Magnetic Susceptibility as a Proxy for Investigating Microbial Mediated Iron Reduction

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Abstract. We investigated magnetic susceptibility (MS) variations in hydrocarbon contaminated sediments. Our objective was to determine if MS can be used as an intrinsic bioremediation indicator due to the activity of iron-reducing bacteria. A contaminated and an uncontaminated core were retrieved from a site contaminated with crude oil near Bemidji, MN and subsampled for MS measurements. The contaminated core revealed enriched MS zones within the hydrocarbon smear zone, which is related to iron-reduction coupled to oxidation of hydrocarbon compounds and the vadose zone, which is coincident with a zone of methane depletion suggesting aerobic or anaerobic oxidation of methane is coupled to iron-reduction. The latter has significant implications for methane cycling. We conclude that MS can serve as a proxy for intrinsic bioremediation by iron-reducing bacteria and for the application of geophysics to iron cycling studies.

INDEX TERMS: 0925 Magnetic and electrical methods, 0416 Biogeophysics, 0463 Microbe/mineral interactions, 0418 Bioremediation.

1.0 Introduction

The Deepwater Horizon spill in the Gulf of Mexico is a reminder of the environmental threat of hydrocarbon contamination and the need for technological advancements for detecting,
monitoring, and remediating hydrocarbon contamination. Due to the relationship between magnetic minerals and redox reactions associated with hydrocarbon seeps, magnetic susceptibility (MS) has been used as a possible tool for oil exploration [e.g., Saunders et al., 1999]. In surficial sediments, MS traditionally is used for climate studies [e.g., Kukla et al., 1988], mapping heavy metal soil contamination [e.g., Hanesch and Scholger, 2002], and identifying sediment sources and transport trends [Ellwood et al., 2006]. MS has recently emerged as a tool for investigating iron cycling mediated by microbial activity [e.g., Porsch et al., 2010; Rijal et al., 2010].

This work investigates MS variations in a hydrocarbon contaminated aquifer where methanogenesis and iron-reduction are the main terminal electron acceptor processes [Baedecker et al., 1993]. Our objective was to determine if MS could be an indicator of the presence of intrinsic hydrocarbon bioremediation by iron-reducing bacteria. Our results suggest enhancements in MS are due to the precipitation of magnetite coupled to iron-reduction as related to; (1) anaerobic oxidation of hydrocarbon compounds within the smear zone and (2) aerobic or anaerobic oxidation of methane within the vadose zone, which provides additional field evidence linking anaerobic oxidation of methane to iron-reduction and suggests significant implications for methane cycling in terrestrial environments.

1.1 Site History

The National Crude Oil Spill Fate and Natural Attenuation Research Site at Bemidji, MN (Figure 1), is a field laboratory for investigating biogeophysical signatures associated with the intrinsic bioremediation of a crude oil spill. In August 1979, a high pressure crude oil pipeline ruptured, releasing 1,700,000 L of crude oil. Oil pooled in low-lying areas (~2000 m²) over a total area of 6,500 m² to the southwest of the pipeline forming the south and north contaminated
plumes. The study site consists of ~20 m-thickness of moderately calcareous silty sand and outwash glacial deposits overlying clayey till of unknown thickness [Bennett et al., 1993]. The north plume has been the focus of intensive geochemical [e.g., Cozzarelli et al., 2010 and references therein] and microbiological studies [e.g., Bekins et al., 2001].

The uncontaminated groundwater is aerobic with dissolved oxygen concentrations between 8 and 9 mg/L, dissolved organic carbon of 2.8 mg/L as C, and low levels of nitrate at 44.8 μg/L as N₂, and sulfate at 2.9 mg/L [Bennett et al., 1993]. Geochemical changes in the subsurface plume have been previously described by Baedecker et al. [1993] and Cozzarelli et al. [2010]. The aquifer is divided in the vicinity of the oil body into anoxic, transition, and background zones. In the anoxic portion, hydrocarbons are oxidized predominantly by iron reduction [Lovley et al., 1989] and methanogenesis [e.g., Baedecker et al., 1993]. Bekins et al. [2001] show two zones of methanogenic activity with CH₄ concentrations greater than 15 mg/L. The vadose zone vapor plume near the oil body has low O₂ concentrations (< 2%) and high levels of CO₂ (>10%) and CH₄ (>15%). Four physiologic microbial populations within the anaerobic portion of the contaminated aquifer suggest the progression from iron-reduction to methanogenesis [Bekins et al. 2001].

2.0 Materials and Methods

2.1 Retrieval of Cores

Two ~5-cm diameter cores were retrieved from contaminated (C1010) and uncontaminated (C1006) locations (Figure 1). In the uncontaminated location two segments of core were collected, representing the vadose and saturated zones. The cores were collected by advancing a core barrel with a polycarbonate liner ahead of a hollow-stem auger. The cores within the smear zone or below the water table were collected with a freezing drive shoe [Murphy and Herkelrath,
1996], preserved in coolers unexposed to the atmosphere, and expeditiously shipped to the laboratory at Oklahoma State University.

2.2 MS Measurements and Iron Analysis

Sub-samples of ~10 grams were taken at 5 to 15 cm intervals along the cores. Sub-samples were oven dried at 50°C for 24 hours then analyzed for MS using a Bartington MS2 magnetic susceptibility meter (1.2566 x 10^{-11} m3/kg sensitivity). Then the same samples were used to quantify iron content. The mass of magnetic particles retained by a bar magnet was measured and the percentage of the iron-rich material per sample was determined.

2.3 Gas data

Vapor wells, 534 (contaminated) and 310 (uncontaminated) (Fig 1) consisted of permanently installed vapor probes generally spaced at 50- to 100-cm depth intervals in the unsaturated zone, from 1 m below land surface to 1 m above the water table. Each probe was constructed of 0.16- to 0.64-cm outside diameter stainless steel tubing with 5 to 10-cm long screens at the bottom. The probes were installed in 10-cm-diameter augered holes, which were backfilled with native sand, and included a bentonite seal between each screened interval. The gas samples were obtained in gas-tight glass syringes using a peristaltic pump and analyzed on-site using a SRI® 8610C Gas Chromatograph (GC). The GC was configured using a 1.0-mL fixed-loop injection and an internal air compressor. Fixed gas (Ar, O2, CO2, and CH4) analyses were determined with a thermal conductivity detector (TCD) and a SRI® CTR-1 double-packed column. Concentrations of gases were quantified in weight percent using gas standards containing mixtures of Ar, O2, CO2, and CH4.

3.0 Results

3.1 Magnetic Susceptibility
The contaminated core shows two zones of higher MS (Figure 2a) versus the uncontaminated core (Figure 3a). The first zone is located from ~423 m elevation at the base of the free hydrocarbon to 426 m at the top of the groundwater table (GWT) where the MS values range from 75 to 299 x10^{-8} \text{m}^3\text{kg}^{-1} reaching a maximum at 423.5 m(Figure 2c). The second zone is between 428-429 m where the MS values range between 88 to 218x10^{-8} \text{m}^3\text{kg}^{-1} to a maximum at ~429 m (Figure 2a). Another zone of high MS is observed at 427.2 m. These high MS zones occur within the vadose zone with no corresponding free or residual hydrocarbon contamination (Figure 2c). The MS of the uncontaminated cores show relatively small variations with no anomalous zones and values ranging from 40 to 145x10^{-8} \text{m}^3\text{kg}^{-1} (Figure 3a).

3.2 Magnetic minerals analysis

The magnetic mineral concentration data in the uncontaminated core show no anomalous concentrations with values ranging from 0.76x10^{-3} \text{ppm to 2.2x10^{-3} ppm} and an average value of 1.48x10^{-3} \text{ppm}. In contrast, the contaminated core shows elevated concentrations between 1.9x10^{-3} \text{ppm to 4.4x10^{-3} ppm}, reaching a maximum at 423.5 m at depths coincident with higher MS (Figure 2b). In the 428-430 m elevation interval, the concentration of magnetic minerals ranges between 1.9x10^{-3} \text{ppm to 4.2x10^{-3} ppm} with a maximum at 429 m. The correlation between MS and magnetite suggest that magnetite is responsible for the MS variations with an R^2 of 0.817 and 0.967 for the contaminated and uncontaminated cores, respectively. An XRD profile (data not shown) suggested that magnetite was the dominant phase.

3.3 Distribution of Ar + O\textsubscript{2}, CH\textsubscript{4} and CO\textsubscript{2}

The 2005 CO\textsubscript{2} gas data increases downward from nearly 0.7\% at ground surface to 6.5\% at 430 m and 12.2\% at 429 m. Ar and O\textsubscript{2} decrease downward to less than 11.9\% at 430 m to 2.9\% at 429 m and are depleted at 428 m. CH\textsubscript{4} shows depletion upwards from about 4\% at 427m to 0\%
These data define a transition zone from ~427 m to 430 m where O$_2$ and CH$_4$ are depleted and CO$_2$ is enriched. The same trends occur in the 2010 data; however, the transition zone has shifted to a lower elevation (Figure 2e). The uncontaminated gas concentration profile shows no variability and gas concentrations are equal to atmospheric values.

**4.0 Discussion and Conclusions**

The MS and magnetic mineral concentration differed between the contaminated and uncontaminated cores. An upper zone, between 428-430 m, and a lower zone, between 423-426 m of enhanced magnetite formation were observed.

**4.1 Mechanisms for Magnetite Formation**

Geochemical and microbial studies suggest iron reduction is an important terminal electron acceptor process occurring within the anaerobic plume [e.g. Baedecker et al., 1993]. In addition, iron-reducing bacteria such as *Geobacter bemidjiensis* sp. and *Geobacter psychrophilus* sp occur in the contaminated aquifer [Nevin et al., 2005]. During iron reduction, Fe(III) is converted to Fe(II):

\[
\text{Fe}_2\text{O}_3 + 3\text{H}_2 \rightarrow 2\text{Fe} \text{(OH)}_2 + \text{H}_2\text{O} \tag{1}
\]

The ferrous hydroxide (Fe(OH)$_2$) can be oxidized by the protons of water to form magnetite and molecular hydrogen:

\[
3 \text{Fe(OH)}_2 \rightarrow \text{Fe}_3\text{O}_4 \downarrow + \text{H}_2 + 2 \text{H}_2\text{O} \tag{2}
\]

**4.2 Smear Zone**

The contaminated core smear zone above the GWT (423.5-425 m) shows the highest MS values and concentration of magnetite recorded (Figure 2a-c). Biogeophysical investigations at other hydrocarbon contaminated sites have documented the highest value of bulk electrical
conductivity within this smear zone and suggested it to be most biologically active [Werkema et al., 2003]. Additionally, MS studies by Rijal et al. [2010] show concentrations of magnetic parameters increasing towards the top of the GWT. Thus, we infer this enriched zone of magnetite is due to the anaerobic oxidation of hydrocarbons by iron-reducing bacteria [Lovley et al., 1989]. Microbial data showed culturable iron reducers were present in the oil layer in 1997 [Bekins et al., 1999].

4.3 Unsaturated zone

The zone of enriched magnetite occurring in the vadose zone (428-430 m) (Figure 2a) can be explained by: (1) naturally occurring higher concentration of magnetite within the sediments in this zone, (2) precipitation of magnetite (eq. 2 and 3) related to past hydrocarbon vapors in the vadose zone, (3) precipitation of magnetite (eq. 2), related to aerobic oxidation of methane (eq. 3) which releases water protons that can oxidize Fe (OH)$_2$ to magnetite,

$$\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$$

or (4) the precipitation of magnetite resulting from anaerobic oxidation of methane (AOM) coupled to iron reduction.

Although there are presently no hydrocarbon vapors present in the vadose zone, vapors were present in the past [Chaplin et al. 2002]. Hence, the zone of enriched magnetite occurring in the vadose zone at the 428-430 m elevation (Figure 2a) could represent a relict process of bacterial oxidation of the hydrocarbon vapors coupled to iron reduction. Nevertheless, we favor hypotheses 3 and 4 for the following reason. The gas data (Figures 2d-e) suggest a transition in the methane and oxygen plots crossing at 428.3 m (2005) and 427.2 m (2010), where the total depletion of methane occurs. Also, culturable methanotroph microbial population numbers peak within this zone [Molins et al., 2010; data not shown]. This has led some to suggest the aerobic
The oxidation of methane within this zone [Amos et al., 2005]. It is possible that the precipitation of magnetite in this zone could be linked to the aerobic oxidation of methane (eq. 2 and 3).

The precipitation of magnetite resulting from the anaerobic oxidation of methane (AOM) coupled to iron reduction is also possible. Whereas AOM coupled to sulfate reduction is documented in marine environments [e.g., Hoehler et al., 1994], AOM coupled to metal reduction is not common and has yet to be definitively demonstrated. There are also no known microorganisms capable of this process [Beal et al., 2009]. Nonetheless, recent studies postulate that AOM coupled to metal reduction is likely to occur [e.g., Beal et al., 2009; Crowe et al., 2011]. Beal et al. [2009] show AOM in the absence of sulfate if birnessite or ferrihydrite is present. Crowe et al., [2011] document that, despite high abundances of Fe (hydr)oxides in lake sediments, more than 50% of antigenic organic matter was degraded through methanogenesis, suggesting methane oxidation and Fe(III) reduction coupling. This mechanism is as follows:

(1) AOM coupled with ferrihydrite (simplified as Fe(OH)₃) reduction [Beal et al., 2009].

\[
\text{CH}_4 + 8\text{Fe(OH)}_3 + 15\text{H}^+ \rightarrow \text{HCO}_3^- + 8\text{Fe}^{2+} + 21\text{H}_2\text{O} \quad \text{(4).}
\]

Part of the Fe^{2+} reacts with the small amounts of O₂ present, thereby precipitating magnetite:

\[
3\ \text{Fe(OH)}_2 + \frac{1}{2} \text{O}_2 \rightarrow 2\ \text{Fe(OH)}_2 \cdot 2\ \text{FeOOH} + \text{H}_2\text{O} \quad \text{(5).}
\]

\[
\text{Fe(OH)}_2 \cdot 2\ \text{FeOOH} \rightarrow \text{Fe}_3\text{O}_4 \downarrow + 2\text{H}_2\text{O} \quad \text{(6).}
\]

The remaining ferrous hydroxide Fe(OH)_2 can be oxidized by water protons to form magnetite and molecular hydrogen (eq. 2).

Geochemical studies at this site show low levels of nitrate (44.8 µg/L as N₂) and sulfate (2.9 mg/L) [Bennett et al., 1993], which, in addition to elevated precipitation of magnetite, supports our argument that this is a zone of methane oxidation coupled with iron reduction. Our study suggests that MS measurements may aid in field investigations of this process. AOM coupled to
iron reduction has significant implications for methane cycling in terrestrial environments. Biogeophysical studies can help guide microbial sampling thereby advancing microbial ecology studies of new microbial species associated with bioremediation.

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**Figure Captions**

Figure 1. Study area showing the location of core samples (circles) and gas data (triangles) at contaminated (closed symbols) and uncontaminated (opened symbols) boreholes. 310 and 534 are vadose zone gas sampling boreholes.

Figure 2. (a) Magnetic susceptibility, (b) concentration of magnetic minerals, (c) hydrocarbon distribution within core, gas concentrations in (d) 2005, and (e) 2010 along the contaminated core (C 1010). HWT: Highest water level, LWT: Lowest water level and WT: Water Table during the time of measurements. Elevation above mean sea level (amsl). The gas data are from location 534 located 10 m from C1010.

Figure 3. (a) Magnetic susceptibility, (b) concentration of magnetic minerals, and (c) hydrocarbon distribution in two 2-m zones along the uncontaminated borehole (C 1006). WT: Water Table during the time of measurements. Elevation above mean sea level (amsl).