# Workshop "Magnetic Methdods in Biogeochemistry – from field to microscopy and Mössbauer spectroscopy"
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Scientific Report by
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By attending the workshop "Magnetic Methods in Biogeochemistry - from field to microscopy and Mössbauer spectroscopy" I hoped to learn more about the possibilities of characterizing the iron mineral phases formed during indirect microbial reduction of Fe(III) minerals via humic substances. In our batch experiments, we study electron shuttling between microorganisms and Fe(III) minerals by humic substances which leads to the reductive dissolution of Fe(III) minerals such as ferrihydrite and the subsequent formation of secondary mineral phases such as the ferrimagnetic mineral magnetite, as well as the further reduction and dissolution of these ferrimagnetic phases. In order to characterize the iron mineral phases formed during these experiments we carried out magnetic susceptibility measurements in combination with µ-XRD, which already gave us a good insight into the formation and dissolution of magnetite. However, in order to characterize our mineral phases and the mineral transformation in more detail, including analysis of grain size, magnetic domain state, oxidation state of the iron and stoichiometric irregularities, we would like to apply further techniques like Mössbauer spectroscopy and TEM. Therefore, attending the workshop was an excellent opportunity to deepen my knowledge of the magnetic techniques we are planning to apply and to become acquainted with new techniques that might offer further possibilities of understanding the (trans)formation processes of iron minerals.

During the workshop we became acquainted with several magnetic techniques including Mössbauer spectroscopy, electron microscopy and rock magnetic methods.

Mössbauer spectroscopy is based on resonant absorption of gamma rays in $^{57}$Fe. The absorption leads to a transition of the nucleus to a higher energy stage which can be measured as decrease in the transmitted gamma rays. Resonant absorption of the gamma rays requires recoil-free emission and absorption, which can only be obtained if the sample material is a solid or a frozen liquid fixed in sample holder to prevent vibration. The resulting Mössbauer spectrum is characterized by hyperfine parameters which are due to different nuclear interactions and give information about the sample material. The most important hyperfine parameters are the isomer shift, which is due to valence, coordination state and ligands of the atom, quadrupole splitting, which is due to non-spherical charge distribution in the nucleus, and hyperfine splitting, which occurs if the sample has an internal magnetic field. The combination of these hyperfine parameters allows the identification of different iron redox states, different iron minerals and may even give some information about the grain size and magnetic orientation of the minerals. During the workshop we learned how to fit Mössbauer spectra and identify the hyperfine parameters in order to gain information about the sample material. We had the opportunity to measure our own samples and analyze the resulting Mössbauer spectra. Thus, we could study the effect of grain size, non-stoichiometry, $Fe^{2+}$ in clay minerals and Co inclusions in magnetite on the Mössbauer spectra.

Five days of the workshop were spent at the Center for Electron Nanoscopy (CEN). There we had the opportunity to see electron microscopes that are amongst the best worldwide in terms of resolution and advanced measuring techniques such as electron tomography, off-axis electron holography or environmental TEM. If an electron beam hits a sample the negatively charged electrons interact with the positively charged nuclei of the sample material and thus are scattered out of the beam. A scanning electron microscope (SEM) detects the electrons that are backscattered through the surface of the sample and the secondary electrons that are knocked out of the sample material due to collisions of the beam electrons with the sample electrons. Thus the sample surface is scanned by the electron beam in order to obtain an image of the surface topography of the sample. In a transmission electron microscope (TEM), on the other hand, the transmission of the electron beam through a thin (<100 nm) sample is measured. In a bright field image the background is bright due to almost complete transmission whereas the sample scatters some of the electrons away from the beam and thus appears darker. For a dark field image, only scattered electrons are detected so that the background appears dark whereas the sample is lighter. The high voltage electron guns of some of the CEN TEMs allow a resolution of up to 0.1 nm and thus the identification of single atoms in the sample lattice. The chemical composition of the sample can by analyzed by energy dispersive X-ray spectroscopy (EDS) or electron energy-loss spectroscopy (EELS). With a focused ion beam (FIB) the sample can be cut and thin slices can be removed from the sample surface thus allowing to obtain a set of images which can be used to construct a three dimensional image. Electron diffraction can be used to identify different minerals due to their different crystal structures. Off-axis electron holography is a technique which is able to visualize the magnetic field lines around a sample of a magnetic material and thus to identify the magnetic domains within the sample. An environmental TEM can tolerate a pressure of up to 10 mbar in the sample chamber in contrast to conventional TEMs which are operated under high vacuum ($10^{-8}$ mbar). Thus, in an environmental TEM hydrated soft and liquid samples can be analyzed. Electron tomography creates a three dimensional image of the sample surface by scanning the sample from different angles and combining the obtained images. During the days spent at CEN, we could observe the application of all these techniques and we could even analyze some of our own samples in the SEM. Thus we received a good impression of the manifold possibilities of electron microscopy.

Rock magnetic measurement techniques are based on the different magnetic properties of different materials. These properties are due to the magnetic moments of the single atoms which are based on the magnetic moment of the nucleus, which is very small, the orbital magnetic moment of the electrons and the spin magnetic moment of the electrons. In materials without unpaired electrons, the net magnetic moment is only due to the orbital magnetic moment of the electrons, as the spins of the paired electrons cancel out each other. This so called diamagnetism is antiparallel to the applied magnetic field and not remanent, i.e. it is only present as long as the external magnetic field is present. Materials with unpaired electrons experience paramagnetism. The spin magnetic moment orients parallel to the external magnetic field resulting in a positive net magnetic moment which is stronger than in diamagnets. If the atoms are close enough to each other to allow overlapping of the orbitals, the interaction of the spin magnetic moments of the single atoms leads to the formation of magnetic domains with parallel orientation. If these ferromagnetic materials are exposed to an external magnetic field, the domains with orientation parallel to the external field enlarge by wall displacement leading to a very strong and remanent magnetization. The different magnetization in an external magnetic field allows the identification of magnetic materials by measurement of the magnetic susceptibility. At low magnetic field, the magnetic susceptibility is completely reversible.

These low field magnetic susceptibility measurements are suitable mainly to identify the presence of para- and ferromagnetic materials. If a higher magnetic field is applied and removed again, magnetic hysteresis can be measured. This gives information about the remanence of the magnetization. The shape of the magnetic hysteresis is characteristic for different ferromagnetic materials and allows the identification of these materials. Further information can be obtained by the combination of different magnetic parameters and by measurement of the frequency dependence and temperature dependence of the magnetic susceptibility.

During the workshop we could also analyze some of our own samples by Mössbauer spectroscopy. The samples I measured were biogenic Fe(III) minerals obtained from the microbial oxidation of Fe(II) chloride by the iron-oxidizing nitrate-dependent strain *Acidovorax sp.* BoFeN1. The aim of these experiments was to compare the abiotic electron transfer from humic substances to biogenic vs. abiogenic Fe(III) minerals. In order to do so, we wanted to characterize the biogenic iron minerals by μ-XRD and Mössbauer spectroscopy. *Acidovorax sp.* BoFeN1 is known to produce mainly goethite, but also partly ferrihydrite. Mössbauer spectra of my biogenic Fe(III) minerals measured at room temperature showed one doublet and several very broad sextets (Figure 1). This indicates a super-paramagnetic or "super-ferromagnetic" compound. Because of the broad signal it is very difficult to obtain a good fit for this spectrum and to draw reliable conclusions about the sample material.
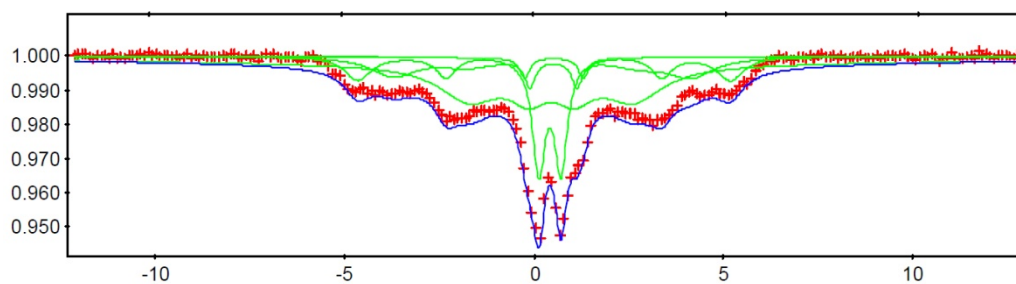


**Figure 1:** Mössbauer spectrum of Acidovorax sp. BoFeN1 minerals at room temperature

 A second spectrum of the same sample was obtained at 80 K (Figure 2). It shows one clear sextet and the hyperfine parameters fit those of goethite. A good fit ($R^2$ = 11.99) can be obtained with only this one sextet indicating that the sample was mainly composed of goethite and no significant second compound was present.
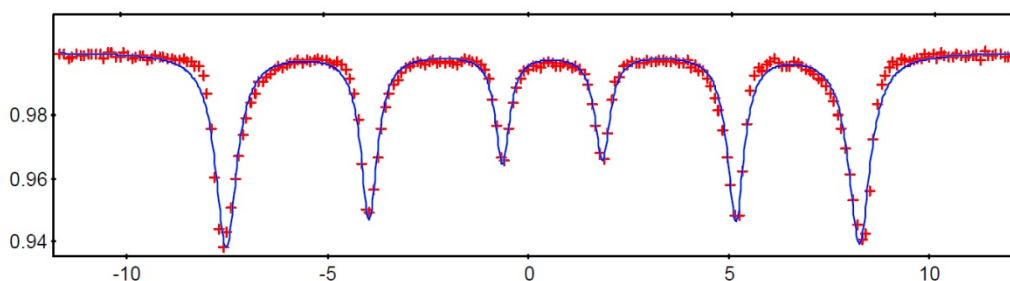


**Figure 2:** Mössbauer spectrum of Acidovorax sp. BoFeN1 minerals at 80 K

The results of the Mössbauer measurements revealed that the biogenic Fe(III) minerals produced by iron-oxidizing nitrate-dependent *Acidovorax sp.* BoFeN1 consisted mainly of goethite. The grain size of this biogenic goethite was mainly below the super-paramagnetic threshold at room temperature. Contrary to our expectations, no traces of ferrihydrite could be found in the samples.