Final report for: Electrochemical sensors for microbial activities in benthic sediments: A sentry for lacustrine P biogeochemistry

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Problem and research objectives

Our goal is to develop a sensor system to detect microbiological activities at a variety of scales. We proposed that zero-resistance ammetry (ZRA) measurements could be used to detect microbiological Fe(III) reduction in aquatic sediments. In ZRA, we measure electric current between sediments at different depths where two different types of microbial respiration are occurring. Specifically, current that develops between oxic (O_2-containing) sediments, where aerobic respiration is occurring, and sediments where Fe(III) reduction is occurring might be used to identify that process. A benefit of the ZRA approach is that it would be a relatively inexpensive and low-power approach to "mapping" microbiological processes at a variety of scales in aquatic sediments. We would eventually like to use this approach to predict harmful algal blooms (HABs) resulting from internal phosphate loading, because microbial Fe(III) reduction dissolved Fe(III) (hydr)oxide minerals, which would otherwise trap phosphate. In other words, electrical current measurements using ZRA sensors positioned in lake sediments could serve as an early warning system for HABs resulting from internal phosphate loading. We envisioned two scenarios in which phosphate could be released from sediments:

1) Reductive dissolution of Fe(III) (hydr)oxide phase to which phosphate had been previously adsorbed
2) Desorption of phosphate from non-redox-active phases by activities of microorganisms (e.g. pH change or production of ligands)

Preliminary work with sediments used for these experiments revealed high concentrations of background phosphate, so in addition to the goals of examining the role of microbial metabolism in phosphate release and the electrochemical signatures of those activities, we needed to determine how much phosphate would adsorb to the phases that we added to experiments. Therefore, the three objectives of our work were:

1) Determine the adsorptive characteristics of sediments used for the microbial activity experiments
2) Determine electrochemical signatures of microbiological activities in sediments
3) Connect electrochemical signatures and microbiological activities to controls on phosphate solubility

Principal findings and results for each objective

We set up experiments where we mimicked different processes in lake sediments by placing sediments from Old Woman Creek estuary on Lake Erie in separate chambers with a synthetic Lake Erie water and Fe(III) minerals. These experiments included sediments from Old Woman Creek Nature Preserve (OWC) and synthetic Lake Erie water (SLEW; 1 mM $CaCO_3$, 0.3 mM Na_2SO_4, 0.04 mM $MgCl_2$, 0.04 mM KCl, and 0.06 mM $NaNO_3$) in ratios of 1 OWC sediment:10 SLEW.

Objective 1 - Determine the adsorptive characteristics of sediments used for the microbial activity experiments

To determine the adsorptive characteristics of background phosphate in OWC sediments, we incubated them in SLEW with varying concentrations of Fe(III) (hydr)oxide and Al (hydr)oxide (Figure 1). Dissolved phosphate concentrations in (hydr)oxide-free incubations were approximately 6 mM (Figure 1). When Fe(III) (hydr)oxides were added, maximal phosphate removal from solution was achieved when 50 mmol/L Fe(III) was added (Figure 1). When Al (hydr)oxides were added, maximal phosphate removal from solution was achieved when 20 mmol/L Al was added (Figure 1). Therefore, 50 mmol/L and 20 mmol/L Fe(III) (hydr)oxide and Al (hydr)oxide were used for all future experiments.

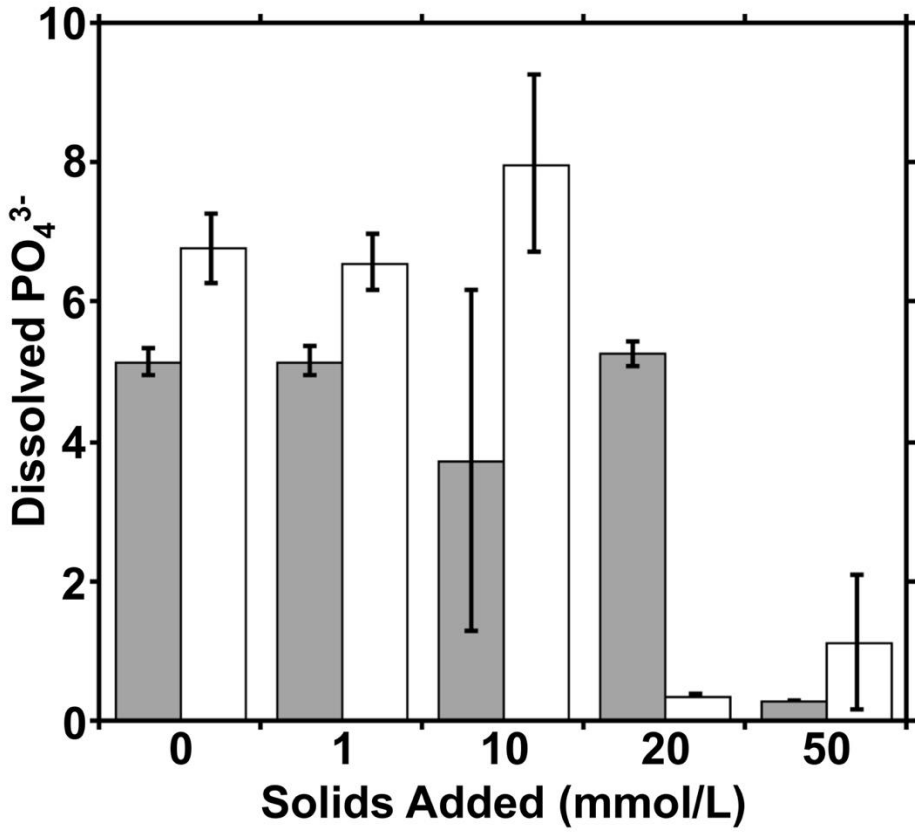

Figure 1. Dissolved phosphate concentrations in OWC sediment suspensions in SLEW amended with solids [$Fe(OH)_3$ (gray columns) or $Al(OH)_3$ (white columns]. Error bars represent standard deviation of triplicate incubations.

Objective 2 - Determine electrochemical signatures of microbiological activities in sediments

To determine electrochemical signatures of microbiological activities, split chamber zero resistance ammetry (SC-ZRA) measurements were made under varying conditions. SC-ZRA incubations entailed two glass chambers containing OWC sediment suspended in sterile SLEW. Where appropriate, sterile control incubations were deactivated by autoclaving before final assembly. The chambers were connected with a cation exchange membrane that was primed in sterile 5% NaCl solution at 40°C for 24 hours prior to use. Sterilized graphite mesh working electrodes (referred to as WE1 and WE2). A reference electrode was placed in the WE1-containing chamber to determine electrochemical potential in that chamber.

A set of control SC-ZRA incubations were done to determine background potentials and whether abiotic processes would yield current. When sediments were deactivated by autoclaving and incubated under oxic conditions in WE1 and anoxic conditions in WE2, no current was observed and the potential stabilized at approximately 0.1 V (Figure 1). Nearly identical patterns were observed when inactive sediments were amended with Fe(III) (hydr)oxide (Figure 1). These results indicate that redox disequilibrium alone is insufficient to induce electrical current between WE1 and WE2. Similarly, when both chambers were incubated under oxic conditions, no current was detected, and the potential in the WE1 chamber was similar to that of the inactive incubations (Figure 1). These results indicate that if chambers are in redox equilibrium, no current will be detected, even if microorganisms are active.

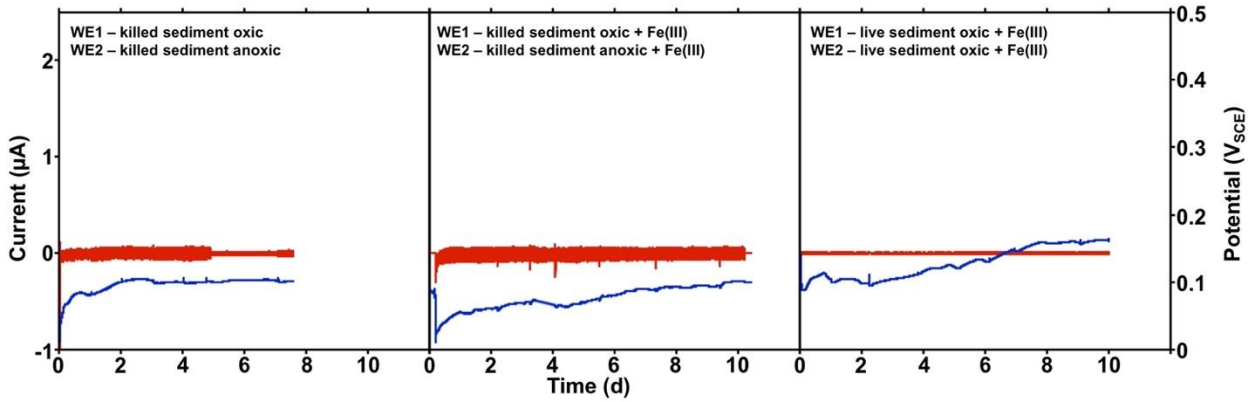

Figure 2. Current (red) and potential (blue) of ZRA incubations containing (left) deactivated OWC sediments under oxic (WE1) and anoxic conditions (WE2), (middle) deactivated and Fe(OH)3-amended OWC sediments under oxic (WE1) and anoxic (WE2) conditions, and (right) non-sterile and Fe(OH)3-amended OWC sediments under oxic conditions in WE1 and WE2 chambers.

Potential and current patterns in non-sterile incubations with the WE1 chamber oxic and the WE2 chamber anoxic differed from those of the control incubations (Figures 2 and 3). Under these conditions, potential in the oxic chamber increased after incubation startup (Figure 3A). At the same time, a positive current developed, indicating electron transfer from the oxic WE1 chamber to the anoxic WE2 chamber (figure 3A). During this period of positive current,

dissolved Fe(II) increased in the WE2 chamber, but not the WE1 chamber, indicating that Fe(III) (hydr)oxide reduction was occurring in the anoxic WE2 chamber, but was inhibited by the presence of O_2 in the WE1 chambrer. Concurrent with Fe(III) reduction, release of phosphate into solution was observed in the WE2 chamber, but not in the WE1 chamber. These results indicate that the development of current from anoxic (and Fe(III) reducing in these cases) sediments can indicate contrasting microbiological processes under differing redox regimes. Additionally, in the cases of these activities, phosphate release resulted from Fe(III) reduction. After ten days of sediment SC-ZRA incubations with Al (hydr)oxide rather than Fe(III) (hydr)oxide, current was negative (not shown), indicating transfer of electrons from the anoxic WE2 chamber to the oxic WE1 chamber. These observations indicate that Fe(III) reduction may give rise to a unique electrochemical signature.

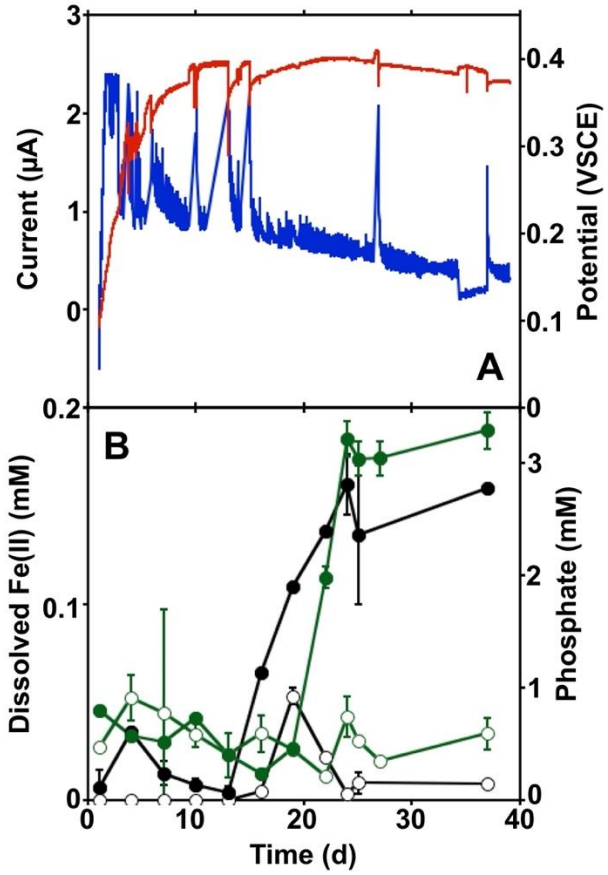

Figure 3. Current (red) and potential (blue) (panel A) and dissolved Fe(II) (black) and phosphate (green) concentrations (panel B) of ZRA incubations containing OWC sediments suspended in SLEW amended with Fe(OH)3. Dissolved Fe(II) amd phosphate concentrations in oxic WE1 and anoxic WE2 are represented by open and closed circles, respectively. Error bars in panel B represent standard deviations of duplicate measurements.

Objective 3 - Connect electrochemical signatures and microbiological activities to controls on phosphate solubility

To differentiate between biologically induced and abiotic processes controlling phosphate solubility, a series of batch incubations were set up, where deactivated or non-sterile OWC

sediments were incubated with SLEW under oxic or anoxic conditions, and without addition of solids or with the addition of Fe(III) (hydr)oxide or Al (hydr)oxide.

In batch incubations without added solids, no increase in total Fe(II) could be detected, nor did the phosphate concentration change, regardless of whether sediments were microbiologically active or inactive, and whether they were incubated under oxic or anoxic conditions (Figure 4D, and G). Similarly, little change in pH was observed in the incubations (Figure 4A). These results indicate that microbiological activities were playing little role in modulating the solubility of phosphate on the absence of added solids that would otherwise serve as adsorbents for phosphate. Little change in pH was observed in Fe(III) (hydr)oxide-amended incubations (Figure 4B). Similar to the SC-ZRA incubations, in batch incubations, Fe(II) accumulated under anoxic conditions, but not under oxic conditions (Figure H). Phosphate accumulated concurrently with Fe(III) reduction, but decreased after reaching a maximum concentration of approximately 1. 5 mM (Figuree 4E). This is likely due to precipitation of newly solubilized phosphate with biogenic Fe(II) (e.g. vivianite). These results indicate that the Fe(III) reducing (and associated phosphate solubilizing) activities observed in SC-ZRA incubations are attributable to microbiological activities. In batch incubations that were amended with non-redox-active Al (hydr)oxide, patterns of pH were similar to those observed in unamended and Fe(III) (hydr)oxide amended incubations (Figure 4C). No evidence of Fe(III) reduction was observed (Figure 4I), but phosphate solubilization occurred after approximately 22 d incubation in both oxic and anoxic incubations, where more phosphate was solubilized under oxic conditions than anoxic conditions (Figure 4F). We hypothesize that this example of phosphate solubilization is attributable to ligand production by microorganisms.

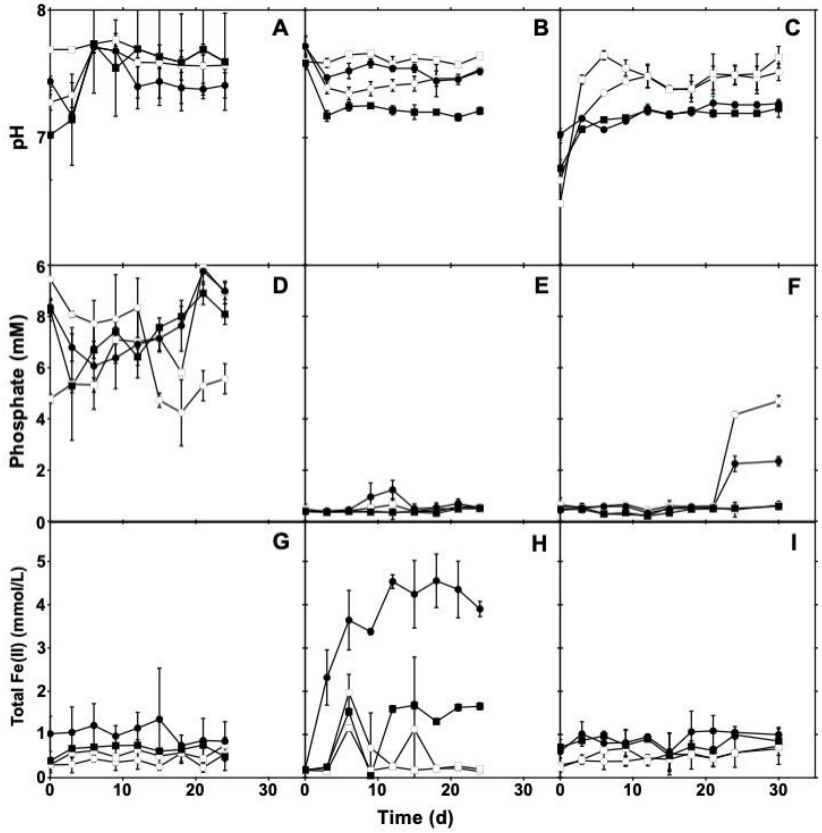

Figure 4. pH (A-C), dissolved phosphate concentration (D-F), and total Fe(II) concentration (G-I) in incubations with OWC sediments and SLEW incubated under oxic (open shapes) and

anoxic conditions (closed shapes). Values of non-sterile incubations are indicated with circles, while heat-deactivated incubations are indicated with squares. Incubations that were not amended with solids are shown in panels A, D, and G; incubations amended with 50 mmol/L $Fe(OH)_3$ are shown in panels B, E, and H; incubations amended with 20 mmol/L $Al(OH)_3$ are shown in panels C, F, and I. Error bars represent one standard deviation of triplicate incubations.

To determine how incubation under oxic or anoxic conditions in SC-ZRA incubations influence microbial community composition, we examined partial 16S rRNA gene sequence libraries from the incubations shown in Figure 3. The most notable difference between the oxic and anoxic side of the SC-ZRA microbial communities was the abundance of Bacillariophyta and Cyanobacteria in the oxic side of the incubations (Figure 5). These are photosynthetic organisms that were likely using ambient light in the laboratory, and production of O_2 by these organisms may have induced the higher potential in these incubations (Figure 3). In the anoxic side of the SC-ZRA, the the most abundant phylotypes were attributable to the Betaproteobacteria and Deltaproteobacteria (Figure 5), both lineages contain taxa capable of Fe(III) reduction. Indeed, the most abundant genera detected in these incubations were *Geobacter* spp. and *Dechloromonas* spp., which are Fe(III) reducing organisms affiliated with the Deltaproteobacteria and Betaproteobacteria, respectively. We also examined microbial communities attached to WE1 and WE2. The communities associated with these electrodes were nearly identical (at the phylum level) to those in the sediment slurries. These results indicate that the microbial communities associated with the electrodes are indicative of the broader microbial community, and the electrochemical signatures that we detected reflect the microbial activities of the sediments (not just the community associated with the electrode).

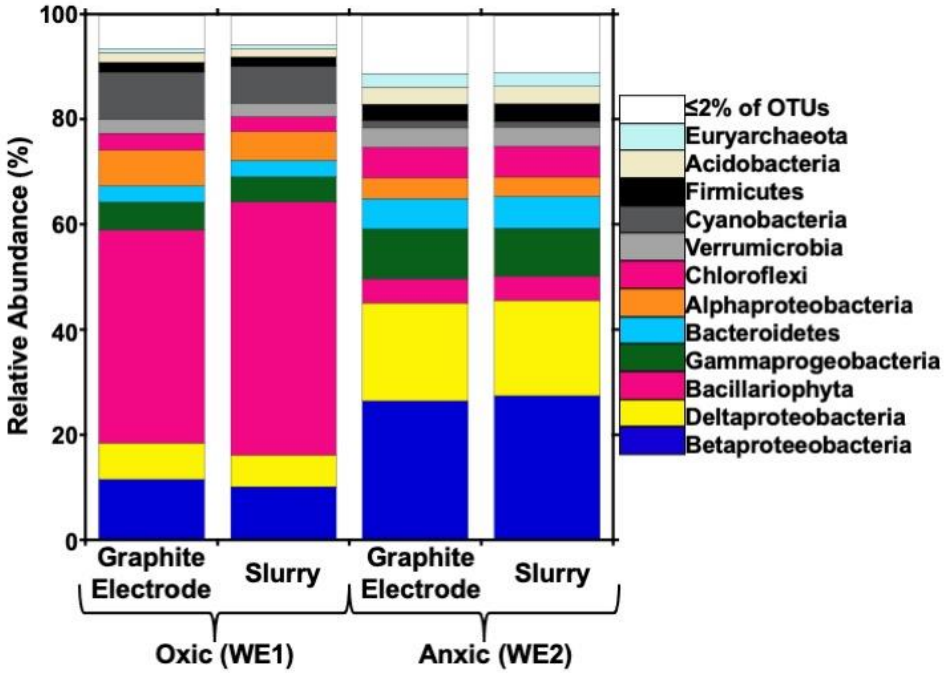

Figure 5. Relative abundances of taxonomic groups (based on 16S rRNA gene sequencing) ZRA electrodes and OWC sediment-SLEW slurries in the oxic and anoxic sides of SC-ZRA incubations amended with 50 mmol/L $Fe(OH)_3$.

Significance

In this work, we examined the dynamics of phosphate adsorption and desorption from redox-active and -inactive (hydr)oxide solid phases. When phosphate was adsorbed to Fe(III) (hydr)oxide, a solid phase susceptible to microbiological redox transformation, it was released into solution by anaerobic Fe(III) reducing bacterial activities, but not when these activities were inhibited under oxic conditions. When phosphate was adsorbed to Al (hydr)oxide, which is not susceptible to microbiological redox transformations, phosphate was released into solution by microbiological activities under both oxic and anoxic conditions, likely the production of organic ligands. These results indicate differing mechanisms of phosphate desorption from (hydr)oxide solid phases. Notably, we were able to electrochemically detect the Fe(III) reducing microbiological activities using the SC-ZRA approach, where electrodes were deployed in sediments with contrasting terminal electron accepting regimes (oxic/aerobic and anoxic/Fe(III) reducing). These contrasting conditions gave rise to contrasting microbial communities. Additionally, comparison of current and voltage patterns between Fe(III) (hydr)oxide and Al (hydr)oxide amended SC-ZRA incubations indicates that Fe(III) reduction may give rise to a unique current and voltage pattern.

Taken together, our results indicate that ZRA-based approaches might be used to detect microbiological activities in sediments. One implication is that the use of ZRA sensors could predict phosphate solubilization in the sediments. Importantly, the microbial communities associated with the electrodes were similar to those of the bulk sediments, indicating that the electrochemical phenomena were not an artifact of the electrodes, but reflective of overall processes in the sediments. Another implication is that ZRA could be used to detect fine-scale contrasts in terminal electron accepting processes that might not be detected by bulk sediment analyses. We are now designing field-deployable ZRA sensors that can detect microbiological activities at a variety of scales (i.e. 0.1 mm – 10 cm). This is part of a US Department of Energy grant to examine scales of distributions of terminal electron accepting processes in OWC.