

CHANGES IN GEOELECTRICAL PROPERTIES ACCOMPANYING MICROBIAL DEGRADATION OF LNAPL

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ABSTRACT

Field geophysical studies have identified anomalously high conductivities within and below the free product zone in soils at sites with "aged" contamination by light, non-aqueous phase liquids (LNAPL). Laboratory experiments and simple numerical modeling studies were conducted to test the hypothesis that these anomalously high conductivities result from products of LNAPL biodegradation. These experiments consisted of 20-L glass vessel reactors with 18 L of inoculated sand and 6 L of pore water. Diesel fuel was added to the top of the sand. These experiments simulated a smear zone with a top layer of LNAPL. Duplicate reactors of the following types were maintained for 120 days; nutrients added (at levels observed in the field), no nutrients added, killed (autoclaved) control with nutrients. The killed control showed no signs of diesel fuel biodegradation. The biologically active reactors showed evidence of diesel fuel biodegradation (e.g., reduced dissolved oxygen, increasing numbers of oil-degrading microbes). Diesel fuel biodegradation was accompanied by increases in the concentrations of volatile organic acids, calcium ion, pore water conductivity, total dissolved solids, surfactant production, and diesel fuel emulsification. These results are complemented by the numerical modeling results, which also showed potential enhancements in aqueous phase conductivity of 3700 $\mu\text{S}/\text{cm}$ when complete mineralization is assumed. However, the more realistic equilibrium models predict enhancements in the 500-1500 $\mu\text{S}/\text{cm}$ range. From this study, two important observations are made that have significant ramifications on the measured geoelectrical properties at aged LNAPL sites. First, microbial degradation of LNAPLs produces a variety of acids that enhance chemical weathering of the aquifer materials, resulting in high TDS content and thereby increasing the conductivities of the pore waters. Second, emulsification of the LNAPL by surfactant production has the potential to change the wetting phase from LNAPL wetted to water wetted, providing electrically conductive paths within LNAPL saturated zones. Both of these observations are consistent with our field investigations where we have reported conductivity values 2-5 times background values from contaminated zones. Finally, our field studies have also shown that the LNAPL saturated zone is conductive and not resistive. Thus, the above laboratory experiment and numerical modeling results demonstrate that LNAPL biodegradation readily explains the temporal changes in conductivity observed in geophysical investigations of impacted aquifers.

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Introduction

The potential for the use of geophysical techniques (mostly geoelectrical methods) for the detection of organic contaminants has been demonstrated frequently by modeling (e.g., Endres and Redman, 1996), laboratory measurements (e.g., Piggott, et al., 1998), controlled spill experiments (e.g., Daniels et al., 1992; Schneider and Greenhouse, 1992; DeRyck et. al., 1993; Redman et al., 1994; Kim et al., 2000); and field studies (e.g., Monier-Williams, 1995; Sauck et al., 1998; Atekwana et al., 2000; Lucius, 2000; Werkema et al., 2000). The efficacy of geoelectrical techniques rests in the high electrical resistivity of LNAPL relative to subsurface solids and pore water, which results in a distinctive geoelectrical signature of the impacted media (Schneider and Greenhouse, 1992; DeRyck et. al., 1993). However, field investigations at sites with "aged" contamination suggest the contrary. At many of these sites, results show unexpectedly higher apparent conductivities (lower apparent resistivities) (e.g., Benson and Stubben, 1995; Gajdos and Kral, 1995; Monier-Williams, 1995; Sauck et al., 1998) and attenuation of GPR signals (often described as "fuzzy", "muted" reflections or "shadow zones") coincident with the impacted zones (Grumman and Daniels, 1995; Maxwell and Schmok, 1995; Bermejo et al., 1997; Sauck et al., 1998; Atekwana et al., 2000). It has been proposed that these anomalously low apparent resistivities may be the result of LNAPL biodegradation over time (Sauck et al., 1998; Sauck 2000).

Recently, Werkema et al. (2000) have reported an orders-of-magnitude increase in microbial abundance in the LNAPL smear zone at a depth coincident with lower apparent resistivity. These results are further substantiated by higher groundwater specific conductance values ($>1100 \mu\text{S/cm}$) from wells within the impacted zones compared to uncontaminated locations ($<300 \mu\text{S/cm}$). This circumstantial evidence links LNAPL biodegradation to anomalously low apparent resistivities. However, to confirm the hypothesis that LNAPL biodegradation can affect subsurface electrical properties, changes in geoelectrical properties must be correlated with biodegradation products over time. This paper describes laboratory experiments and numerical modeling studies designed to correlate temporal changes in diesel fuel degradation products with changes in pore water conductivity, under aerobic and anaerobic conditions.

MATERIALS AND METHODS

Experimental Reactors

The reactors consisted of 20-L glass vessels filled with 18 L of sand from a hydrocarbon-impacted site. The soil was washed several times to remove soluble constituents, then mixed together with fresh diesel fuel in a 10-gallon portable cement mixer. De-ionized water was then added to the soil after it was placed in the reactor to ensure that the sand was saturated. As such, the experimental reactors simulated a saturated LNAPL-smear zone. A slotted PVC tube extending the entire depth of the reactor allowed pore water samples to be collected and probes to be inserted into the reactors. Duplicate reactors of the following three types were maintained for 120 days; nutrients added, no nutrients added, and killed (autoclaved) without nutrients. The carbonate content and organic carbon of the sand mixture were measured using the gravimetric methods (*Standard Methods of Soil Analysis*, 1996). Nutrients were mixed into the soil in solid form (NH_4Cl , KNO_3 , K_2HPO_4 -Aldrich, Milwaukee, Wisconsin) to provide maximum concentrations

upon dissolution, similar to those observed at the test site (10 mg/L NO₃-N, 10 mg/L NH₄-N, and 5 mg/L PO₄-P).

Biogeochemical Analyses

Dissolved oxygen (DO), pH, and specific conductance were measured *in situ* by inserting the respective probe into the slotted PVC tube. Pore water samples were withdrawn to measure conductivity, volatile organic acids (VOA), emulsified diesel fuel, and biosurfactant concentrations. The samples were first filtered through a 0.45 µm Whatman filter paper to remove suspended solids and non-emulsified diesel fuel. VOAs were measured by chromatographic separation (*Standard Method* 5560-B, Eaton et al. 1995) and reported as mg/L as acetic acid. Calcium ion concentrations were measured using the EDTA titrimetric method (*Standard Method* 3500-Ca D, Eaton et al. (1995)). Emulsified diesel fuel concentration and surfactant concentrations were measured as described by Cassidy et al. (in press). The detection limit for diesel fuel was 0.5 mg/L. Surface tension decreases with increasing surfactant concentration until the critical micelle concentration (CMC) is reached, after which surface tension remains constant with increasing surfactant concentration. Therefore, surfactant concentrations can be measured relative to the CMC by diluting samples until the surface tension shows an increase. Surface tension was measured in increasing dilutions (1/1, 1/2 and 1/3, etc.) until it rose above 3 dynes/cm. Surfactant concentration is reported as the dilution resulting in an increase in surface tension, expressed as times the CMC (x CMC). Dissolved solids (DS) were measured on filtrate at the end of 120 days. Total, volatile (i.e., organic), and fixed (i.e., mineral or inorganic constituents) DS were measured using the gravimetric techniques (*Standard Methods* 2540-B and 2540-C) described by Eaton et al. (1995).

Microbial Quantification

The top 10 cm of the sand in the reactors was sampled every 10 days using a hollow stainless steel core (3.0-cm ID). Two cores were taken from different locations in each reactor per sampling event. Both cores were mixed thoroughly and 4 sub-samples were used to perform quadruplicate quantification of oil-degrading microorganisms. Cells were extracted from each 10-g sub-sample using a modification of the procedure described by Van Elsas and Smalla (1994). Sodium pyrophosphate (9.5 mL of a 0.1% solution) was added and the samples were shaken for 30 minutes at 28°C, followed by centrifuging at 1000 X g for 10 minutes. A 100-µL aliquot was taken and the appropriate serial dilutions were made in 1.5-mL micro-centrifuge tubes. Dilutions were then plated onto oil agar 2 (OA2), which was prepared using Noble agar (Difco) and diesel fuel as the hydrocarbon source, according to the method described by Walker et al. (1975). The plates were wrapped in parafilm during the first week of incubation to avoid desiccation and incubated for another 4 weeks at 28°C. After 5 weeks the colonies on each plate were counted and the colony forming units (CFU) per gram of soil were calculated.

Numerical Modeling

A simple numerical model was used to estimate the biogradation effects on pore water conductivity. Complete aerobic mineralization of toluene and benzene were initially assumed in the computation of the pore water composition; these models considered the effects of the presence of calcite. As a next step, carbonate equilibrium was incorporated

into the computation of the pore water composition. In all cases, the limiting factor was the amount of dissolved oxygen available in the pore water. Kohlrausch's Law (Marion and Lando, 1974) was used to determine the resulting aqueous phase conductivity.

RESULTS

Several first-order results can be demonstrated from the laboratory experiments (Figures 1-4). Figure 1 shows the counts of oil-degrading microorganisms represented as

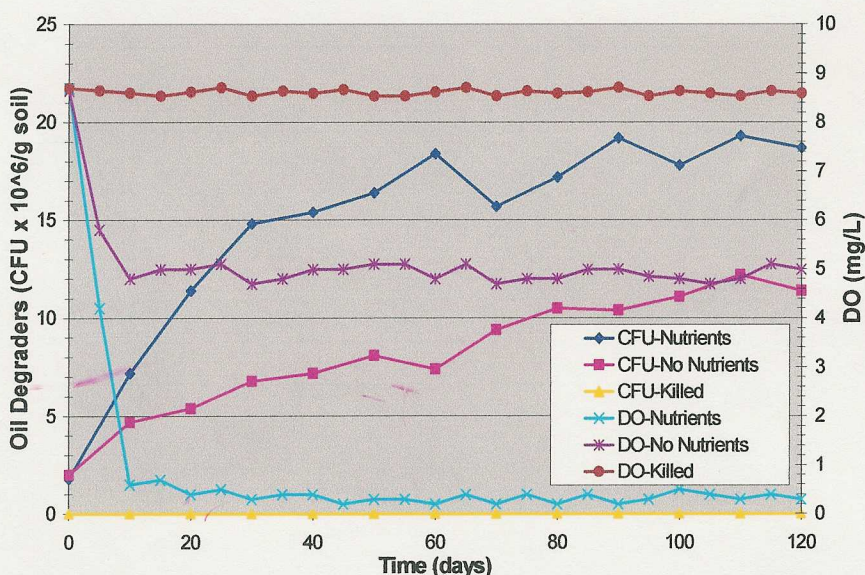


Figure 1. Plot of oil-degrading microorganisms represented as colony forming units (CFU) and dissolved oxygen (DO) with time.

colony forming units (CFU) and dissolved oxygen (DO) with time. The killed controls showed no CFU and no decrease in DO from the saturation concentration of 8.5 mg/L throughout the entire experiment. In contrast, the biologically active reactors showed a significant increase in CFU and a decrease in DO within the first 5 to 10 days. The greatest increase in CFU and decrease in DO was observed in the reactors with added nutrients. The active reactors with nutrients showed an increase in the number of oil-degrading microbes from approximately 2×10^6 CFU/g soil to approximately 19×10^6 CFU/g soil after 120 days. Similarly, the DO in these reactors decreased from 8.5 mg/L to less than 0.5 mg/L within 20 days, and remained at this concentration throughout the experiment (Table 1).

The results for organic acids (VOA) and calcium concentrations are shown in Figure 2. The calcium ions are suggested to result from dissolution of the carbonate sands used in the experiments. As expected, the killed controls showed non-detectable levels of VOA and a slight increase in calcium ion concentration (40 mg/L). In contrast, significant changes were observed in biologically active reactors, and the greatest changes occurred in the reactors with added nutrients. After a 20-day lag period, VOA accumulated in the active reactors with added nutrients to a concentration of approximately 30 mg/L as acetic acid on day 120 (Table 1). This change is complemented by significant increases in calcium ion concentrations up to 220 mg/L. The reactors without added nutrients also showed below detectable VOA levels during the entire experiment but showed an increase in calcium ion concentrations up to 105 mg/L.

| Measurement | Nutrients | No Nutrients | Killed Control |
|-----------------------|-------------------------------|-------------------------------|-------------------------------|
| Oil-degraders (CFU/g) | $1.7 \times 10^6 \pm 0.4$ (4) | $1.1 \times 10^6 \pm 0.3$ (4) | $2.4 \times 10^2 \pm 0.6$ (4) |
| Total DS (mg/L) | 2130 ± 64 (8) | 758 ± 46 (8) | 416 ± 28 (8) |
| Fixed DS (mg/L) | 1618 ± 54 (8) | 602 ± 35 (8) | 416 ± 28 (8) |
| Volatile DS (mg/L) | 512 ± 36 (8) | 156 ± 31 (8) | 0 |
| Total DS/Conductivity | 0.72 | 0.69 | 0.64 |

Table 1. Measurements in the reactors at the conclusion of the 120-day experiment. mean \pm standard deviation (number of measurements).

However, these values are significantly lower than those measured for the reactors with added nutrients. We suggest that the increase in calcium ion concentrations in both the

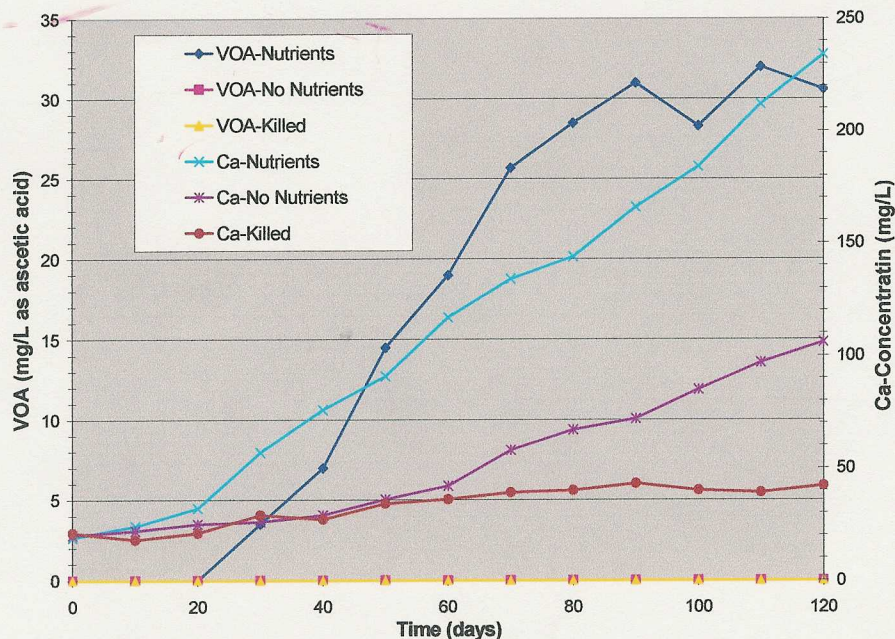


Figure 2. Plot of volatile organic acids (VOA) and calcium concentrations.

In the field, we have measured significant increases in VOA from contaminated locations with values up to 16 mg/L compared to below detectable levels from uncontaminated locations.

killed reactors and reactors with no nutrients may result from normal geochemical dissolution processes common in uncontaminated aquifers. However, these processes will be significantly enhanced in the presence of VOA as demonstrated by the reactors with nutrients.

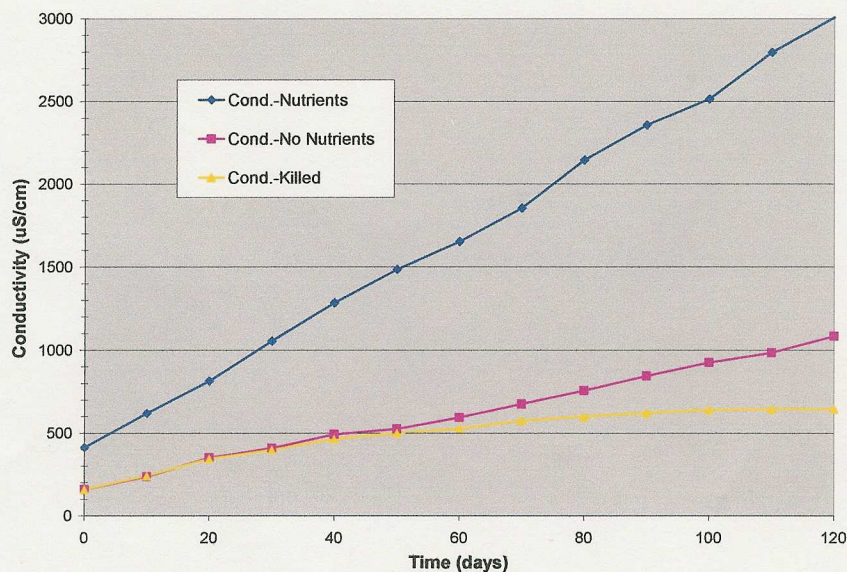


Figure 3. Measurements of bulk conductivity with time.

approximately 600 $\mu\text{S/cm}$, and then remained at this value until the end of the experiment. In reactors without added nutrients, specific conductance increased steadily over the 120 days to a final value of approximately 1100 $\mu\text{S/cm}$. The specific conductance in the reactors with added nutrients increased more rapidly to a value of approximately 3000 $\mu\text{S/cm}$ over the duration of the experiment. Increases in specific conductance are accompanied by increases in dissolved solids (TDS). The results for the dissolved solids (DS) measurements of pore water samples at the end of the 120-day experiment are listed in table 1. As expected, the TDS value from the reactors with nutrients added is significantly higher (1917 mg/L) than values from the killed and reactors with no nutrients added.

These results are complemented by the numerical modeling results. Numerical modeling based on complete aerobic mineralization found an aqueous phase conductivity enhancement

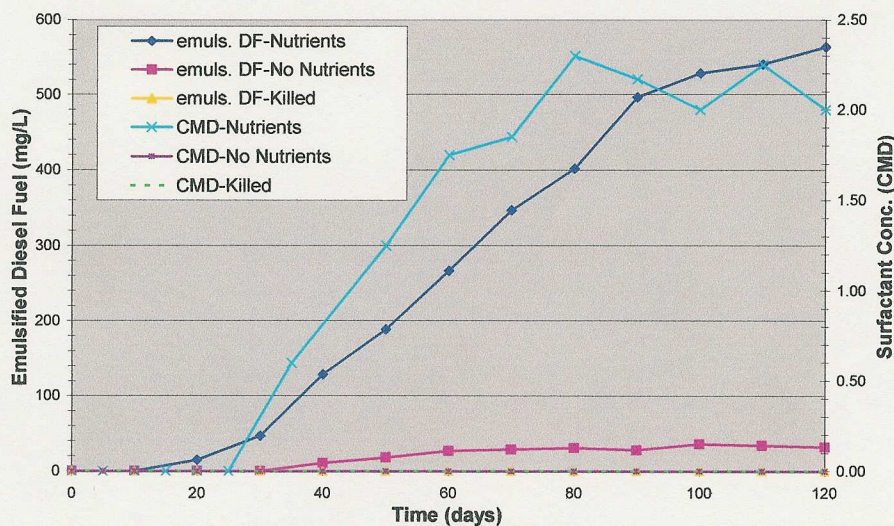


Figure 4. Concentrations of surfactant and emulsified diesel fuel.

Figure 3 shows the measurements of the specific conductance. The conductivity of the pore water at time zero was approximately 100 $\mu\text{S/cm}$.

Conductivity measurements in the killed control reactors increased steadily over the first 80 days to a value of

enhancement of approximately 3800 $\mu\text{S/cm}$ when calcite was not present; this enhancement lowered to about 1900 $\mu\text{S/cm}$ in the presence of calcite. The more realistic models incorporating

carbonate equilibrium showed a conductivity enhancement in the range of 500-1000 $\mu\text{S}/\text{cm}$ for the typical pH values of groundwater.

The concentrations of surfactant and emulsified diesel fuel are shown in Figure 4. Surfactant concentrations were zero for the entire period in the killed control reactors. Measured concentrations of emulsified diesel fuel in the killed controls were all below 4 mg/L, which is consistent with a published range in values of 2 to 4 mg/L for aqueous diesel fuel solubility at 20°C (Testa and Winegardner, 1991). Considerable increases in both surfactant and emulsified diesel fuel concentrations were observed in the biologically active reactors. The greatest increase was observed in the active reactors with added nutrients. Moreover, the time profiles of surfactant and emulsified diesel fuel concentrations were remarkably similar for the reactors with added nutrients. In the reactors with added nutrients, the concentrations of surfactant and emulsified diesel fuel began to increase from background levels after 25 days. In the reactors without added nutrients, surfactant concentrations increased only slightly from background levels but were not accompanied by any increase in the emulsified diesel fuel concentrations.

DISCUSSION AND CONCLUSIONS

The results strongly suggest that diesel fuel was biodegraded in the biologically active reactors, and that adding nutrients enhanced rates of biodegradation. The significant increase in CFU of oil-degrading microbes and the decrease in DO relative to the killed controls (Figure 1) clearly show that aerobic microbial activity occurred in the biologically active reactors. Diesel fuel concentrations in the sand were not measured, nor were individual microorganisms identified. However, the organic content of the soil was less than 0.005% (by weight), indicating that there was no other carbon source in the sand besides diesel fuel that could have supported the observed biological activity. The results also demonstrate that the microbial degradation of diesel fuel caused considerable changes in pore water biogeochemistry.

The specific conductance and total dissolved solids (TDS) concentration of pore waters in the biologically active reactors increased by orders of magnitude relative to background values in the killed controls during the 120-day experiment (Figure 3, Table 1). Concentrations of total and fixed DS, and conductivity values increased somewhat in the killed controls (Table 1), likely due to dissolution of the highly soluble nutrients added ($(\text{NH}_4)_2\text{PO}_4$) and other minerals. The reactors without added nutrients showed concentrations of total and fixed DS somewhat higher than those observed in the killed controls, despite not having added nutrients to contribute to the electrolyte concentration. These results suggest that biodegradation reactions in the reactors without added nutrients were responsible for the increase in dissolved solids. The high fixed DS concentration in the reactors with added nutrients relative to the values for the other two reactor types is likely due to the dissolution of minerals. This would be consistent with the high VOA concentration (Figure 2), and the abundance of carbonates (8% by weight). The production of organic acids accompanying microbial colonization of soil surfaces has been shown to enhance mineral dissolution (Cozzarelli et al., 1990, 1994, and 1995; Bennett et al., 1993; Eganhouse et al., 1993; Hiebert et al., 1995; Baedecker et al., 1993; McMahon et al., 1995). As a result, the dissolved solids concentration in the pore water increases (Bennett et al., 1993; Hiebert et al., 1995; McMahon et al., 1995). Therefore,

the outcome of this mechanism is a significant increase in the conductivity of the contaminated zone.

At our field sites we have observed higher concentrations of VOA from contaminated locations and below detectable levels from background locations. This is consistent with field observations of concomitant higher groundwater conductivities 2-5 times greater in the contaminated locations compared to background regions. Thus our field observations are consistent with observations in the laboratory experiment. On this basis, it is suggested that LNAPL biodegradation can explain the occurrences of decrease bulk resistivities and attenuation of GPR reflections observed by field geophysical investigations at sites where significant biodegradation of the LNAPL has occurred.

Furthermore, the results clearly show that surfactants were produced in excess of the critical micelle concentration (CMC) during microbial growth on diesel fuel (Figure 4). When present at concentrations above the critical micelle concentration, surfactants can produce microemulsions of LNAPL in water, with micelle-contained LNAPL droplets having diameters less than $0.1\mu\text{m}$. The resulting increase in LNAPL surface area available for microbial uptake can increase biodegradation rates by orders of magnitude (Desai and Banat, 1997). Biosurfactants are produced under both aerobic and anaerobic conditions (Desai and Banat, 1997). Because surfactants contain a hydrophilic moiety, they contribute to the dissolved solids of waters. More importantly is the fact that the emulsion of LNAPL resulting from biosurfactant production would replace LNAPL-wetted solids with water-wetted solids, at least partially, providing conductive paths in the LNAPL saturated zone. This observation is very significant and would further contribute to the increased conductivity observed within the LNAPL saturated zone at our field sites. Therefore, such a change in wetting properties has the potential to tremendously impact both resistivity and GPR measurements. Moreover, pore water samples from our field site that provided the inoculum for the reactors in this study also showed indications of biosurfactant production similar to those reported here. Although we have inferred that the decrease in apparent resistivities within the LNAPL saturated zone observed in the field may be related to the biosurfactant production and emulsification of the LNAPL, at this time we cannot fully explain these relationships. We believe that this is significant enough to warrant further studies.

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