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Comparison of surficial CO_2 efflux to other measures of subsurface crude oil degradation

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ABSTRACT

At a spill site near Bemidji, Minnesota, crude oil at the water table has been undergoing anaerobic biodegradation for over 30 years. Previous work at this site has shown that methane produced from biodegradation of the oil migrates upward and is oxidized in a methanotrophic zone midway between the water table and the surface. To compare microbial activity measurement methods from multiple locations in the oil body, surficial carbon dioxide efflux, methanogen and methanotroph concentrations, and oil degradation state were collected. Carbon dioxide effluxes over the oil body averaged more than four times those at the background site. Methanotrophic bacteria concentrations measured using pmoA were over 10^5 times higher above the oil-contaminated sediments compared with the background site. Methanogenic archaea measured using mcrA ranged from 10^5 to over 10^7 in the oil and were below detection in the background. Methanogens correlated very well with methanotroph concentrations ($r = 0.99$), *n*-alkylcyclohexane losses as a proxy for degradation state ($r = -0.96$), and somewhat less well with carbon dioxide efflux ($r = 0.92$). Carbon dioxide efflux similarly correlated to methanotroph concentrations ($r = 0.90$) and *n*-alkylcyclohexane losses ($r = -0.91$).

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1. Introduction

Subsurface microbial activity at contaminated sites has been measured using a variety of techniques including molecular methods, contaminant mass loss rates, and production of degradation products (Weiss and Cozzarelli, 2008). However molecular methods do not always clearly correlate with other measures of activity (e.g., Röling, 2007). In this study, we examined the relationship among biodegradation populations, contaminant mass losses, and degradation products in the vadose zone at a crude oil spill site near Bemidji, Minnesota.

The crude oil source zone at the study site constitutes a coupled reaction system in which methane (CH_4) is produced in the oil body at the water table and oxidized in the overlying vadose zone. The entire supply of methane diffusing upward from the crude oil contaminant source is oxidized to carbon dioxide (CO_2) and water in the vadose zone. Thus, methane oxidation rates should be directly proportional to methane flux from the underlying oil, assuming steady-state microbial concentrations. Methane flux is difficult to infer from gas profiles because of variations in sediment diffusivities (Molins et al., 2010). However, because the methane is completely oxidized, CO_2 efflux measured at the surface can be related to the methane flux from the source despite subsurface reactions and dissolution. Previous studies have established that surficial CO_2 efflux at the Bemidji site varies with location (Sihota et al., 2011), suggesting spatial variations in degradation rates of the underlying contaminant source.

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We selected a range of sites with varying surficial CO_2 efflux and measured four parameters expected to reflect methane production and oxidation rates: surficial CO_2 efflux, population densities of methanogens and methanotrophs using molecular methods, and crude oil degradation state. Microbial concentrations were measured using quantitative Polymerase Chain Reaction (qPCR), a method to determine microbial concentrations using function-specific DNA fragments (Heid et al., 1996) that has been shown to correlate with the Most Probable Number (MPN) growth method (Lavender and Kinzelman, 2009; Wright et al., 2007). qPCR has been shown to be less labor-intensive than the MPN method, but perhaps more importantly, qPCR data have been shown in some cases to correlate with reaction rates at other sites (Braker and Conrad, 2011; Wang et al., 2011). Thus, we measured concentrations of methanogens in the oil body to assess whether these correlate with variations in crude oil degradation rate. Also, methanotroph concentrations in the vadose zone were measured, since methane oxidation was expected to vary with methane production. Finally, the degradation state of the crude oil is a cumulative measure of degradation rates that have occurred since the spill in 1979 (e.g., Peters et al., 2007), so we present analyses of currently degrading hydrocarbon homologues from oil samples at each location.

2. Materials and methods

2.1. Site description

The study site, located near the town of Bemidji, MN, USA, was contaminated in 1979 when a high pressure pipeline ruptured spilling about 1.7×10^6 L (10,700 barrels) of crude oil. Despite a major cleanup effort, about 25% of the spilled oil infiltrated into the surficial aquifer and is now present in the unsaturated zone and at the water table (6–8 m below the surface) (Essaid et al., 2011). Fig. 1 shows a plan view map of the site near the largest subsurface oil body. The direction of groundwater flow is to the northeast. The surficial aquifer is an ~20-m-thick unconsolidated glacial outwash deposit of mostly sand and gravel with some local silt layers (Bennett et al., 1993). The microbial population within the sediments containing residual oil is dominated by fermenters and methanogens (Bekins et al., 1999). Previous results from the site showed that methane from methanogenic degradation of the subsurface oil diffuses upward where it mixes and reacts with oxygen from the surface at 2–4 m depth to create an active methanotrophic zone (Amos et al., 2005; Sihota and Mayer, 2012). Fig. 2 shows a conceptual model depicting the production of methane in the subsurface oil and upward diffusion through the vadose zone to the methanotrophic zone.

Biodegradation of *n*-alkanes under methanogenic conditions occurs at the Bemidji site (Bekins et al., 2005). Other studies have shown methanogenic biodegradation of *n*-alkanes at other sites (Siddique et al., 2006) as well as in laboratory microcosms (Anderson and Lovley, 2000; Zengler et al., 1999). Multiple lines of evidence indicate that methanogenic conditions have existed in the Bemidji oil body since 1988. These include aqueous data (Cozzarelli et al., 2001), gas data (Amos et al., 2005; Chaplin et al., 2002) and microbial data (Bekins et al., 1999, 2001, 2005). These methanogenic conditions are stable because oxygen in soil gas and recharge are consumed by methane oxidation in the vadose zone and do not reach the oil body at the water table

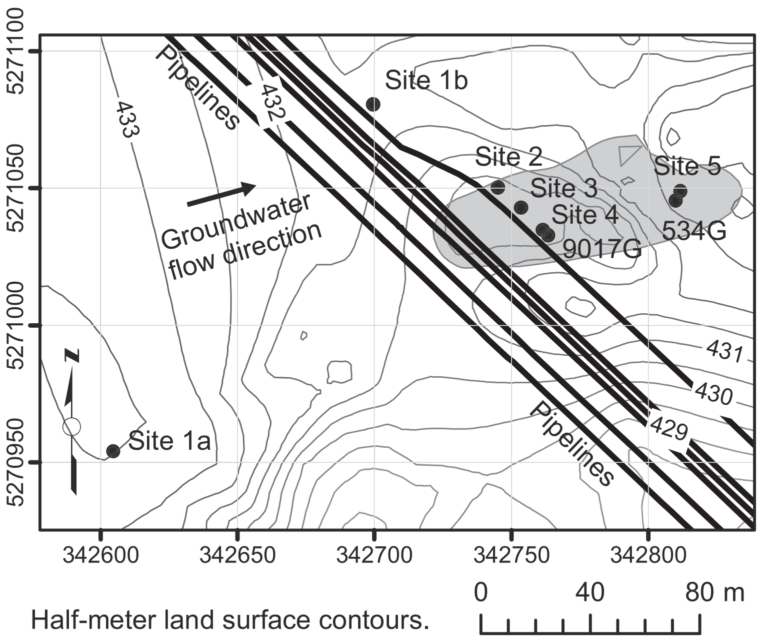

Fig. 1. Plan view of the Bemidji site showing surface topography (light lines with elevations above sea level (NAVD88) in meters), locations of the oil body (gray shading), pipelines (dark lines), wells (9017G, 534G) and sample sites (Sites 1a, 1b, 2, 3, 4, and 5), and direction of groundwater flow (arrow). The coordinate system is NAD 1983 UTM Zone 15 N.