. In the top lid two holes were punched, one hole 1.27 cm diameter to fit a size 1 rubber stopper and another hole 2.54 cm diameter to fit a size 5 rubber stopper. A 0.508 cm hole was cored through the size 5 rubber stopper and the Teflon rod with electrodes was pressed through the stopper and held tight.

To establish the different reaction zones along the length of the tubes, sterilized agar layers were poured around the electrode rod. The layer convention was as follows: 15 ml of 1.5% agar solution; 1 ml overnight biological culture of *S. alga* BrY combined with 7 ml 0.2% agar + nutrients and ferric iron; 20 ml 1.5% agar. Control cells received no inoculum. To seal the cells, a strip of parafilm was wrapped

around the inside of the rubber stopper and the stopper was placed snugly in the mouth of the test tube. Another strip of parafilm was wrapped around the edge of the test-tube and stopper, making an airtight seal. Aluminum foil was wrapped around the outside of the cell to shield the contents from surrounding light. The bottom-most wire of the probe served as the reference electrode.

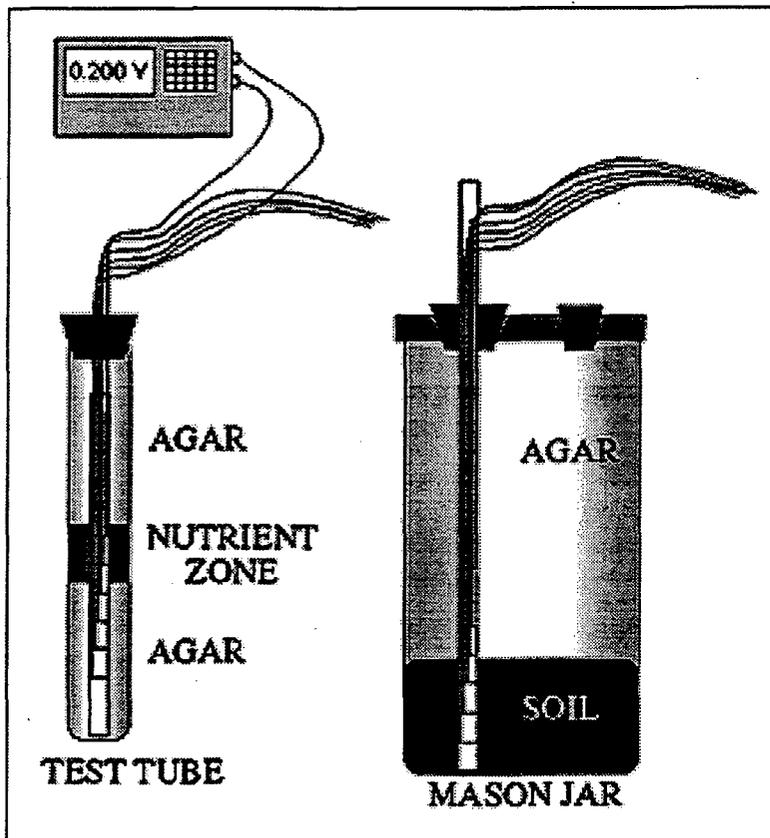


Figure 2. Experimental Apparatus.

A soil volume of 250 ml was placed in the bottom of the Mason jar. The electrode rod attached to the stopper and lid was pressed into the soil. The stopper and lid were adjusted along the length of the rod so the top of the lid formed a tight seal with the lip of the jar. The screw cap was screwed down tight. To ensure an air tight seal around the stopper and rod a 100% silicon sealant was generously applied around the edges. The entire apparatus was sterilized by autoclave. Through the 1.27 cm hole in the lid of the Mason jar, the inoculum and sterile nutrient solution were pipetted over the soil. The soil was then capped with 400 ml 1.5% agar. Control cells received no inoculum. The wire in the agar layer located 10 cm from the bottom of the jar served as the reference electrode.

Chemical Compositions

All of the nutrient zones were mixed with a 0.2 % agar (Difco Bactoagar) to make a soft gel. Ferric chloride (Matheson), ferric citrate (Sigma), and sodium citrate (Mallinckrodt) experiments used trypticase soy broth (TSB, Becton Dickinson) as the carbon and nutrient source. Soil experiments used lactic acid solution (Sigma) as the carbon source.

Ferric Chloride was tested at three concentrations: 12.33 mM, 6.165 mM, and 3.083 mM. Ferric Citrate was tested at five concentrations. In the first set of ferric citrate experiments, initial pH was not adjusted to neutral so the starting pH was variable depending on the concentration of the ferric citrate (56 mM, pH 3.85; 28 mM, pH 4.5; 14 mM, pH 5.6; 7 mM, pH 6.2; 3.5 mM, pH >6.2). Ferric Citrate was also tested at three concentrations at neutral pH. Ferric Citrate with MOPS(4-Morpholinopropane sulfonic acid, Sigma) solutions were titrated to pH 6.9-7.0 with NaOH. This solution was then filter sterilized (0.45 μ m Nalgene filter) and added to autoclaved TSB-agar suspension. A sodium citrate suspension (7 mM, 1g MOPs/l, 3.0% TSB, 0.2% agar, neutral pH) was tested as a control with no ferric iron for bacterial respiration. This solution was titrated to pH 6.9-7.0 with NaOH, filter sterilized (0.45 μ m Nalgene filter) and added to autoclaved TSB-agar suspensions.

A soil sample was obtained from near Savannah River, Georgia, USA. This highly oxidized soil contained a high percentage of iron oxide minerals as well as kaolinite and quartz sand. For the soil test tube experiments, Savannah River soil slurry was made by adding 100g of soil to 100 ml of a 0.2% agar plus sodium citrate medium. This medium contained 10.4 g/l MOPS, 2.5 g/l NaHCO₃, 1.5 g/l NH₄Cl, 0.06 g/l NaH₂PO₄, 0.10 g/l KCl, 2ml of 60% lactic acid solution, 10 ml of vitamin solution and 10 ml of trace mineral solution. The soil and agar suspensions were autoclaved. The nutrient solution was filter sterilized (0.45 μ m Nalgene filter). For the Mason jar soil experiments, 30 ml of filter sterilized sodium citrate medium was pipetted over 250 ml of autoclaved soil. An overnight culture was then pipetted over the soil, followed by 20 ml of sodium citrate medium. This was then capped with a sterilized 1.5% agar suspension.

Inoculum

S. alga BrY cells were transferred from plates into 50 ml of TSB. This suspension was incubated at 30°C and shaken overnight. Serial dilution plate counts and analogous spectrophotometric readings for absorption at 600 nm showed that the inoculum contained ~ 10⁹ cells/ml. The test tube experiments received 1 ml of inoculum. The first of the two Mason jar experiments received 2 ml and the second received 0.5 ml of inoculum.

Electrical Measurements

The readings were taken at constant ($20^{\circ}\text{C} \pm 2^{\circ}$) room temperature. Electrical measurements were made with a digital nanovoltmeter, manufactured by Keithley, Model 181 (input impedance of $10\text{ M}\Omega$; accuracy of $\pm 0.15\%$ of displayed reading + 1 mV). The stripped portion of reference electrode wire was connected to the reference terminal of the voltmeter. The remaining wires were sequentially connected to the other voltage input, completing the circuit and enabling a potential difference reading. Initially, readings were taken every two to four hours for the test-tube experiments, and one to two times daily for the Mason jar experiments. After electrical activity rates slowed, the readings were taken at longer time intervals.

Results

Ferric Chloride Experiments

The results of the FeCl_3 experiments are shown in Figure 3. Figure 3a shows a typical profile of measurements at four different times, taken along the length of one of the inoculated cells. The measurements taken from the sterile control cell are shown for comparison. Notice that there is a significant signal in the ferric chloride zone of the inoculated cell (-450 mV). The corresponding signal in the sterile control cell is much weaker (-100 mV). Figures 3b, c, and d show the change in electrical potential with time in the ferric chloride zone for three different concentrations of ferric chloride. The inoculated tubes reach a maximum signal sometime after 4 h, stay at a plateau, then slowly return to less negative potentials. The sterile control signals are relatively constant and stay within a range of 0 to -100 mV .

The results for the three experiments are summarized in Table 1. The inoculated cells with the lowest concentration of ferric chloride (and the highest pH) showed the strongest electrical signal, and reached the strongest signal at the earliest time. Since these experiments were not repeated at neutral pH, we could not determine whether the variations in signal were caused by differences in ferric chloride concentration or variations in pH.

Table 1 Results of Ferric Chloride Experiments

FeCl_3 , g/l	Initial pH	Inoculated Cell, strongest electrical potential, mV	Time of strongest electrical potential	Average change in electrical potential, mV/h	Control Cell, range of electrical potential in FeCl_3 zone, mV
0.5	6.9	-482	4 h	-120.5	-11.4 to -44.0
1.0	6.5	-443	7.5 h	-59.0	-31.6 to -95.6
2.0	5.8	-293	21 h	-13.9	-18.9 to -79.3

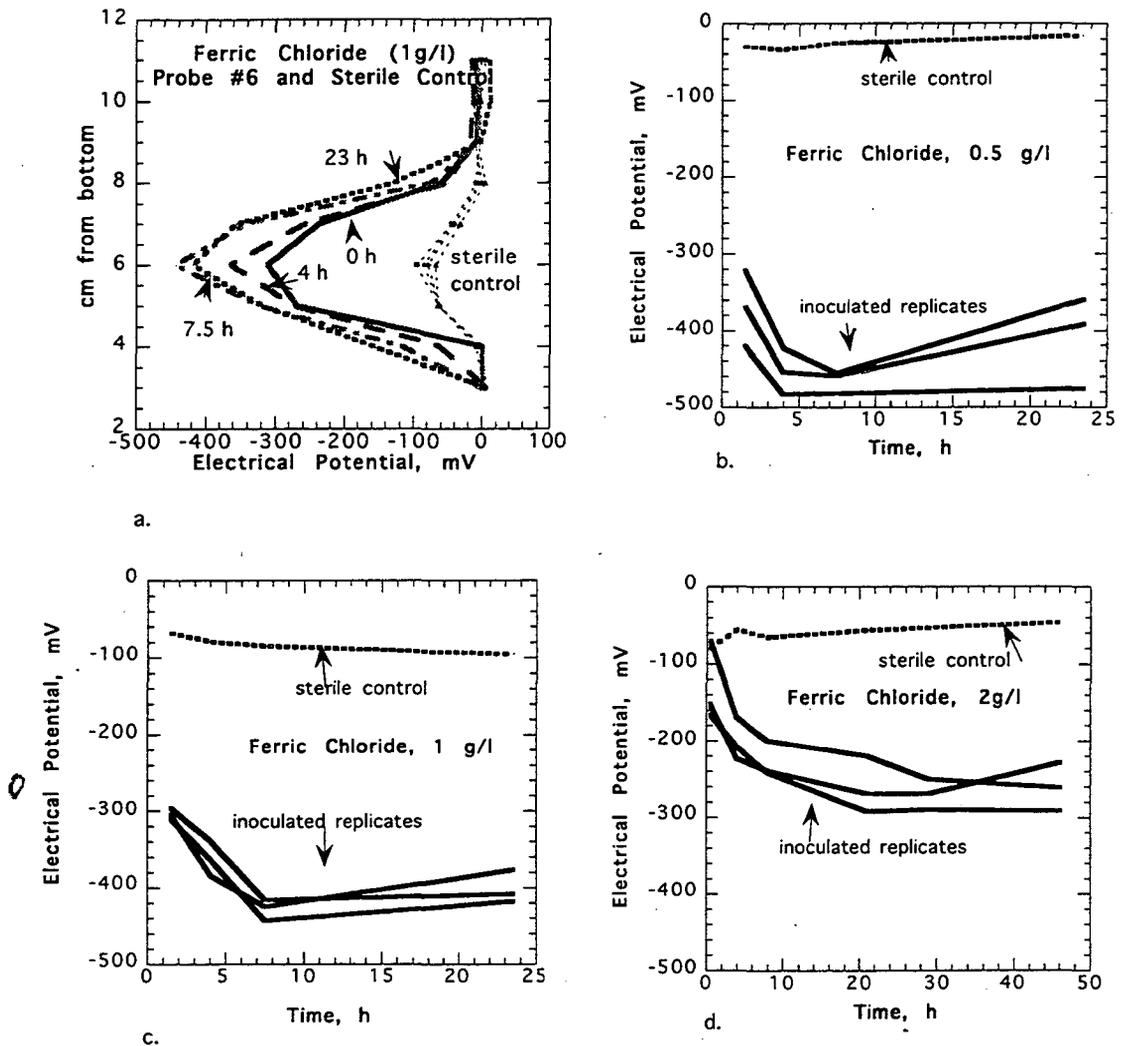


Figure 3. Results of Ferric Chloride experiments.

Ferric Citrate Experiments

We performed several experiments at different concentrations of ferric citrate. Initially, the solutions were not adjusted to an initial neutral pH. Later experiments were titrated to pH 7. Summary plots of the reaction kinetics of inoculated cells and the uninoculated controls are shown in Figure 4 for experiments with pH = 7. All 4 of these experiments showed a very strong electrical signal in the inoculated zone, and a much weaker, nearly flat electrical signal in the sterile control. The replicates in Figure 4c (3.4 g/l) showed a much wider spread than those in the other

experiments. This may have been caused by a variation in inoculum size or distribution. The information for all of the experiments is summarized in Table 2. There does not appear to be any correlation between ferric iron concentration and magnitude of electrical signal. The variations may be the result of differences in the initial size of the bacterial population.

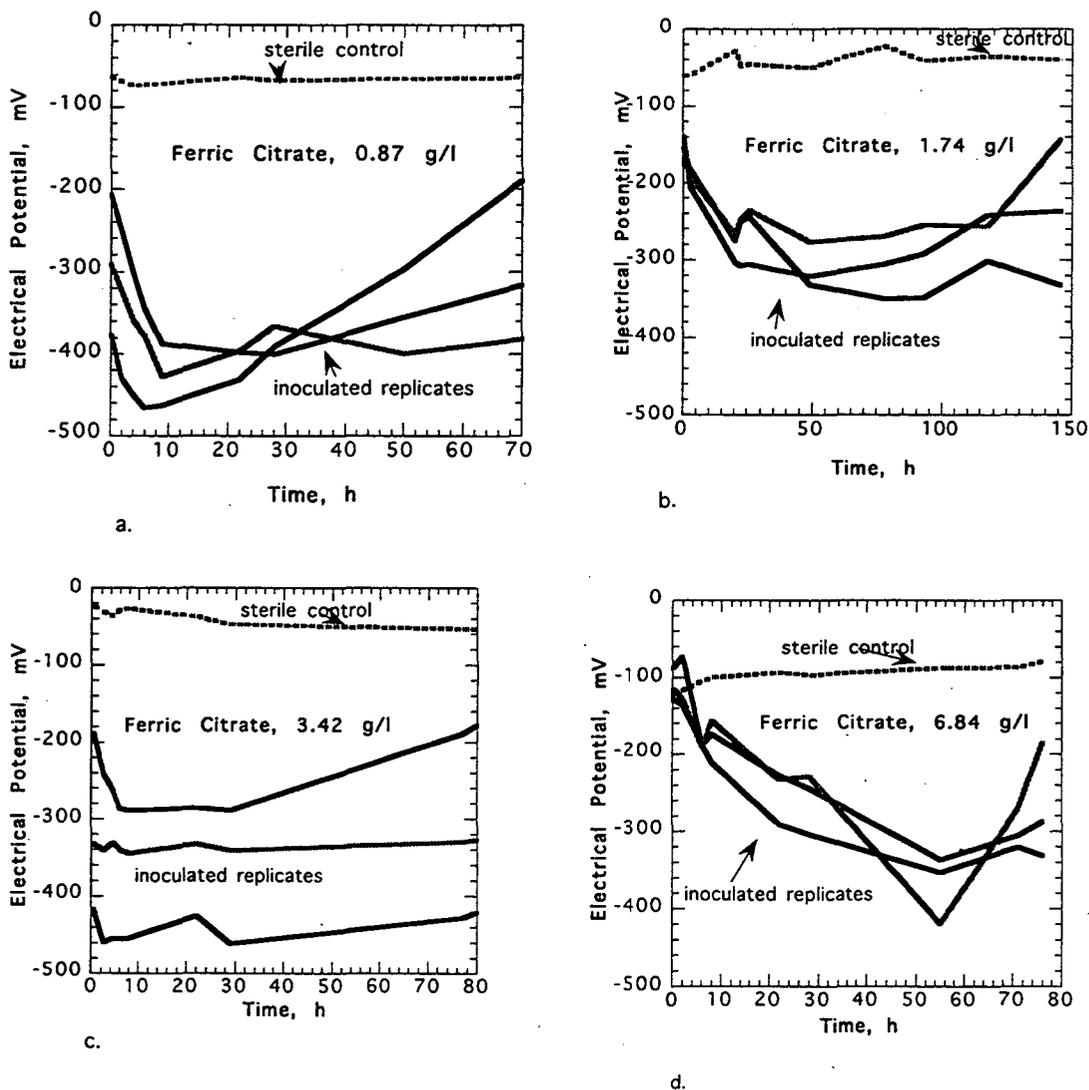


Figure 4. Results of Ferric Citrate experiments with pH = 7.

Table 2. Results of Ferric Citrate Experiments

Ferric Citrate, g/l	Date of Inoculation	Initial pH	Inoculated Cell, strongest electrical signal in FeCl ₃ zone, mV	Time of strongest Signal, h	Average change in electrical potential, mV/h	Control Cell, range of electrical response in FeCl ₃ zone, mV
0.87	6/3/97	> 6	-466.3	6	-77.7	-30.7 to -74.8
1.74	5/26/97	6.2	-460.0	26	-17.7	5.3 to -106
1.74	8/7/97	7	-327.4	78	-4.2	-14.1 to -61.1
2.16	8/14/97	7	-400.7	5	-80.1	10.5 to -110.7
3.4	5/15/97	5.6	-260.8	24	-10.9	11.0 to -138.0
3.4	5/20/97	5.6	-426.0	20	-21.3	7.9 to -73.3
3.4	6/26/97	7	-459.6	2	-229.8	10.4 to -128.9
6.8	5/15/97	4.5	-340.0	98	-3.5	17.3 to -103.0
6.8	5/20/97	4.5	-106.0	50	-2.1	8.0 to -26.8
6.8	6/20/97	7	-419.2	55	-7.6	6.1 to -121.8
13.7	5/14/97	3.85	-115.1	25	-4.6	5.9 to -89.0

Two additional control experiments are shown in Figure 5. One of these experiments used a sodium citrate medium to test the electrical response in the absence of ferric iron. The other control was a ferric citrate medium with gold electrodes to test the electrical response of more stable electrodes. Both of these experiments were adjusted to an initial pH of 7.0. The electrical response for the inoculated sodium citrate layer was very rapid and strong (Figure 5a). This signal quickly decayed after five hours, unlike the ferric chloride and ferric citrate experiments, where the strong signal persisted for more than 50 hours.

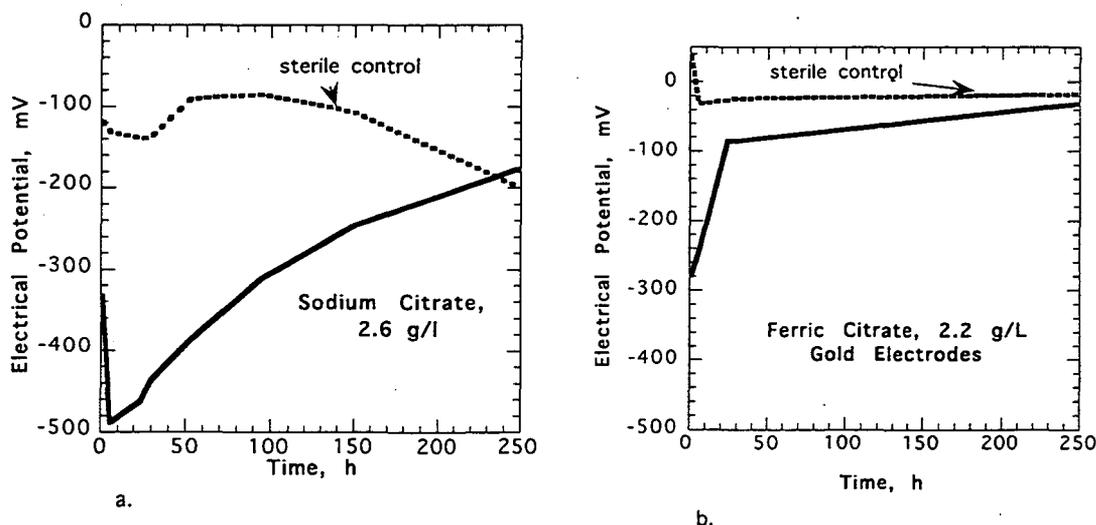


Figure 5. Sodium citrate and gold electrode control experiments.

Figure 5b shows the results of the experiment with gold electrodes. The gold electrodes were not sheathed with plastic, but were coated with a spray. This spray might have degraded over the first 30 h of the experiment because, after this period, the voltmeter wouldn't stabilize.

Soil Experiments

The results of the soils experiments are summarized in Figure 6 and Table 3.

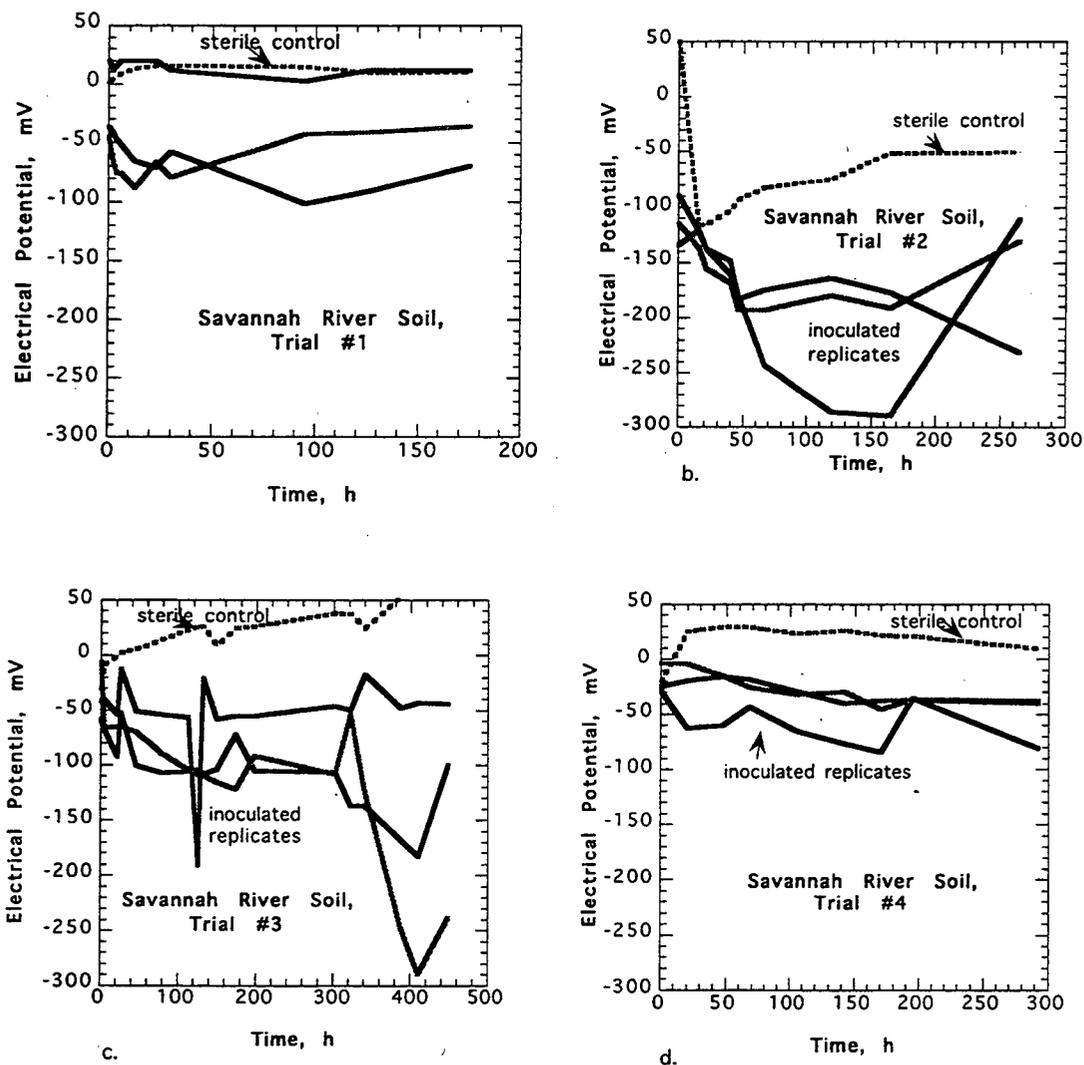


Figure 6. Results of Savannah River soil experiments.

The first two experiments (Figures 6a and 6b) were performed in test tubes, and the other two (Figures 6c and 6d) in Mason jars. One of the tubes appeared to have no microbial activity (Figure 6a). One of the tubes (Figure 6b) and one of the jars

(Figure 6c) showed a response of nearly -300 mV. The electrical responses recorded for the inoculated soils were generally slower to reach a maximum, more erratic, and less intense than those in inoculated soluble iron media.

Table 3. Results of Soil Experiments

Type of Container	Inoculated cell, strongest electrical signal, mV	Time of strongest signal, h	Average change in electrical potential, mV/h	Control Cell, range of electrical response, mV
Test Tubes #1	-102.1	90	-1.1	23.1 to -19.2
Test Tubes #2	-266.3	165	-1.6	65.1 to -116.6
Mason Jars #1	-290	405	-0.7	109.5 to -45.1
Mason Jars #2	-100	195	-0.5	55 to -62.4

Discussion

We have demonstrated that strong electrical signals resulting from microbial activity can be detected by vertical probes in ferric iron media, in media without ferric iron, and in ferric iron-rich soils at two different scales. These signals are distinctly different from those measured in uninoculated control cells. There are many different processes occurring in the inoculated cells (not occurring in the uninoculated cells) which could be contributing to the difference in signal. These include consumption of O₂, generation of CO₂, removal of carbon and nutrients, generation of bacterial products, reduction of ferric iron to ferrous iron, biological corrosion of electrodes, and migration of ions due to changes in redox potential. We could address the relative effects of these different processes by designing a more sophisticated monitoring system (use of ion-specific electrodes; simultaneous measurement of pH, redox indicator, Fe(II), O₂ and bacterial growth in the reaction layer; analysis of change in surface topography of the electrodes; automated data collection, etc.). However, the simpler experimental design does satisfy our experimental objectives.

Electrical responses in soils were more erratic and took longer to reach a maximum negative potential than those in soluble ferric iron media (Figure 7). The experiments performed in soils differed in several ways from those performed in soluble iron solutions. First, the iron in the soils is chemically bound to mineral surfaces. Thus, the iron is less accessible to the bacteria. Second, the presence of surfaces produces microenvironments where the bacteria may collect, causing localized zones of chemical reactions. For the soil experiments, concentrations of reduced iron might initially accumulate close to the mineral grains, rather than in a homogeneous iron suspension. Bacteria are attracted to surfaces, and in the experiments without soils the bacteria may have accumulated on the surfaces of the probe and electrodes. Third, the electrode contact with the ferric iron is better in the soluble iron medium. Also, we used a defined mineral medium with lactate as the carbon source for the soil experiments, instead of the rich TSB broth. The soil

experimental conditions were closer to those which might exist in the field, where lactate can occur as a fermentation product under anaerobic conditions.

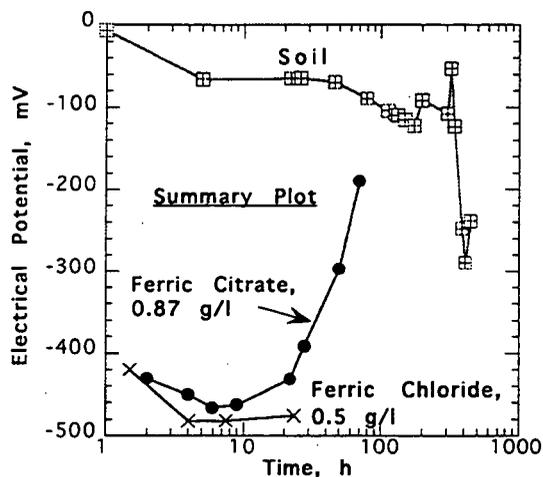
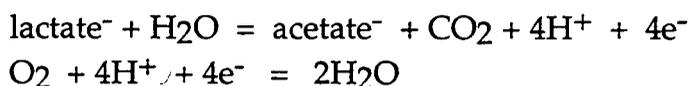


Figure 7 Summary plot of experiments with greatest electrical signal in the inoculated layer.

The electrical signals in the soils were stepped. One explanation for this is trapped oxygen. The soils were not de-aired prior to the experiments, so oxygen was trapped in the pore spaces after the agar and nutrients were poured. This would lead to intermittent degassing of oxygen, and intermittent iron respiration. The consumption of O_2 occurs during aerobic respiration. This is coupled to the oxidation of the carbon source. In the soil experiments, the carbon source was lactate- ($CH_3CHOHCOO^-$) which was probably oxidized to acetate- (CH_3COO^-) and CO_2 :



The combined coupled reaction is:

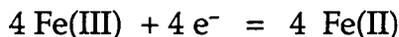


Therefore, one mole of O_2 is used for every mole of lactate⁻ consumed. As the O_2 is used up, it is replaced by CO_2 . This alters the chemistry of the solution in several ways. The redox potential of the solution changes as the O_2 is depleted, the partial

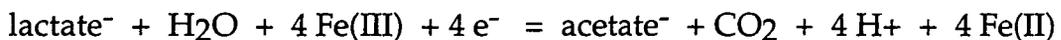
pressures of the CO₂ and O₂ change, and the pH is affected because of the solubility of CO₂ and its conversion to HCO₃⁻.

The rate of O₂ utilization depends on many factors, including size and species of bacterial population, type of carbon used, growth stage of the bacteria, diffusion rate of oxygen, ionic strength of the medium, pH of the medium, temperature, solution viscosity, and mechanical mixing (1). The rapid decay in signal observed in the Na-citrate experiment suggests that oxygen in the test tubes may have been used up after 5 hours, leading to a rapid and steady decline in microbial activity. There was no ferric iron for respiration; once the oxygen was used up, the bacteria could no longer perform respiration.

After the O₂ is depleted, iron-respiring bacteria can use Fe(III) as an alternative electron acceptor for respiration. The first equation is the same as above for lactate⁻ oxidation to acetate⁻ and CO₂. The second reaction is



The combined reaction is:



Therefore, 4 moles of Fe(III) are needed for every mole of lactate⁻ consumed. The redox potential is now affected by the ratio of Fe(III) to Fe(II). We did not directly measure iron reduction. The observation that electrical responses remained strong in the solutions with ferric citrate and ferric chloride after the O₂ was used up suggests that iron reduction was occurring in these experiments. Some of the tubes showed a distinct darkening of the iron-containing zone during the course of the experiment. This was further evidence that iron reduction was occurring.

Scaling up to a Field Experiment

The last two soils experiments were performed in Mason jars, a much larger system than the tubes, with 35 times the volume of soil in the inoculated layer. The scale did not seem to affect the magnitude of the electrical response. The trends of the electrical potential transients suggest that the soil experiments had not yet reached a maximum negative potential.

The electrical measurements we recorded are the differences in electrical potential between electrodes at fixed locations along the length of the probe. This is not entirely the same as the conventional geophysical technique known as self potential: self-potential borehole probes record the difference in electrical potential between a moving electrode in the borehole and a stationary electrode at the surface. Those recorded electrochemical potential signals result from the difference in

salinity (or other property affecting electrical properties) between the drilling mud and the formation fluid (18). As the probe encounters strata with different properties, the signal changes, allowing the vertical mapping of the different lithologic units. We used a stationary probe embedded in layered systems without drilling mud and monitored changes within single layers over time.

If this technique were applied under field conditions, the system would be open instead of closed; a drillhole might allow the introduction of O₂ into the anaerobic zone. However, if the system were initially anaerobic, there would be many biological as well as chemical sinks for any O₂ seeping into the contaminated zone, and we would expect that the system would remain anaerobic because the O₂ utilization rate would exceed the O₂ source.

If further experiments were designed to test the applicability of this approach to field measurements, it might be useful to construct an intermediate-scale laboratory experiment in large (~1.5 meter tall) plastic containers. The bottom and top layers could be filled with some kind of insulating material with an inner sealed layer of soil with ports for injecting nutrients and bacteria. A vertical well could be cored through the three layers. Then, after inoculating the soil zone with bacteria and nutrients, an actual self-potential probe could be tested on this system by performing time lapse (repeated) measurements during a period in which bacterial population and activity were known. Our preliminary results suggest that we should be able to monitor microbial activity at an intermediate scale. Another possibility would be to bypass the intermediate scale and make self-potential measurements at a field site undergoing bioremediation, with laboratory measurements on natural field materials to calibrate the response.

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**ERNEST ORLANDO LAWRENCE BERKELEY NATIONAL LABORATORY
ONE CYCLOTRON ROAD | BERKELEY, CALIFORNIA 94720**

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