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## Evidence for microbial enhanced electrical conductivity in hydrocarbon-contaminated sediments

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[1] Bulk electrical conductivity of sediments during microbial mineralization of diesel was investigated in a mesoscale laboratory experiment consisting of biotic contaminated and uncontaminated columns. Population numbers of oil degrading microorganisms increased with a clear pattern of depth zonation within the contaminated column not observed in the uncontaminated column. Microbial community structure determined from ribosomal DNA intergenic spacer analysis showed a highly specialized microbial community in the contaminated column. The contaminated column showed temporal increases in bulk conductivity, dissolved inorganic carbon, and calcium, suggesting that the high bulk conductivity is due to enhanced mineral weathering from microbial activity. The greatest change in bulk conductivity occurred in sediments above the water table saturated with diesel. Variations in electrical conductivity magnitude and microbial populations and their depth distribution in the contaminated column are similar to field observations. The results of this study suggest that geophysical methodologies may potentially be used to investigate microbial activity.

**INDEX TERMS:** 0925 Exploration Geophysics: Magnetic and electrical methods; 0915 Exploration Geophysics: Downhole methods; 1831 Hydrology: Groundwater quality. **Citation:** Atekwana, E. A., E. A. Atekwana, D. D. Werkema, J. P. Allen, L. A. Smart, J. W. Duris, D. P. Cassidy, W. A. Sauck, and S. Rossbach (2004), Evidence for microbial enhanced electrical conductivity in hydrocarbon-contaminated sediments, *Geophys. Res. Lett.*, 31, L23501, doi:10.1029/2004GL021359.

### 1. Introduction

[2] Bacteria play an important role in the acceleration of silicate mineral weathering by directly colonizing mineral surfaces or indirectly by the production of organic and inorganic acids that enhance the weathering of minerals [e.g., Hiebert and Bennett, 1992; McMahon et al., 1995]. Thus, microbial processes that modify the physical properties of sediments and rocks may potentially be detected using

geophysical methodologies. We have previously documented from field investigations that sediments impacted by petroleum hydrocarbons may develop regions of higher bulk electrical conductivity ( $\sigma_b$ ) in contrast to the expected lower  $\sigma_b$  due to petroleum compounds [Werkema et al., 2003; Atekwana et al., 2004]. In a laboratory experiment conducted by Abdel Aal et al. [2004a, 2004b], induced polarization measurements showed that changes in electrical properties of diesel amended sediments undergoing biodegradation were concurrent with increase in oil degrading microbial population numbers, decrease in diesel, decrease in terminal electron acceptors ( $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ) and an increase in  $\text{Ca}^{2+}$  from mineral weathering. Except for the Abdel Aal et al. [2004a, 2004b] study, there are only a few studies [e.g., Ntarlagiannis et al., 2004] from controlled laboratory experiments demonstrating the link between bioactivity and changes in geophysical properties. The role of microbes in altering the physical properties of rocks and sediments is not fully understood and is only now being recognized [Knight, 2001]. There is a need for more studies addressing this bioactivity-geophysics relationship, as biogeophysical approaches may have broad implications for investigating geomicrobiological processes.

[3] In this study, we extend the work of Werkema et al. [2003] by investigating changes in electrical conductivity during microbial mineralization of hydrocarbons in a mesoscale column experiment that partially simulates field conditions. The results from this study show depth stratification of microbial population concomitant with higher  $\sigma_b$  in contaminated sediments, suggesting that  $\sigma_b$  changes are related to bio-activity.

### 2. Material and Methods

[4] The experimental columns were 80 cm long and constructed of 31 cm diameter polyvinyl chloride pipe (PVC). The columns were filled with autoclaved fine to medium-grained sands obtained from a field site in Carson City, Michigan, USA. The sands are predominantly quartz with minor amounts of feldspars and carbonates. The bacteria used in this experiment was obtained by shaking contaminated sands collected from the Carson City site in 25% Bushnell-Haas (BH) medium (Becton Dickinson, Detroit, MI) in the ratio of 1:9, respectively. Culturing experiments revealed mainly *Rhodococcus* species (e.g., *Rhodococcus opacus*) in the sediments.

[5] The columns partially simulated typical field conditions with a water saturated zone, a transition zone, and a vadose zone. The uncontaminated column, designed to simulate background conditions, contained water, nutrients

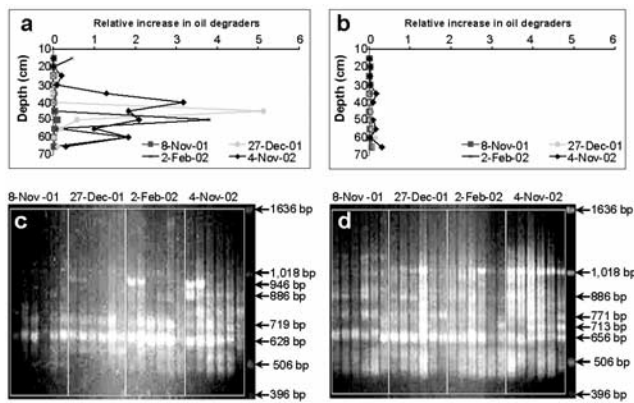
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**Figure 1.** Temporal changes in microbial populations capable of diesel mineralization: (a) contaminated column and (b) uncontaminated column. Microbial community dynamics determined from ribosomal DNA inter spacer region analysis (RISA): (c) contaminated column and (d) uncontaminated column. The relative MPN numbers were determined as the change in microbial populations capable of diesel mineralization compared to total microbial community. Boxes in the RISA profiles (c, d) denote individual sampling events with six lanes in each box representing sediment depths of 15, 25, 35, 45, 55, and 65 cm from left to right. The far right lane shows the size marker with rDNA fragments of 1636, 1018, 506 and 396 base pair (bp) length. Other numbers refer to sizes in base pairs of specific ribosomal DNA fragments.

(25% BH), and bacteria. The contaminated column was similar to the uncontaminated column, but amended with 4 l of diesel. The columns were initially filled with fluid (25% BH + bacteria) for calibration purposes. Pre-fabricated 2.5 cm diameter sediment cores to be used for microbial analyses were placed in the columns prior to filling with sand. The sediment cores consisted of sand (same as in the columns) packed in slotted PVC liners. The sediment cores were positioned on the opposite side of the column such that the closest core was ~17 cm from the electrodes. Sand was added to the fluid filled columns. This procedure allowed for even sand distribution and minimal air pockets. In the uncontaminated column, fluid was drained to maintain a water level at 45 cm depth. In the contaminated column, diesel was introduced at the top of the fully saturated sand column and the water level lowered to 45 cm. This resulted in diesel sorbed (residual phase) on the sand from 0 to 32 cm, sands contaminated with free phase diesel from 32 to 45 cm, and dissolved phase diesel contamination in the water saturated zone below 45 cm. The columns were maintained at room temperature (~23–25°C).

[6] The  $\sigma_b$  was measured from steel electrodes installed along the length of the column using a 2 cm Wenner array (four equally spaced electrodes with potential electrodes nested inside current electrodes). Microbial population numbers and community structure were determined from the sediment cores placed in the columns during initial set up. The cores were removed from the columns and the sediment was sub-sampled aseptically every 5 cm for analyses. Microbial population numbers capable of mineralizing diesel were determined by the Most Probable

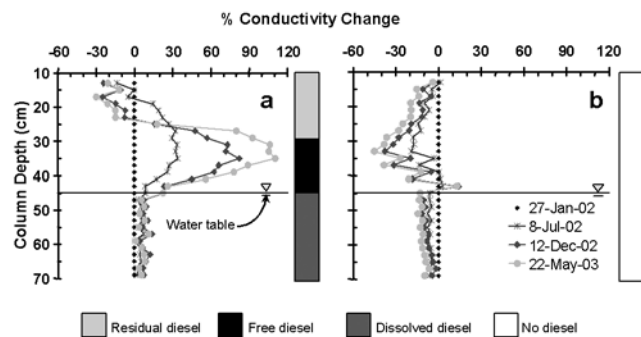
Number technique (MPN) [Wrenn and Venosa, 1996], and the microbial community structure was determined by the ribosomal DNA intergenic spacer analysis (RISA) [Garcia-Martinez et al., 1999]. Approximately 75 ml of pore fluids were extracted for chemical analyses using a syringe fitted with a 0.45  $\mu\text{m}$  filter at discrete depths of 45, 50, 60, and 65 cm through a rubber septum installed along the full vertical length of the column. This caused water levels to decline to a depth of ~55 cm during sampling events. The columns were subsequently recharged with 25% BH medium from stock solutions through an inlet at the bottom to maintain a constant water level at ~45 cm. The fluctuations in water level during sampling and recharge caused a hydrocarbon “smear zone” (45–55 cm) as the free phase diesel coated the sands. Microbial mineralization of diesel was assessed by measuring dissolved inorganic carbon (DIC) [Hunkeler et al., 2002] using the method of Atekwana and Krishnamurthy [1998]. Microbial enhanced weathering of sediments in the column was determined by measuring dissolved  $\text{Ca}^{2+}$ .

### 3. Results and Discussion

#### 3.1. Oil Degrading Microbial Population Numbers and Microbial Community Structure

[7] The relative increase in oil degrading microbial population numbers was determined by dividing the ratio of oil degraders to the total heterotrophic bacteria population for each sampling period to that at the start of the experiment. A higher relative increase in oil degraders was measured in the contaminated column (Figure 1a) compared to the uncontaminated column (Figure 1b). The increase in microbial numbers may be related to excess organic carbon substrate available in the free and residual phases above the water saturated zone and as dissolved phase in the water saturated zone [Bekins et al., 1999]. A five-fold increase in microbial numbers in the contaminated column was observed in the second month (Dec-01) in a narrow zone at 45 cm, which decreased in peak magnitude to 3-fold and increased to a broader depth zone (30–65 cm) in later months. This spatial (i.e., depth) increase is likely due to microbial succession (continuous change and adaptation of microbes to changing conditions) typically observed in organic contaminant plumes [Bekins et al., 1999]. The higher microbial population numbers could have been enhanced by periodic hydrocarbon supply to the water saturated zone (45–55 cm) as water levels in the contaminated column declined during pore fluid sampling. Sediments in the uncontaminated column (Figure 1b) show relatively low microbial population numbers throughout all depths, with only a slight increase over time and no apparent depth stratification.

[8] The microbial community structure showing different micro-organisms in the columns is presented as RISA profiles (Figures 1c and 1d). The contaminated column (Figure 1c) shows fewer rDNA fragments (specific sized DNA fragments (measured in base pairs) seen as discrete alignment of lighter areas (bands) on the images) than the profiles from the uncontaminated column (Figure 1d). Temporal changes in the microbial community structure are demonstrated by two prominent fragments of 628 and 719 base pair (bp) size visible in the profiles at all sampling



**Figure 2.** Temporal percent change in bulk conductivity ( $\sigma_b$ ): (a) contaminated column and (b) uncontaminated column. Four time periods have been plotted to illustrate temporal changes in  $\sigma_b$ . Also shown is the vertical distribution of diesel.

times from the contaminated column (Figure 1c). Beginning at the two-month (Dec-01) and intensifying at the four (Feb-02) and twelfth-month (Nov-02) samples, fragments of 946 bp size are observed in the samples from the top (15 and 25 cm depth) of the columns. RISA profiles with fewer fragments, some of which show greater intensity representing a few dominant bacterial strains, would be expected from a microbial community disturbed by environmental stress, in which a few members adapt, specialize (e.g., for diesel degradation), and thrive [Ranjard *et al.*, 2000; Haack and Bekins, 2000]. The RISA profiles of the uncontaminated column (Figure 1d) show a higher number of fragments that are also more uniform in their density compared to the profiles from the contaminated column (Figure 1c), indicating a more uniform and less specialized microbial community.

### 3.2. Bulk Conductivity Changes

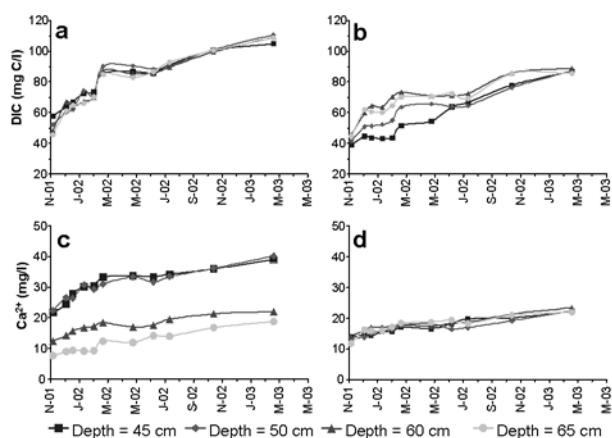
[9] To determine the relative magnitude of  $\sigma_b$  change, we calculated the temporal percent change  $(100(\sigma_{bti} - \sigma_{btb}) / \sigma_{btb})$  for select time intervals  $t_i$  relative to the baseline value  $t_b$  (time at which the  $\sigma_b$  stabilized). It took three months from the start of the experiment for  $\sigma_b$  in the columns to equilibrate ( $\sigma_{btb}$ ). A relative decrease in  $\sigma_b$  was observed in the uncontaminated column (Figure 2b) within the unsaturated zone (10–45 cm). Decreasing water saturation (drying out of the sediment) with time can explain this observation, since the sediments were initially fully saturated and the columns were recharged from the bottom after extracting fluids for chemical analyses. The same drying out effect is observed in the contaminated column (Figure 2a) at 10–25 cm where decreases in  $\sigma_b$  were measured. In the uncontaminated column, the decreasing trend in  $\sigma_b$  continued into the water saturated zone, albeit to a lesser extent. This may be attributed to bacterial utilization of ions in the nutrient medium [e.g., Barker *et al.*, 1997]. Over time, the contaminated column (Figure 2a) revealed a more than 100% increase in  $\sigma_b$  in the unsaturated zone (25–45 cm) compared to the uncontaminated column which showed a decrease with time (Figure 2b). The much higher  $\sigma_b$  in the contaminated column is contrary to the expectation that diesel saturated sediments should be less conductive [Mázac *et al.*, 1990]. In the water saturated zone of the contami-

nated column (below 45 cm), the increase in the  $\sigma_b$  is less than 10% of the baseline value.

### 3.3. Geochemical Changes

[10] DIC in the water saturated zone of the columns was used to verify microbial mineralization of diesel, and  $\text{Ca}^{2+}$  was used to investigate mineral weathering (Figure 3); both of which are related to changes in  $\sigma_b$ . Higher DIC was measured in the contaminated column (up to 110 mg/l; Figure 3a) than in the uncontaminated column (up to 80 mg C/l; Figure 3b), which we attribute to microbial mineralization of diesel and the production of  $\text{CO}_2$  [e.g., Hunkeler *et al.*, 2002]. DIC production in the uncontaminated column may have resulted from microbial mineralization of natural organic carbon in the sands.  $\text{CO}_2$  produced during diesel degradation forms carbonic acid which enhances weathering of minerals such as feldspars and carbonates in the sands. The contaminated column (Figure 3c) also showed a greater temporal increase in  $\text{Ca}^{2+}$  (change of  $\sim 20$  mg/l) and higher overall concentration (up to 40 mg/l) than the uncontaminated column which showed an 8 mg/l increase (Figure 3d). Also, the higher  $\text{Ca}^{2+}$  at the 45, 50, and 60 cm depths (Figure 3c) was coincident with the depths where the highest microbial population numbers were observed (Figure 1a). The  $\text{Ca}^{2+}$  concentrations at the 65 cm depth in the contaminated column (Figure 3c) is similar to values in the uncontaminated column (between 10 and 20 mg/l; Figure 3d). We infer from the  $\text{Ca}^{2+}$  data that mineral weathering was enhanced in the contaminated column due to higher biological activity which increased the ionic content of pore fluids and thus the  $\sigma_b$ .

[11] DIC and  $\text{Ca}^{2+}$  were not measured in the unsaturated zone and in the residual and free phase diesel layer (25–40 cm) in the contaminated column due to lack of pore fluids and the presence of free-phase diesel. However, we speculate that the higher  $\sigma_b$  in this region compared to the water saturated region (Figure 2a) may suggest microbial degradation of diesel and enhanced weathering of minerals which increased the  $\sigma_b$  [Sauck, 2000]. We believe this to be likely true as the conductivity and microbial zonations observed in our experimental columns are similar to observations in the field and thus replicate and validate field



**Figure 3.** Temporal concentration of dissolved inorganic carbon (DIC) and  $\text{Ca}^{2+}$  (a) and (c) contaminated column and (b) and (d) uncontaminated column.



observations. *Werkema et al.* [2003] reported higher  $\sigma_b$  and *Abdel Aal et al.* [2003] report higher induced polarization parameters coincident with residual and free phase hydrocarbon contaminated zones than in the water saturated zone with dissolved phase hydrocarbon. In the maximum  $\sigma_b$  zones, *Atekwana et al.* [2004] document a higher percentage of oil degrading microbial populations and *Duris et al.* [2003] show from RISA analyses fewer fragments indicative of a highly adapted microbial community similar to our observations (Figure 1c; 628, 719, and 946 bp).

[12] If the relative increase in  $\sigma_b$  is an indirect measure of microbial activity or the extent of biologically mediated alterations of the geoelectrical properties of the sediments and pore-fluids, then our data suggest that the residual and diesel saturated sediment layer was the most biologically active and the most biogeochemically altered. This finding is significant since most field geochemical studies of organic contaminant degradation are primarily focused in the water saturated zone [e.g., *Hunkeler et al.*, 2002] with little work done within the residual and free phase hydrocarbon contaminated sediment layers using traditional water chemical analyses. Thus, the increase in  $\sigma_b$  within the free diesel and residual diesel saturated layers observed in this study and reported in previous work [*Werkema et al.*, 2003] may provide insights into biogeochemical changes where traditional water chemical analyses cannot be conducted due to the relatively high diesel saturation and underscore the importance of this study.

#### 4. Conclusions

[13] The purpose of this investigation was to further our understanding of the processes responsible for the higher  $\sigma_b$  observed at some hydrocarbon contaminated sites. Results from a mesoscale laboratory column experiment shows that higher  $\sigma_b$ , higher numbers of oil degrading microbial populations, shifts in microbial community structure, and elevated concentrations of cations occur within the contaminated column. The greatest increase in  $\sigma_b$  occurred in the depth zones where the diesel contamination was in the free and residual phase, similar to field observations. The similarity of our laboratory results (i.e., stratification in the  $\sigma_b$  response and microbial population distribution and community structure) and observations from the field suggest the mesoscale laboratory experiment was able to replicate field observations. We infer from our results that the mechanism for the high  $\sigma_b$  observed in hydrocarbon contaminated sediments is in part related to enhanced mineral weathering from metabolic by-products due to microbial degradation of the hydrocarbon. The results from this study suggest that geophysical methodologies may potentially be used to investigate microbial activity and/or geomicrobiological processes. However, more research is needed to further our understanding of microbial-rock or sediment interactions and geophysical properties.

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