An electrode-based approach for monitoring *in situ* microbial activity during subsurface bioremediation

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Abstract

Current production by microorganisms colonizing subsurface electrodes and its relationship to substrate availability and microbial activity was evaluated in an aquifer undergoing bioremediation. Borehole graphite anodes were installed downgradient from a region of acetate injection designed to stimulate bioreduction of U(VI); cathodes consisted of graphite electrodes embedded at the ground surface. Significant increases in current density ($\leq$50 mA/m^2) tracked delivery of acetate to the electrodes, dropping rapidly when acetate inputs were discontinued. An upgradient control electrode not exposed to acetate produced low, steady currents ($\leq$0.2 mA/m^2). Elevated current was strongly correlated with uranium removal but minimal correlation existed with elevated Fe(II). Confocal laser scanning microscopy of electrodes revealed firmly attached biofilms, and analysis of 16S rRNA gene sequences indicated the electrode surfaces were dominated (67-80%) by *Geobacter* species. This is the first demonstration that electrodes can produce readily detectable currents despite long-range (6 m) separation of anode and cathode, and these results suggest that oxidation of acetate coupled to electron transfer to electrodes by *Geobacter* species was the primary source of current. Thus it is expected that current production may serve as an effective proxy for monitoring *in situ* microbial activity in a variety of subsurface anoxic environments.

Introduction

There is a pressing need to monitor microbial activity in subsurface environments because of its influence on groundwater chemistry and its role in controlling contaminant fate and transport. Many of the approaches for estimating rates of microbial processes in near-surface environments are not applicable to the subsurface. For example, it is common to measure rates of microbial processes in short-term incubations of cores of aquatic sediments injected with radiolabeled tracers of organic substrates or electron acceptors [1]. However, such an approach is not readily feasible for subsurface environments, due to drilling costs and difficulty in obtaining undisturbed cores, and because radiotracer measurements can significantly overestimate rates of metabolism in subsurface environments [2].

This has led to the search for *in situ* measurements that can serve as a proxy for microbial activity. One of the most successful has been measuring concentrations of dissolved hydrogen to determine which anaerobic terminal electron-accepting processes predominate in subsurface zones of interest [3]. With this method, it is possible to establish the terminal electron-accepting process, which often cannot be determined with standard geochemical measurements. Unfortunately, hydrogen measurements provide no indication of the rates of the microbial processes.

In some instances, it is possible to estimate rates of microbial processes in the subsurface by monitoring changes in relevant groundwater constituents at sampling points along groundwater flow paths [4] or in push-pull studies [5]. However, these approaches require extensive sampling and analytical expertise. Furthermore, these approaches do not work when trying to estimate rates of multiple anaerobic respiratory

processes. For example, monitoring the accumulation of Fe(II) in groundwater greatly underestimates Fe(III) reduction rates because most of the Fe(II) produced during Fe(III) oxide reduction remains in solid phases or sorbed to mineral surfaces [6, 7]. Such considerations indicate that novel approaches to the *in situ* measurement of anaerobic processes in subsurface environments are required.

Acetate is expected to be an important intermediate in the metabolism of complex organic matter in anoxic subsurface environments [8] and thus rates of acetate metabolism are likely to approximate rates of anaerobic respiration. Furthermore, both acetate and simple organic compounds, such as lactate and ethanol that are fermented to acetate, are frequently added to groundwater to stimulate anaerobic respiration during bioremediation of metals and chlorinated solvents [9-12]. Some microorganisms, most notably members of the family *Geobacteraceae*, can oxidize acetate with electron transfer to electrodes, producing current [13-15]. In natural systems, *Geobacteraceae* colonize graphite electrodes buried in anoxic sediments, producing current from the oxidation of acetate and products released from the fermentation of organic matter [13, 16]. Current levels are expected to be proportional to the rate of acetate production up to the point where additional increases in acetate concentration do not yield additional increases in current production due to limitations in the rate of electron transfer at both anode and cathode [17, 18].

These considerations suggest that the rate of current production from graphite electrodes in the subsurface might serve as a proxy for monitoring rates of microbial activity. This possibility was suggested on the basis of the linear dependence between current and acetate concentration (when ≤2.3 mM) observed during column experiments

using *Geobacter sulfurreducens* [17]. These results corroborated those obtained using more conventional microbial fuel cells in pure culture laboratory studies [14], which show a similar dependence between acetate availability and current production.

There are many unknowns to the functioning of an electrode deployed in the subsurface that are dependent upon the activity of natural microbial consortia. For example, studies with laboratory and benthic microbial fuel cells have emphasized the importance of close proximity between anode and cathode to promote optimal proton transfer and maximum current output [19], suggesting that substantial distances between subsurface anodes and air-coupled cathodes may not allow for significant current production. Furthermore, the ability of aquifer microorganisms to colonize electrodes and produce current has not previously been evaluated. Finally, the current production capacity of subsurface microorganisms varies significantly, even among *Geobacter* species [15], and potentially confounding current production from reduced metabolic end products, such as Fe(II) and sulfide, interacting with electrodes must also be assessed.

This last concern is largely alleviated during remediation scenarios where organic carbon amendments are designed to stimulate subsurface microbial activity. Such is the case at Department of Energy study sites near Rifle, Colorado and Oak Ridge, Tennessee where organic carbon additions to groundwater have repeatedly demonstrated the ability to remove uranium from groundwater by stimulating the activity of metal reducing microorganisms, such as *Geobacter* [9, 11, 12, 20]. Here we report on current production using subsurface electrodes during *in situ* bioremediation at the Rifle site and demonstrate a clear correspondence between current levels and both the availability of acetate and the removal of uranium from groundwater. These results serve as the first

validation that such an approach may be employed as part of field bioremediation activities capable of providing real time, semi-quantitative information relevant to optimized uranium bioremediation.

Materials and Methods

Site Description

A comprehensive description of the Rifle site has been presented elsewhere [12, 21]. Briefly, the site is located on a flood plain in Northwestern Colorado (USA) consisting of an aquifer comprised of approximately 6.5 m of unconsolidated sands, silts, clays and gravels deposited by the adjacent Colorado River. Elevated concentrations of uranium (1-1.5 μM) in groundwater result from residual contamination by mill tailings.

Groundwater amendment, sampling, and transport properties

Acetate amendment experiments were conducted over consecutive summers within the same experimental gallery (Fig. S1). In both cases, upgradient groundwater was pumped into a storage tank and amended with sodium acetate and bromide to achieve aquifer concentrations of 5 mM and 1.5 mM, respectively. Injection occurred within ten boreholes and lasted 31 and 110 days during 2007 and 2008, respectively. Amended groundwater was continuously injected at a rate of 16 L per injection well per day. Both experiments involved short-duration intervals (6-8 days) bracketing the first and second amendment tanks where neither acetate nor bromide was injected referred to as a groundwater flush.

Groundwater samples were obtained from monitoring wells up and downgradient from the injection galleries (Fig. S1). Acetate, bromide, and sulfate were measured using an ion chromatograph (ICS-1000, Dionex) equipped with an AS-22 column. Fe(II) and

dissolved sulfide were filtered (0.2 μm) and measured immediately using the 1,10 Phenanthroline and Methylene Blue colorimetric methods, respectively (Hach Company, Loveland, CO). Dissolved uranium was quantified using kinetic phosphorescence analysis (Chemchek Instruments, Richland, WA).

An analytical solution of the convection dispersion equation (CDE) was fit to the bromide breakthrough curves observed at D03 and D09 during the 2007 experiment using established approaches [22]; the two injection peaks resulting from the groundwater flush were combined in a single fitting procedure using superposition. Injection tank bromide concentrations, injection volumetric flux, groundwater levels, and estimates of hydraulic conductivity were used to estimate bromide concentrations in the vicinity of the injection gallery during the first and second peak, after which the final CDE fit was obtained by including the exact timing and concentration ratio between first and second peak.

Sensor design, deployment, and current monitoring

Anodic electrodes were installed within boreholes (5 cm; inner diameter), while cathodes consisted of air-coupled electrodes embedded at the ground surface (Fig. 1). All electrodes were composed of graphite cylinders (3 cm diameter), with baffles machined into the base of each to increase surface area (220 cm^2). All connections were made with watertight connectors using marine-grade wire screwed into holes drilled into the graphite and sealed with silver and marine epoxy. The borehole and surface electrodes were connected using 560 Ω resistors, and charge balance and completion of the circuit occurred through the sediments and pore fluid. Current was calculated at 30-minute intervals using the voltage drop across the resistor and measured with a high impedance voltmeter.

Electrodes were deployed within the same upgradient (U03) and downgradient monitoring boreholes (D03 and D09) during the consecutive field experiments (Fig. S1). The 2007 experiment utilized electrodes deployed at a single depth located 5 m below ground surface (bgs). The 2008 experiment utilized electrodes deployed at depths of 4, 5, and 6 m bgs in D03 and D09; the control electrode in U03 was located at 5 m bgs. The two monitoring wells were located 2.5 and 8.5 m, respectively, downgradient from the region of acetate injection.

To maintain integrity and expedite colonization, electrodes were placed within permeable housings (Fig. 1) and surrounded by site sediments; nylon mesh was used to retain sediments within the housing. Sediment porosity (30%) yielded a graphite surface area exposed to the pore fluids of 66 cm^2. Prior to acetate amendment, the contact resistance between anode and cathode ranged from 1-2 kΩ. Electrodes were removed from D09 for confocal laser scanning microscopy and microbial community analysis during both experiments. In 2007, the single electrode was removed after 29 days; in 2008, two electrodes were removed 261 days after beginning acetate injection.

Confocal laser scanning microscopy and 16S rRNA microbial community analysis

The spatial extent and thickness of biofilms associated with the borehole electrodes was examined with confocal laser scanning microscopy. After recovery from the boreholes, electrodes were placed at 4°C and shipped by overnight courier for analysis. Prior to analysis, loosely bound material was removed by gentle washing with isotonic buffer [23]. Duplicate biofilm samples were fluorescently stained with either the LIVE/DEAD BacLight bacterial viability kit (L7012, Molecular Probes, Inc., Eugene, OR) or a generic nucleic acid binding stain (Syto9; Molecular Probes) and examined as

described [15]. The image stacks were representative of the entire electrode as each image was obtained from twenty random locations across the surface.

Biofilm samples for microbial community analysis were frozen on dry ice and shipped by overnight courier for analysis. They were rinsed with sterile isotonic buffer prior to analysis to remove sediments. A sterile razor blade was used to remove a mixture of biomass plus graphite before extraction of DNA from the sample, PCR amplification, and cloning of the 16S rRNA gene as described [24]. Assembly of the clone library was derived from a total of 48 clones, and at least half the electrode surface was used for the analysis.

Results and Discussion

Current Production Associated with Acetate Amendment

An electrode deployed in well upgradient from the region of acetate injection (U03) produced continuous, low levels of current (Fig. 2). In contrast, electrodes deployed in downgradient wells D03 and D09 produced significant amounts of current in the presence of elevated acetate concentrations. The maximum current densities of ca. 40 mA/m^2 produced by the D03 and D09 electrodes were higher than the ca. 10 mA/m^2 and 25 mA/m^2 produced in freshwater [24] and marine [16] sediment microbial fuel cells (MFC's), respectively, and somewhat lower than laboratory-scale *Geobacter sulfurreducens* MFC's fed equivalent concentrations of acetate [14].

The initial increase in current from the D03 and D09 electrodes corresponded with the initial appearance of acetate (Fig. 2). This was particularly evident for D03, which had higher acetate concentrations. Bromide breakthrough data indicated that upgradient consumption was the primary cause of diminished acetate delivery to D09

(Fig. 3). Maximum current densities in D03 and D09 were comparable even though acetate concentrations were significantly higher in D03 at peak current. This suggests that at high acetate concentrations factors other than acetate availability may limit current production, as previously observed in laboratory incubations [15, 17, 25, 26]; the inefficient reduction of molecular oxygen at the cathode was another likely contributing factor [18].

Although the D09 electrode was removed for microbial analysis, the D03 electrode was retained for longer-term analysis of current production. During the groundwater flush, acetate concentrations rapidly fell with a corresponding drop in current (Fig. 2). Current rebounded when acetate was reintroduced, reaching a maximum current density comparable to that observed prior to the flush. This was despite the fact that acetate did not rebound to pre-flush levels, further emphasizing the observation that factors other than acetate availability likely limit maximum current output. When amendment ceased and acetate dropped below detectable limits, current densities declined to levels slightly higher than those observed at the upgradient control.

Additional insight into the temporal current patterns was gained from analysis of the CDE fits, which exhibited good (D03) to fair (D09) correspondence with breakthrough of bromide (Fig. 3). The fits suggest that conservative transport (as bromide) and current production are not related in a simple linear fashion. Such a relationship would not be expected, however, as current production may not be treated in an identical fashion to bromide breakthrough, given that it results from microbial activity stimulated by transport of a non-conservative species (acetate) to the electrode. In comparing the D03 electrode response to the CDE fit (Fig. 3C), a clear lag (4-5 days)

existed between delivery of amendments during the first peak and onset of current flow. This lag – also observed at D09 – likely corresponded to the time required for colonization of the electrode and onset of electron transfer tied to acetate oxidation. In contrast, current production associated with the second injection peak began with no discernible lag and began before arrival of maximum amendment concentrations (Fig. 3C). Such an effect would be expected given the existence of an affixed and pre-conditioned microbial community capable of immediate current production following resumption of acetate delivery.

The temporal change in current density in response to changes in acetate concentration was remarkably similar to previously described changes in the metabolic activity of *Geobacter* species during a previous field experiment at the Rifle site [27]. In that study, monitoring transcript levels of the *Geobacter*-specific citrate synthase gene (*gltA*) indicated rates of metabolism that (a) increased sharply when acetate was introduced into the system, (b) decreased when acetate additions were temporarily disrupted, (c) increased again when acetate additions were resumed, and (d) declined when acetate inputs were stopped.

One concern in using current as a proxy for microbial activity is the potential for abiotic current production from reduced species, such as Fe(II) and dissolved sulfide. Although sulfide was not detected during the 2007 field experiment, aqueous Fe(II) did accumulate during acetate amendment (Fig. 2). In contrast to the clear relationship between acetate availability and current, there was no clear relationship between Fe(II) and current. For example, the dip in current from day 19-22 was associated with the greatest increase in Fe(II), and high concentrations of Fe(II) from day 45 onward

corresponded to periods of low current density. In contrast to Fe(II), there was a strong relationship between current levels and the removal of U(VI) from the groundwater (Fig. 2). As current densities reached high levels, U(VI) declined substantially. When current declined, U(VI) concentrations rebounded to influent levels.

The field-derived relationships between Fe(II) and current production were corroborated by laboratory experiments in which inoculated (*Geobacter sulfurreducens*) and uninoculated fuel cells were poised at +300 mV (vs. Ag/AgCl) and supplemented with 5 mM $FeCl_2$. Whereas acetate addition to the inoculated fuel cell generated a steady-state current of 12 mA, no additional increase in current was detected following Fe(II) addition (data not shown). Similarly, no increase in current was observed after Fe(II) addition to the uninoculated control. These results suggest that elevated concentrations of Fe(II) are insufficient to enable significant current production above background levels. Rather, current production during microbial Fe(III) reduction appears to result primarily from electron transfer to electrodes by electrode-respiring microorganisms suggesting sensitivity of the method to microbial activity rather than accumulation of a reduced by-product.

Several possibilities for the apparent lack of Fe(II)-mediated current production exist. Graphite fuel cells optimized for the abiotic oxidation of Fe(II) have demonstrated that large current densities are achievable at high ionic strength and low pH (2-4 M H_2SO_4) [28]. In contrast, the circumneutral pH and low ionic strength conditions of the laboratory and field experiments may have minimized rates of Fe(II) oxidation. Indeed, rates of electron transfer for Fe(II)/Fe(III) couples on glassy carbon surfaces decrease by 10-100 fold with decreases in ionic strength from 1 to 0.01 M [29], with the latter value

characteristic of our studies. Just as an enzymatic catalyst enables charge transfer during acetate amendment, the presence of a similar catalyst – or catalytic effect, such as electrode pretreatment – may be required to enable high rates of Fe(II)-mediated electron transfer. Even under ideal conditions using polished and/or hydrogenated glassy carbon electrodes, measured electron transfer rate constants for different iron compounds vary dramatically, with rate constants for compounds relevant to our field and laboratory experiments (e.g. $Fe^{2+/3+}$) three orders of magnitude lower than those of more reactive iron couples (e.g. $Fe(CN)_6^{3-/4-}$) [29]. The unpolished and untreated graphite electrodes used here would thus be expected to only marginally enable the Fe(II) oxidation process. Lastly, adsorption of non-catalytic, passivating organics to the electrodes could have further degraded their capacity for mediating significant rates of Fe(II) oxidation.

Geobacter species were the most abundant subsurface microorganisms during the active phase of U(VI) removal with *Geobacter* 16S rRNA gene sequences accounting for 76-98% of the sequences recovered in groundwater samples [21]. This finding is consistent with the concept that *Geobacter* species are responsible for acetate-stimulated U(VI) reduction at the Rifle site and the finding that *Geobacter* species are consistently the most abundant microorganisms in groundwater during such field studies [9, 12, 27, 30, 31]. Analysis of the 16S rRNA gene sequences extracted from the D09 electrode - harvested as current density peaked and U(VI) removal commenced - revealed a predominance of *Geobacter* species (Fig. 4), which accounted for 67% of the sequences recovered. The more minor sequences were distributed in the gamma (16%), beta (12%), and alpha (2%) Proteobacteria and Firmicutes (2%). The *Geobacter* sequences on the electrode were most similar (96% similarity) to those of *Geobacter* strain M18, which

was recovered in culture from the Rifle site and has a 16S rRNA gene sequence matching one that predominates in the groundwater during uranium bioremediation. These results suggest that the *Geobacter* species colonizing the electrode were similar to those involved in U(VI) reduction. If so, the physiological responses of *Geobacter* species predominating on the electrode may be similar to those of *Geobacter* species primarily responsible for U(VI) reduction.

Confocal laser scanning microscopy revealed that the biofilms were 25-50 μm thick (Fig. 5). Based on background fluorescence, the stained images were not significantly influenced by the presence of fluorescent minerals (Fig. S2). The biofilms were morphologically similar to those formed by *G. sulfurreducens* on graphite surfaces in laboratory studies [15, 32]. When treated with the LIVE/DEAD BacLight viability kit, 30-40% of the cells in the biofilm stained green, suggesting viability (Fig. 5A). It has been proposed, however, that some cells staining red with this kit may also be viable [33], suggesting that a higher proportion of cells were capable of contributing to current production. The dense accumulation of cells observed using the general nucleic acid binding stain (Fig. 5B) suggests this is a possibility. Previous studies with *G. sulfurreducens* have suggested that cells at distances up to 75 μm from the electrode surface may contribute to current production, possibly via electron transfer through conductive filaments [32, 34], outer-surface *c*-type cytochromes and/or multicopper proteins [24, 31, 35], or a combination of mechanisms [36].

Current Production Following Long-Term Acetate Amendment

Electrode-based monitoring during acetate amendment was repeated in 2008 and a similar correspondence between groundwater acetate concentrations and current was

observed (data not shown). Unfortunately, changes in injection approach and decreases in aquifer permeability made continuous and uniform delivery of acetate problematic, thus confounding reliable interpretation of current patterns during acetate amendment.

The 2008 experiment did provide the opportunity to evaluate long-term current production following prolonged organic input. After acetate concentrations fell to levels below detection, the D03 and D09 electrodes yielded low but steady current densities of 3-6 mA/m^2 for 100+ days (data not shown). These lower values suggest a slower rate of microbial metabolism during this period than was observed during periods of high acetate concentration, consistent with the lower availability of acetate. However, current densities were significantly higher than the 0.05-0.2 mA/m^2 values observed in the upgradient control well not exposed to acetate. This is consistent with the concept that following prolonged periods of acetate addition there are substantial quantities of moribund biomass that can promote prolonged microbial metabolism [20].

Two electrodes deployed in D09 were removed after 261 days of current production. The maximum biofilm thickness was 5-10 µm and ca. 10% of the cells in the biofilm stained green with the viability stain (Fig. S3). This thinner biofilm was consistent with the lower current output and the general correspondence expected between biofilm thickness and current production [32]. As with the 2007 electrode, *Geobacter* species predominated, accounting for more than 80% of the sequences (Fig. S4). The predominant *Geobacter* species were most closely related to the subsurface isolates *G. psychrophilus* and *G. chapellei*, which were isolated from an acetate-impacted aquifer [37] and a deep subsurface site [38], respectively. This contrasts with the predominance of *Geobacter* species most closely related to strain M18 on the 2007

electrode suggesting growth of different *Geobacter* species in the subsurface may be favored under different environmental conditions.

Implications for future monitoring studies.

These results demonstrate that electrodes deployed in anoxic subsurface environments can serve as electron acceptors for microbial metabolism even when anode-cathode separations are large. This is the requisite first step for ultimately using electrode-based approaches for quantifying rates of microbial metabolism in the subsurface. In the interim, monitoring temporal changes in current appears to be a useful tool for ensuring appropriate concentrations of electron donor are being supplied to a desired location, such as occurs during the remediation of chlorinated solvents, perchlorate, and other metal and radionuclide contaminants that rely on electron donor amendments to stimulate desired metabolic processes.

Now that is it is known that sufficient current can be produced from subsurface electrodes to readily detect microbial metabolism *in situ* - at least at the elevated levels associated with the introduction of allochthonous organic compounds - this raises the possibility of monitoring rates of microbial metabolism in aquifers contaminated with organics such as petroleum hydrocarbons or landfill leachate. These are contaminants that *Geobacter* species and other organisms can oxidize with the reduction of Fe(III), and hence their oxidation coupled to electrodes might also be expected [13]. To further expand the applicability of the method, studies are warranted at sites having different lithological properties (e.g. low permeability silts and fractured rock) and where bioremediation activities targeting other metals and radionuclides (e.g. chromium and

technetium) via stimulation of metal-reducing microorganisms are underway (e.g. Hanford, Washington and Oak Ridge, Tennessee).

The predominance of *Geobacter* species on the electrodes, coupled with the known ability of *Geobacter* species to effectively couple the oxidation of organic compounds to electron transfer to electrodes, suggests that *Geobacter* species played an important role in the current production at the Rifle site. The current production patterns in response to changes in acetate availability were similar to previously documented responses in metabolic rates of *Geobacter* species at the Rifle site during similar fluctuations in acetate concentration [27, 31]. Furthermore, high currents were associated with the rapid removal of U(VI) from groundwater, an activity previously attributed to *Geobacter* species reducing soluble U(VI) to insoluble U(IV) [12].

Additionally, colonization of subsurface electrodes at the Rifle site by *Geobacter* species was similar to the abundance of *Geobacter* species found on the anodes of sediment microbial fuel cells [13, 16, 24], as well as the colonization of anodes by *Geobacter* species in laboratory systems capable of producing high current densities when inoculated with sewage and amended with acetate [39-41]. However, a wide diversity of microorganisms are capable of electron transfer to electrodes via a variety of mechanisms [36], and it remains quite possible that in other subsurface environments microorganisms other than *Geobacter* species could be the primary current producers.

Although current production from subsurface electrodes may eventually be used to quantify rates of subsurface microbial metabolism, it cannot unambiguously discern the terminal electron accepting process associated with those rates. By coupling hydrogen measurements - which can identify the terminal electron accepting process

associated with current measurements - the possibility exists of gaining a more complete understanding of subsurface anaerobic microbial processes than is presently available with other approaches.

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Supporting Information. This document contains supporting information consisting of four figures intended to supplement the material presented in the manuscript. These include the electrode layout, as well as additional confocal laser scanning microscopy and microbial community analysis results.

Brief

We describe a novel electrode-based technique for monitoring the *in situ* activity of microorganisms in anoxic subsurface environments.

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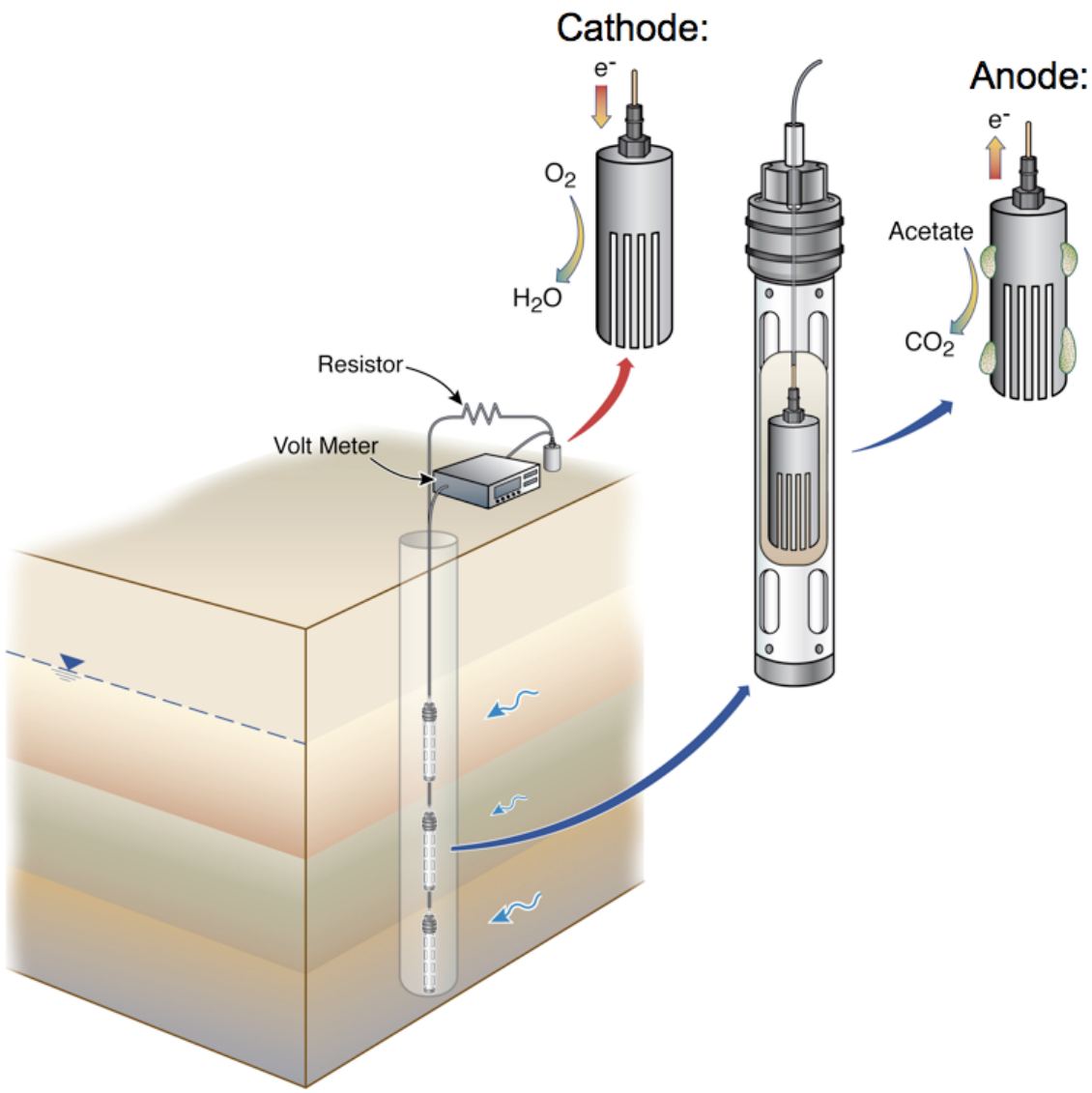

Figure 1. Illustration of the sensor design for evaluating subsurface microbial activity via current production from subsurface electrodes. Anodes consist of graphite electrodes embedded within a sand pack (encapsulating mesh not shown) located within the aquifer, while air-coupled cathodes are embedded in the soil.

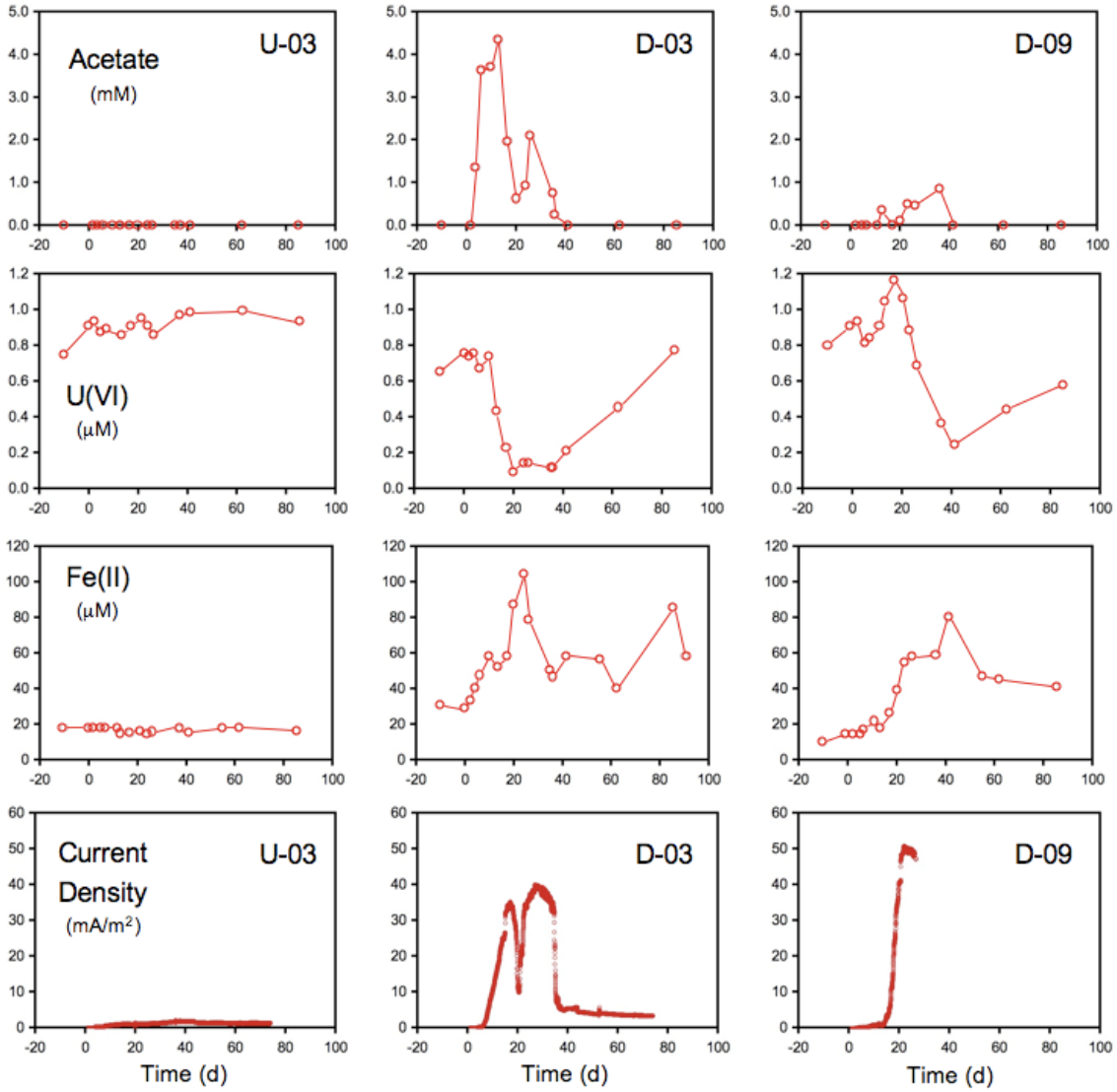

Figure 2. Temporal changes in acetate, uranium, Fe(II), and current density at wells U03, D03, and D09 during the 2007 experiment. The D09 electrode was removed for microbial analysis on day 29; the 6-day acetate-free groundwater flush started on day 12.

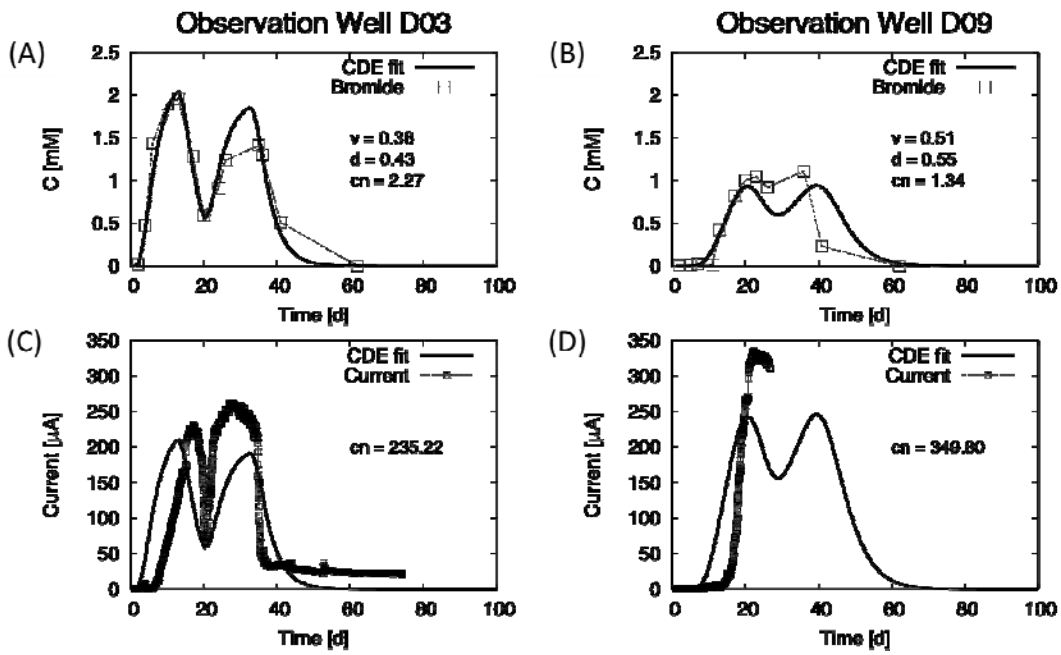

Figure 3.: Comparison of conservative transport behavior and current production for D03 and D09: (A) and (B) measured bromide concentrations and fitted convection dispersion equation (CDE) including estimates from the fitting procedure (v=velocity in m/d, d=dispersivity in m and cn=bromide concentration at the injection in mM). (C) and (D) current production versus the CDE fits.

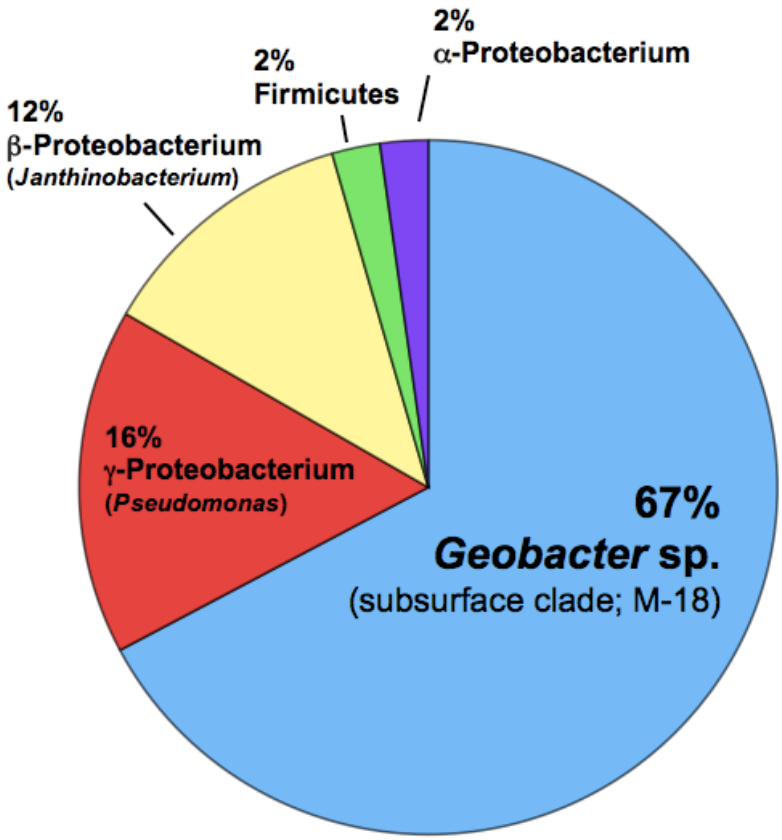

Figure 4. Microbial community composition based on 16S rRNA gene sequences extracted from the D09 electrode on day 29 during the 2007 experiment; *Geobacter* clones were 96% similar to clade M18.

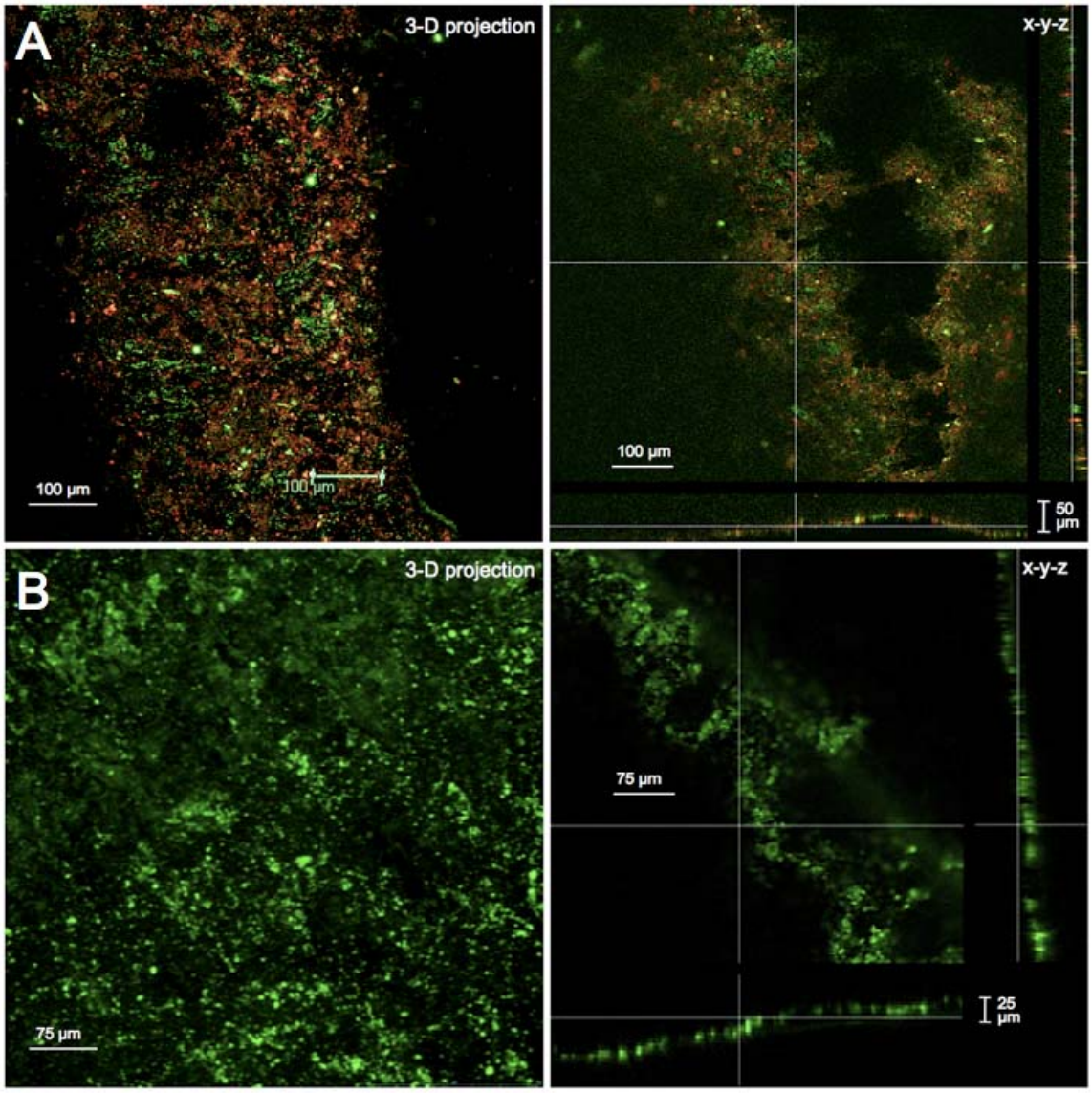

Figure 5. Confocal laser scanning microscopy (3-D projections and cross sections) of the D09 (5 m bgs) electrode recovered during peak current flow (day 29) in 2007. (A) Results using the LIVE/DEAD BacLight viability kit, with putative viable cells exhibiting green fluorescence and putative moribund cells staining red. (B) Results using the Syto9 general nucleic acid binding stain.