The Effects of LNAPL Biodegradation Products on Electrical Conductivity Measurements

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ABSTRACT

Field geophysical Studies have identified anomalously high conductivities in and below the free product Tone at many sites with aged contamination by light. non-aqueous phase liquid\ (LNAPL). Laboratory experiments were conducted to test the hypotheses that these anomalously high conductivities can result from products of LNAPL biodegradation. Soil from a hydrocarbon-impacted site with anomalously high conductivities was washed repeatedly IO remove soluble constituents, re-contaminated with diesel fuel (DF), and the port\ tilled with water to simulate a saturated smear Tone. Nutrients were provided at level? observed at the site. which resulted in anaerobic conditions due to DF biodegradation. Within 121) days. the increase in specific conductivity from microbial activity was 2, 100 μS/cm. caused by an increase in total dissolved solids (DS) of* over 1,700 mg/L. The increase in DS was due to mineral (mostly carbonate) dissolution and to the production of organic acids and biosurfactants. Under aerobic conditions (i.e., without added nutrients) products of DF biodegradation increased the total DS and conductivity by 340 mg/L and 440 μS/cm. respectively. The results show that products of LNAPL biodegradation can drastically increase the conductivity at impacted sites.

Introduction

The collection. preparation. and analysis of ground water samples at contaminated sites constitute a major portion of the total cost for remediation (Granato and Smith, 1999) Geophysical surveys using resistivity and ground penetrating radar (GPR) are convenient, non-invasive tools 10 detect and map subsurface contamination with light, nonaqueous phase liquid\ (LNAPL). Recent reports suggest that LNAPL biodegradation can change biogeochemical properties sufficiently to have a significant impact on resistively and GPR measurements (Sauck, 2000, Werkema *et al.* 2000: Atekwana *et al.* 1998, 1999). If the geophysical responses caused by these Microbially-induced changes in pore water biogeochemistry can be better understood geophysical measurements could possibly be used to monitor contaminants and their breakdown products in the subsurface. This could allow resistivity techniques to be used as a surrogate or ground-water sampling and analysis to achieve lower cleanup costs.

Typical products of LNAPL biodegradation ale acids and biosurfactants. Carbonic and organic acids are produced during LNAPL biodegradation (Cozzarelli *et al.* 1990, 1994 1995; Eaganhouse *et al.* 1993; Hiebert *et al.* 1995: Baedecker *et al.* 1993; McMahon *et al.* 1995). These products increase conductivity directly by increasing the dissolved solids (DS) concentration, and indirectly by promoting mineral dissolution (Hiebert *et al.* 1995; McMahon *et al.* 1995). High DS concentrations in LNAPL-impacted zones have been invoked to explain anomalously low bulk electrical resistivity (Sauck *et al.* 1998; Bermejo *et al.* 1997). However. it has not yet been shown that temporal changes in geoelectrical properties of* pore water occur due to LNAPL biodegradation.

Biosurfactants are produced by many genera of soil micro-organisms during growth on NAPL (Alexander. 1994; Miller. 1995: Desai and Banat. 1997). When present at concentration above the critical micelle concentration (CMC). Surfactants product microemulsions of NAPL m water. Biosurfactant are produced by aerobic and anaerobic microorganisms (Desai and Banat. 1997: Cooper *et al.* 1980; Mclnerney *et al.* 1990). Biosurfactants increase DS concentrations. Perhaps more importantly. emulsion of NAPL resulting from biosurfactants could promote a change in conditions from LNAPL-wetted to water-wetted. This can increase the contact area between water and solids. providing more nutrients and promoting further biogeochemical changes. Hence, biosurfactant production he> the potential to impact both resistivity and GPR measurements tremendously. While biosurfactants have been linked with NAPL biodegradation in mixed soil reactors (Cassidy, in press). in situ biosurfactant production and NAPL emulsification have not been demonstrated.

The efficacy of resistivity surveys rests in a high electrical resistivity of LNAPL relative to subsurface materials. This "insulating layer" model has been verified in short-term laboratory and controlled spill experiments (Schneider and Greenhouse. 1992). However, investigations at numer-

ous sites with aged contamination show that the LNAPL smear zone has a lower resistivity (higher conductivity) than the bulk formation (Atekwana *et al.*, 1998, 1999; Benson and Stubben, 1995; Gajdos and Kral, 1995; Sauck. 1998). It has been hypothesized that anomalously low apparent resistivities are the result of LNAPL biodegradation (Atekwana *et al.* 1999: Sauck, 2000). However, to confirm this hypothesis changes in geoelectrical properties must be co! related with biodegradation products over time. This paper describes laboratory experiments designed to correlate temporal changes in the concentrations of diesel fuel (DF) degradation products with changes in specific electrical conductivity. under aerobic and anaerobic conditions. The production of biosurfactants and the resulting emulsification of DF were also monitored.

Materials and Methods

The soil was obtained from a hydrocarbon-impacted site described by Atekwana *et al.*. (1999) and Werkema *et al.*. (2000). The contaminated soil was washed five times with deionized water to remove soluble constituents. Fresh, no. 2 diesel fuel (DF) was mixed into the soil. The reactor-x consisted of 20-L plastic vessels. Approximately 18 L of soil was packed into each reactor, and deionized water was added to bring the water level up to the surface of the soil. A visible NAPL layer was present after adding water. The reactor setup was designed to simulate conditions in the saturated smear zone. A slotted, fully-penetrating PVC tube allowed composite pore water samples to be drawn and probes to be inserted into the saturated zone.

Duplicate reactors of three types were maintained for 120 days; one with added nutrients, one without added nutrients. and one "killed" (autoclaved) control without nutrients. Nutrients (4 mg/L NO_3-N, $_4$ mg/L NH,-N. and I mg/L PO,-P) were added to the reactors with the deionized fill water. These nutrient concentrations arc similar- to those observed at the site. Addition of nutrients resulted in anaerobic conditions within 10 days. The reactor with added nutrients was labeled "anaerobic." Anaerobic conditions also predominate at the bite. The reactor without added nutrients maintained aerobic conditions and was labeled "aerobic."

Dissolved oxygen (DO), pH. and conductivity were measured in situ. Pore water samples were drawn to quantify volatile organic acids (VOA), aqueous DF concentration. surface tension (ST), biosurfactant concentration, and C^{2-} concentrations. The samples were first passed through a 0.45-μm Whatman filter paper to remove suspended solids and non-emulsified DE Emulsified DF passes through this filter and is measured as aqueous DE Volatile organic acids (VOA) and Ca^{2-} concentrations were quantified with *Standard Methods* 5560-B and 35000 D. respectively (Eaton *et al*. 1995). ST and concentrations of aqueous DF
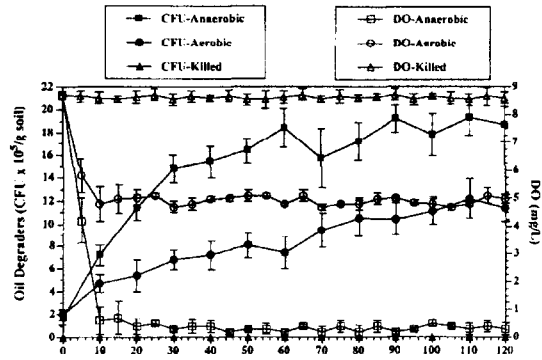

Figure 1. Average concentrations of oil degrading mi croorganisms and DO with time.

and biosurfactants. were measured according to Cassidy (in press). Biosurfactant concentration was measured using critical micelle dilution, which provides units of "times the critical micelle concentration" (x CMC). Dissolved solids (DS) were measured on filtrate at the end of I20 days. Total. inorganic. and organic DS were measured using Standard Methods 2540-B & C (Eaton *et al.*, 1995). The concentration of DF-degrading microbes was quantified as described by Werkema *et al.* **[2000].**

Results and Discussion

Figure 1 shows the average measurements of oil-degrading microorganisms and DO with time. The killed controls showed no decrease in DO from the saturation concentration of 8.5 mg/L throughout the entire experiment. The killed reactors also had microbial concentrations that were essentially zero throughout the experiment. This shows that autoclaving was successful ill killing the micro organisms. In contrast, the biologically active reactors showed significant increases in microbial concentrations and decreases in DO within the first 5 to 10 days. These results show that there was considerable aerobic microbial activity in the biologically active systems. It can be concluded that this microbial activity was driven by DF biodegradation, since DF was the only major food source available. The greatest increase in CFU and decrease in DO was observed in the anaerobic systems, because adding nutrients promoted more biological activity than was possible in the aerobic systems. The anaerobic reactors showed an increase in the number of oil-degrading microbes from roughly 2 X10^5 CFU/g to 1.9 X 10^6 CFU/g after 120 days. This represents an increase of nearly an order of magnitude. DO in the anaerobic reactors decreased to less than 0.5 mg/ L within 10 days, and remained at this concentration thereafter. The aerobic reactors showed an increase in the number of oil degrades from roughly 2 x 10^5 CFU/g to
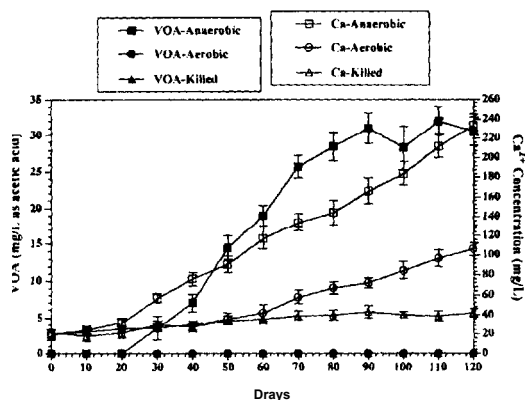
Figure 2. Average concentrations of VOA and Ca²⁺ with time.

Figure 3. **Average values of surface tension, aqueous DF concentration, and biosurfactant concentration with time.**

to 1.3 X 106 CFU/g during the experiment. The DO in the reactors without added nutrients reached a steady state concentration of approximately 5 mg/L, which is considered aerobic.

The anaerobic reactors were designed to simulate conditions observed in the subsurface at the hydrocarbon impacted site from whence the soil was obtained (Werkema *et al.*, 2000) and at many such sites. Nutrients were provided at concentrations observed at the site, where the presence of sufficient nutrients and hydrocarbons have resulted in anaerobic conditions (*i.e.,* DO < 0.5 mg/L) caused by microbial activity (Werkema PI al., 2000). Anaerobic conditions exist in the saturated zone at nearly all LNAPL impacted site& (Alexander, 1994). Nitrate (NO³⁻), which is present at the site and was added to the anaerobic systems. promotes the growth of denitrifying bacteria that degrade DF by providing a necessary electron acceptor. It is important to remember that aerobic reactions occur in anaerobic systems along with anaerobic reactions. which is what continually consumes oxygen and maintains anaerobic conditions. Therefore, both aerobic and anaerobic reactions took place in the anaerobic systems. The aerobic reactors were maintained as a control to observe the effects of nutrients at the site on biogeochemistry and conductivity. and to simulate a nutrient-starved, aerobic site. Killed reactors provided an abiotic system for comparison with the biologically active reactors.

The average values for measurements of VOA and Ca²⁺ concentrations are plotted in Fig. 2. VOA levels were zero throughout the experiment in the killed and aerobic systems. However, VOA concentrations in the anaerobic systems began to increase on day 20 and stabilized at values between 30-35 mg/L as acetic acid between days 90 and 120. Since VOAs are biodegradable, an accumulation of VOA indicates that rates of production exceed rates of deg-
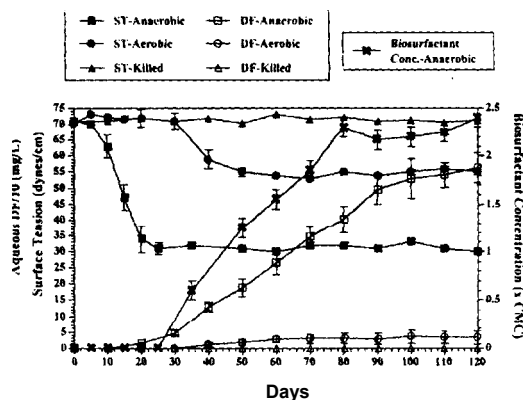
radation during that time. Concentrations of Ca" started at a background value of approximately 25 mg/L and increased with time in all the reactors. Increases in Ca" concentrations with time were probably to due to dissolution of carbonates, which comprised approximately 2% (by weight) of the soil. The lowest levels of Ca²⁺ at the end of the experiment (40 mg/L) were observed in the killed reactors, which represent\ Ca²⁺ concentrations achieved by carbonate dissolution in the absence of microbial activity. In contrast. the Ca²⁺ concentration in the aerobic and an-aerobic systems reached levels of 105 mg/L and 235 mg/ L, respectively.

The results in Fig. 2 show that microbial activity promoted the dissolution of carbonates, most pronounced under anaerobic conditions. The greater dissolution of carbonates achieved in the anaerobic systems is consistent with the accumulation of VOA and the overall enhanced micro-bial activity in these reactors compared with the aerobic reactors. While VOA was not detected in the aerobic reactors, it may have been present in low levels. CO_2 is another common product of microbial activity (not measured in this study), and its production is proportional to overall microbial activity (aerobic and anaerobic). Considering that more microbial activity was observed in the anaerobic systems than the aerobic ones, and that both aerobic and anaerobic reactions took place in the anaerobic systems, it is likely that more CO_2 was produced in the anaerobic systems than the aerobic ones. CO_2 and organic acid production has been demonstrated at LNAPL-impacted sites (Cozzarelli *et al.* 1990, 1994, 1995; Eaganhouse *et al.*, 1993; Hiebert *et al.* 1995: Baedecker *et al.* 1993; McMahon *et al.* 1995) and is known co enhance the dissolution of carbonate\ and other minerals.

Figure 3 shows the average values of biosurfactant

related measurements: ST and concentrations of aqueous

DF and biosurfactants. Killed controls showed no significant decrease in surface tension from 72 dynes/cm (the value for distilled water al 25#C) throughout the entire experiment. In contrast. ST measurements in the anaerobic reactors decreased from 72 dynes/cm to approximately 30 dynes/cm after 20 days. and remained al these levels until the end of the experiment. ST in the aerobic systems decreased after day 30 IO values between SO and 55 dynes/ cm, where they remained for the remainder of the experiment. Aqueous DF concentrations were zero for the first 20-X) days in all the reactors, and remained zero throughout the experiment in the killed reactors. The aqueous concentration of DF is roughly 5 mg/L (Testa and Winegardner, 1991). hut DF sorbs readily to soil, which explains the initial absence of DF in the aqueous phase. Aqueous DF concentration\ in the aerobic systems increased after day 30 to final values of nearly 5 mg/L. Aqueous DF concentrations in the anaerobic systems increased dramatically after day 20. reaching values of 550 mg/L (over 100 times the aqueous solubility). Biosurfactant concentrations in the killed and aerobic systems (not shown) were below the CMC throughout the experiment. However, biosurfactant concentrations in the anaerobic reactors increased after day 20 to levels over 2 times the CMC during the last 40 days of the experiments.

The results in Fig. 3 show that biosurfactants were produced in the anaerobic systems to levels over twice the CMC. The drop in ST to 30 dynes/cm observed in the anaerobic reactors coincided with biosurfactant concentrations above the CMC. and is a clear indication of surfactant concentrations above the CMC (Zajic and Seffens, 1984; Desai and Banat. 1907). Common metabolic products (e.g., organic acids) are not able to reduce ST to 30 dynes/cm. even at concentrations above 10% (Zajic and Seffens. 1984). indicating that the low ST reached in the anaerobic systems was not due to the accumulation of such products. Another unmistakable sign of biosurfactant concentration observed in the anaerobic systems was the increase in aqueous; DF concentrations coinciding with increasing biosurfactant concentrations after day 20. Aqueous DF measurements two orders of magnitude greater than the solubility limit is explained by emulsification of DE Emulsified NAPL droplets are le\s than 0.1 +m in diameter (Miller, 1995). and can pass through the 0.4%-μm filter, whereas non-emulsified NAPL cannot. Biosurfactant are biodegradable and sorb readily to soil (Miller, 1995: Desai and Banat. 1997). so the accumulation of biosurfactants in the anaerobic systems indicates that the rate of production exceeded rates of biodegradation and sorption. While biosurfactant concentrations above the CMC were not measured in the aerobic systems. ST values decreased and aqueous DF concentrations increased noticeably. This suggests that biosurfactants may have been in excess of the CMC in
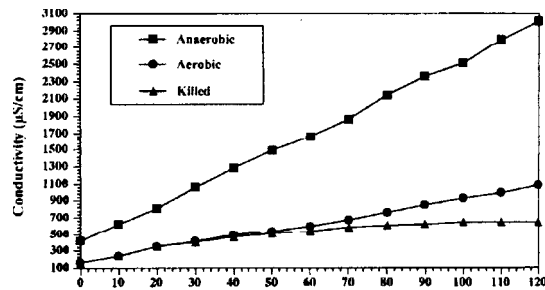

Figure 4.   **Average values of specific electrical conductivity with time.**

some pores. but that the concentrations dropped to below the CMC by dilution during sampling. Increasing aqueous DF concentrations with time in the aerobic systems support this. Numerous aerobic and anaerobic species produce biosurfactants (Zajic and Seffens, 1984: Desai and Banat, 1997). However. this study is the first to demonstrate in situ biosurfactant production accompanying microbial growth on NAPL.

Figure 4 shows the average values of specific conductivity measurements in pore water r:lmple\ over time. Conductivity increased in all reactors, hut the increase was by far the greatest in anaerobic systems. The initial conductivity of the anaerobic systems began at approximately 420 μS/cm. which was considerably higher than m the killed and aerobic reactors because of the added nutrients. Conductivity then increased in the anaerobic systems to over 3,000 μS/cm after 120 days. The initial conductivity in the killed and aerobic reactors was approximately 150 μS/cm. Conductivity increased to final values of roughly 650 μS/cm and l.100 μS/cm in the killed and aerobic systems. respectively. Since conductivity increases in the killed reactors are strictly from abiotic processes. subtracting these values from those observed in the biologically active systems gives an estimate of the increases in conductivity due to DF biodegradation processes in those systems. After 120 days in the killed reactors, conductivity increased 500 μS/cm (650 μS/cm- 150 μS/cm). Subtracting this value from the conductivity increases observed after 120 days in the aerobic systems (2,600 μS/cm) and anaerobic systems (950 μS/cm). yields an increase in conductivity of the aerobic and anaerobic systems of approximately 2,100 μS/cm and 450 μS/cm, respectively. From this analysis it is clear that specific conductivity increased roughly 4.5 times more in the anaerobic reactors than in the aerobic ones. This IS consistent with enhanced microbial activity, greater VOA production and carbonate dissolution. and enhanced production of biosurfactants in the anaerobic systems relative to the aerobic ones (Figs. 1-3).

Table 1. Measurements of dissolved solids in the pore waters from the three systems at the conclusion of the 120-day experiment.

Measurement	Anaerobic	Aerobic	Killed
Total DS (mg/L)	2130 ± 64 (8)[a]	758 ± 46 (8)	416 ± 28 (8)
Inorganic DS (mg/L)	1518 ± 54 (8)	602 ± 35 (8)	416 ± 28 (8)
Organic DS (mg/L)	612 ± 36 (8)	156 ± 31 (8)	0
Inorganic DS/Organic DS	2.5	3.9	NA
Total DS/Conductivity	0.72	0.69	0.64

[a] mean ± standard deviation (number of measurements). NA = not applicable.

Bulk conductivity was not measured in the reactors. However, specific conductivity is the major variable affecting bulk conductivity measurements, as described by Archie's Law. Using a soil porosity of 0.4 (common for un consolidated sands) and other commonly used values for Archie's Law the ratio of specific conductivity to bulk conductivity is 10 (Telford *et al.*, 1990). This means that the increase in specific conductivity observed in the anaerobic systems due to DF biodegradation processes was 210 μS/cm (2,100 S/cm/10). This represents a significant increase in bulk conductivity measured in the field. Furthermore, the reactors in this study were only operated for 120 days. These results clearly show that biodegradation of DF can have a significant effect on bulk conductivity measurements at NAPL-impacted sites.

Table 1 lists results from the dissolved solids (DS) measurements of pore waters from the three systems at the end of the 120-day experiment. Total DS concentrations ranged from 416 mg/L in the killed controls to 2,130 mg/L in the anaerobic reactors. The value in the killed control (416 mg/L) was strictly due to abiotic processes, and subtracting this value from the total DS concentration in the anaerobic and aerobic systems gives an indication of the increase in those system due to DF biodegradation processes. This analysis yields an increase in total DS due to DF biodegradation in the anaerobic and aerobic systems of 1,714 mg/L and 342 mg/L, respectively. The organic DS concentration in the killed controls was zero, which is supported by the lack of microbial activity and lack of associated products (Figs. 1–3). The ratios of inorganic DS to organic DS concentrations in the anaerobic and aerobic reactors were 2.5 and 3.9, respectively. The lower ratio for the anaerobic system is explained by the greater concentrations of biosurfactants and emulsified DF (Fig. 3). The ratio of total DS concentrations to conductivity in the reactors on day 120 ranged from 0.64 to 0.72. These ratios are within the range of 0.55–0.75 reported for a survey of natural groundwaters (Hem, 1970).

The results clearly show that products of DF biodegradation in the reactors increased with increasing microbial

activity and were greater for the anaerobic systems (with added nutrients) than for the aerobic ones (without added nutrients). DF biodegradation resulted in an increase in inorganic DS due to mineral dissolution and in organic DS due to organic acid and biosurfactant production. The increase in DS resulted in increases in conductivity. The anaerobic reactors simulated subsurface conditions at a LNAPL-impacted site with anomalously high conductivities (Atekwana *et al.*, 1999; Werkema *et al.*, 2000). The results from this study show that products of LNAPL biodegradation can explain those anomalous resistivities.

Conclusions

The results show that LNAPL biodegradation, under aerobic and anaerobic conditions, can substantially change pore water biogeochemistry, producing dramatic increases in electrical conductivity. These results help explain anomalously high conductivities reported in recent geophysical investigations at several LNAPL-contaminated sites. It has been known for some time that LNAPL biodegradation produces CO_2 and can produce organic acids, which enhance mineral dissolution. However, this study is the first to link these biogeochemical processes to enhanced pore water conductivity. This is also the first study demonstrating in situ biosurfactant production and LNAPL emulsification. The results suggest that biosurfactant production can accelerate a change in conditions from NAPL-wetted to water-wetted solids over time. The resulting increase in water/solids contact area could affect resistivity and GPR measurements.

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