ESF Short Visit Grant – Final Report

Applicant: Dr. Denise M. Akob, Friedrich Schiller University Jena, Germany

Host: Dr. Martial Taillefert, Georgia Institute of Technology, Atlanta, GA USA

Title: Fe(III)-complexes and the iron cycle in slightly acidic environments

Report:

The purpose of my visit to the lab of Dr. Martial Taillefert was to build collaboration that can lead to a better understanding of iron cycling and Fe(II)-oxidizing bacteria (FeOB) in slightly acidic environments. We focused our work on aerobic FeOB cultivated from two circumneutral environments, a peatland and an acid mine drainage (AMD)-impacted creek, where FeOB have been shown to compete with chemical oxidation at pH 4.0-5.5. At the peatland site, FeOB can play an important role in the carbon cycle by providing biogenic Fe(III) as source for Fe(III)-reducing bacteria, thereby suppressing methanogenesis. At AMD sites, FeOB can affect the fate and transport of metal-contaminants by coprecipitating metals along with Fe(III)-oxide precipitates. In order to better understand iron cycling and FeOB in these environments, we need to determine next whether soluble Fe(III)-complexes, *i.e.*, colloidal or chelated Fe(III), play a role as intermediates, facilitate the oxidation of Fe(II), and affect the transport of Fe(III) from oxic to anoxic zones. The aims of my visit were to learn about voltammetric techniques for measuring aqueous geochemistry and to plan future collaborations to measure *in situ* Fe(II)/Fe(III) gradients and Fe(III)-complexes and to measure the effect of FeOB on metal contaminants.

During my visit, we used voltammetric methods to (1) assess the presence of soluble Fe(III)-complexes in biogenically formed Fe(III)-oxides from peatland FeOB cultures and (2) determine the soluble concentrations of toxic heavy metals (i.e., Cd) after growth of FeOB. The initial part of the lab work involved learning how to prepare and calibrate the voltammetric microelectrodes to quantify dissolved oxygen, Fe^{2+}, Mn^{2+}, and ΣH_2S. Simultaneously, these microelectrodes can be used to qualitatively detect organic-Fe(III) complexes, usually produced as intermediates in the oxidation of Fe^{2+} in the presence of dissolved organic compounds (Taillefert et al., 2000) or reduction of iron oxides by iron-reducing bacteria (Taillefert et al., 2007; Jones et al., 2010), as well as molecular clusters of FeS that have been shown to be intermediates in the precipitation of $FeS_{(s)}$ in sulfidic environments (Taillefert et al., 2000; Carey and Taillefert, 2005). Voltammetric microelectrodes consisted of a 100 μm diameter gold wire housed in 3 mm diameter PEEK

tubing or 30 cm long glass electrodes pulled to a tip of 5 cm long and 0.5 mm diameter and connected via a copper conducting wire to a potentiostat. Gold mercury (Au/Hg) microelectrodes were prepared by first polishing the 100 μm gold surface with diamond pastes of 15, 6, 1, and 0.25 μm, plating mercury at -0.1 V in a $Hg(NO_3)_2$ solution, and polarizing the electrodes at -9 V for 90 s to form a good amalgam between the Au and Hg. Finally, electrodes were tested for quality and calibrated for dissolved oxygen O_2 by linear sweep voltammetry, then Mn^{2+} by cathodic square wave voltammetry in degassed 0.02 M NaCl. Both the O_2 (MDL ≈ 4 μmol L^{-1}) and Mn^{2+} (MDL ≈ 15 μM) calibrations were run from -0.1 to -1.75 V with a scan rate of 200 mV s^{-1} in 0.02 M NaCl. A conditioning potential of -0.1 V for 10 s was applied to all measurements to clean the surface of the microelectrodes between measurements. The Mn^{2+} calibration curves were used to elucidate the concentrations of other species with the pilot ion method (Brendel and Luther, 1995). All measurements were performed with a computer-operated DLK-100A potentiostat (Analytical Instrument Systems, Inc.) connected to the Au/Hg microelectrode, a platinum counter electrode, and an Ag/AgCl reference electrode fabricated as described previously (Brendel and Luther, 1995). These microelectrodes were used to investigate the aqueous chemistry of biogenic Fe(III) oxides collected from FeOB gradient tubes.

Biogenic Fe(III) oxides were collected from three culture originating from the peatland site that had been amended or not with humic acids extracted from the peat (Table 1). Square wave voltammetry scans revealed a peak for soluble Fe(III) [~ -0.2 V], a suspected thiol peak [~ -0.6 V], and a peak for Fe(II) [-1.44 V] in Samples #1-3 (Figure 1). Fe(II) voltammetric signals were higher for samples 1 and 2 than sample 3, while Samples #1 and #3 had larger peaks for soluble Fe(III) indicating that the addition of humic acids probably stabilized Fe(III) by complexation during the oxidation process as has been shown previously with humics obtained from the International Humic Substance Society. Sample #3 also contained several unknown peaks, which were found by their potentials to be consistent with soluble Fe(III) species of different composition or age. The suspected thiol peak at -0.6 V was investigated further by varying the conditioning time applied to the electrode at -0.1 V before each scan. This experiment revealed that the unidentified peak preconcentrates on the electrode surface at positive potentials, a typical characteristic of thiol compounds (Figure 2). In order to confirm that the peak is indeed produced from humic acids further work is needed. We are planning to evaluate the voltammetric signal of the humic acid extract alone and to test further the effect of humics on biogenic and abiotic formation of Fe(III).

All 3 samples displayed a high concentration of Fe(II) within the Fe(III) oxide ring of the gradient tube. This was surprising as in this region of the gradient tube, Fe(II) should be oxidized biologically or abiotically as oxygen diffuses into the media. In addition, the samples were collected and shipped under oxic conditions. Therefore, we expected all Fe(II) to be oxidized and no voltammetric signal to be present during the measurements. To further understand these observations, we quantified Fe(II) in Samples #2 and #3 using both electrochemical methods and the ferrozine assay (Figure 3). We observed for Sample #2 that the Fe(II) concentration determined by voltammetry exceeded that determined by ferrozine analysis, suggesting that Fe(II) is complexed by an organic ligand that enhances the electrochemical signal (Figure 3A). Such behavior has been observed before during the reduction of iron oxides by *Shewanella* species and was attributed to the reduction of organic-Fe(III) intermediate complexes produced by *Shewanella* to solubilize iron oxides during anaerobic respiration (Taillefert et al., 2007; Jones et al., 2010). In Sample #3, we observe very little Fe(II) with both methods although we had observed soluble Fe(III). This suggested that humic acids stabilized soluble Fe(III) complexes. In addition, because no Fe(II) was observed in Sample #2, we can infer that humic acids are likely increasing the rate of Fe(II) oxidation. The results of these analyses have led us to speculate that Fe(II) may be stabilized by a ligand produced by the actively growing FeOB from the peatland. Future experiments will be designed to test this hypothesis.

The effect of biological Fe(II) oxidation on heavy metals was investigated in enrichment cultures originating from an AMD-impacted creek. We collected biogenic Fe(III) oxides from gradient tubes amended with Co, Cd, and Ni (Table 1). During the trip we were only able to perform preliminary voltammetry using a hanging mercury drop electrodes (HMDE) system (Metrohm VA663 and μAutolab potentiostat) on the Cd-amended cultures, as the speciation of Cd can directly be analyzed by voltammetry while only total dissolved Co and Ni can be analyzed voltammetrically. This preliminary work showed that aqueous Cd can be quantified by HMDE in the presence of Fe(III) oxides. In addition, a higher concentration of Cd was detected in cultures amended with an environmental additive to promote growth of unique FeOB species (biofilm extract) than without extract amendment. This suggests that organisms cultivated in the presence of the biofilm extract differ in their ability to complex Cd and remove it from solution during iron oxidation. This work is on-going to quantify the concentration of Cd in the cultures and develop methods for determining metal speciation in these cultures.

A primary aim of my visit to Dr. Martial Taillefert's lab was to plan future collaborations for understanding iron cycling in slightly acidic pH environments. During my stay we developed a plan for Martial to visit the Friedrich Schiller University Jena in August of 2010. In August we will measure *in situ* Fe(II)/Fe(III) gradients in a peatland in Northern Bavaria to determine the contribution of soluble Fe(III)-complexes to iron cycling at this site. In addition, we are planning to further explore the effect of humics on biogenic and abiotic formation of Fe(III) by analyzing additional FeOB and chemical control cultures. To further understand the effect of FeOB on metal contaminants we will develop methods to measure the speciation of heavy metals and determine the fate of metals after biogenic Fe(II) oxidation. In particular, we would like to assess whether heavy metals are complexed during Fe(II) oxidation and stabilized in solution, incorporated in the cell biomass (potentially as detoxification mechanism), or adsorbed to Fe(III)-oxides.

References

Brendel P. J. and Luther III G. W. (1995) Development of a gold amalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, O2, and S(-II) in porewaters of marine and freshwater sediments. Environmental Science and Technology 29(3), 751-761.

Carey E. A. and Taillefert M. (2005) The role of soluble Fe(III) in the cycling of iron and sulfur in coastal marine sediments. Limnology and Oceanography 50(4), 1129-1141.

Jones M., Fennessey C. M., DiChristina T. J., and Taillefert M. (2010) Shewanella oneidensis MR-1 mutants unable to produce soluble organic-Fe(III) are impaired in Fe(III) respiratory capability. Environmental Microbiology 12(4), 938-950.

Taillefert M., Bono A. B., and Luther III G. W. (2000) Reactivity of Freshly Formed Fe(III) in Synthetic Solutions and (Pore)Waters: Voltammetric Evidence of an Aging Process. Environmental Science and Technology 34, 2169-2177.

Taillefert M., Beckler J. S., Carey E. A., Burns J. L., Fennessey C. M., and DiChristina T. J. (2007) Shewanella putrefaciens produces an Fe(III)-solubilizing ligand during anaerobic respiration on insoluble Fe(III) oxides Journal of Inorganic Biochemistry 101(11), 1760-1767.

Table 1. FeOB culture source of biogenic Fe(III)-oxides.

Site	Name	Culture	Amendment
Peatland	Sample #1	Enrichment	+ 1% humic acid extract[1]
	Sample #2	Isolate CL21	+ 0.5 mM $FeCl_2$[2]
	Sample #3	Isolate CL21	+ 1% humic acid extract
AMD-impacted creek	Co+BE	Enrichment	10 mM Co + biofilm extract[3]
	Ni+BE	Enrichment	10 mM Ni + biofilm extract
	Cd+BE	Enrichment	10 mM Cd + biofilm extract
	Cd	Enrichment	10 mM Cd

[1]Humic acids extracted from peat samples.
[2]$FeCl_2$ was added as a growth factor.
[3]Biofilm extract was derived from the AMD-impacted creek and added as a growth factor.

Figure 1. Square wave voltammetry scans of peatland biogenic Fe(III)-oxides from Samples #1-3. Samples #1 and #3 contained humic acids. From left to right: a peak for soluble Fe(III) at ~-0.2 V, a suspected thiol peak at ~-0.6 V, and a peak for Fe(II) at -1.44 V.

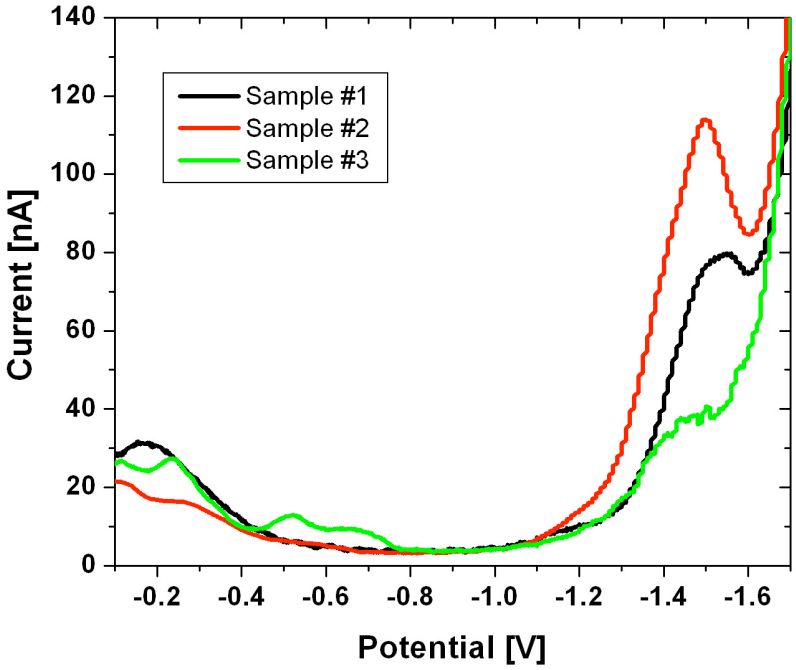

Figure 2. Voltammetry scans of Sample #1 obtained by varying the time of conditioning at -0.1 V before scanning cathodically (from -0.1 V to -1.75 V).

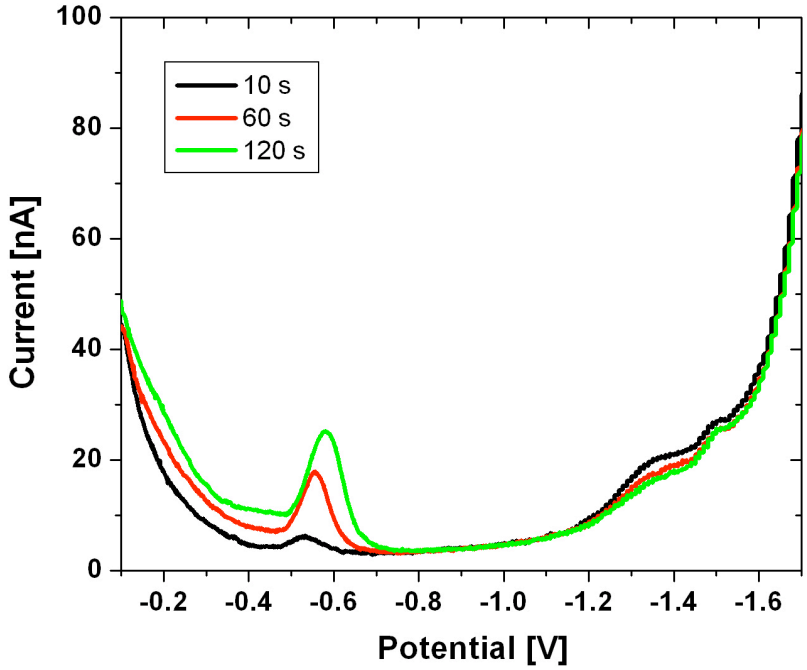

Figure 3. Quantification of Fe(II) using voltammetry and the ferrozine assay in Samples #2 and #3. Note: electroactive Fe(III) complexes cannot be quantified because they are of unknown chemical composition; therefore, concentrations are reported as current intensities.