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Low temperature soil petroleum hydrocarbon degradation at various oxygen levels

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ABSTRACT

A laboratory incubation study was conducted on a petroleum-contaminated soil from Macquarie Island in sub-Antarctic Australia to develop a target O_2 level for bioventing. The soil was amended with NH_4NO_3 (175 mg N kg^{-1} soil) and ^{14}C-hexadecane (250 mg kg^{-1} soil) and placed in sealed respirometry chambers. The headspaces in the chambers were adjusted to 0, 1, 2.6, 5.2, 10.5, and 20.9% O_2. Each chamber was connected to an NaOH CO_2 trap and to an O_2 feed line (except the 0% O_2 chambers were connected to an N_2 feed line). Chambers were supplied with O_2 in response to pressure drop resulting from CO_2 trapping. Soils were incubated at 6 °C for 12 weeks. O_2 consumption and petroleum degradation were maximized in chambers with 10.4% O_2. There was a slight decline in both O_2 consumption and petroleum degradation at 20.9% O_2. As O_2 concentrations declined below 10.4% O_2 both O_2 consumption and petroleum degradation declined markedly. ^{14}C collected in the CO_2 traps did not follow this pattern, but was greater in the 1% O_2 chambers than in 2.6 or 5.2% O_2 chambers. Nitrogen remaining at the conclusion of the study indicated that nitrate was completely consumed in the 0, 1, 2.6, and 5.2% O_2 chambers. nC_{17}:pristane and nC_{18}:phytane ratios in the soil at the conclusion of the incubation were significantly lower in the 10.4% O_2 chambers than in those with 20.9% O_2, and more petroleum hydrocarbons were consumed in the 10.4% chambers. Preferential degradation of pristane and phytane in the presence of limited O_2 may be the result of denitrification, evidenced by lower residual nitrate levels in the 10.4 than the 20.9% O_2 environment. Ten percent O_2 is suggested as a target for O_2 enhanced bioremediation.

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1. Introduction

Microbial oxidation of organic carbon and petroleum hydrocarbon soil contaminants is favored by the presence of molecular oxygen. Hydrocarbon oxidation processes generally require oxygenases (Atlas and Bartha, 1987) and typically occur more rapidly as oxygen levels increase (Atlas and Cerniglia, 1995).

The relationship between aerobic hydrocarbon biodegradation and oxygen consumption is stoichiometrically variable depending, in part, on the degree of hydrocarbon oxidation and the proportion of carbon and oxygen used for formation of biomass. In the absence of the formation of biomass the following equation has been used to relate oxygen consumption and hydrocarbon degradation

$$CH_2 + 1.5O_2 \rightarrow CO_2 + H_2O$$

and suggests that about 3.4 g oxygen per g hydrocarbon are required for complete mineralization to CO_2 and H_2O (Huesemann and Truex, 1996).

In waterlogged soil low concentrations of O_2 may be rate-limiting for hydrocarbon biodegradation (Atlas and Cerniglia, 1995; Rayner et al., 2007). As soil moisture increases, air in soil pores is displaced. The rate of diffusion of air into and through soil is approximately proportional to the square of the air-filled porosity, and is therefore greatly limited in waterlogged soil (Reddy and Patrick, 1983; Turco and Sadowsky, 1995). Troquet et al. (2003) identified O_2 diffusion as a limiting factor in soil bioremediation. As a consequence of reduced gas diffusion in wet soils, O_2 levels are reduced and levels of CO_2 elevated because diffusion of respired CO_2 is restricted in poorly aerated soils.

Zhou and Crawford (1995) reported that biodegradation of petroleum in gasoline contaminated soil was most rapid when microcosm headspace was 8% O_2, and slowed considerably in both higher and lower O_2 concentrations. The degradation rate at 8% O_2 was more than twice that they observed at a near atmospheric O_2 level (18% O_2). According to Wuerdemann et al. (1994), an oxygen concentration of 5% or lower was limiting to hydrocarbon biodegradation in soil from a former gasworks site. Sierra and Renault (1995) further indicated that microbial mineralization of nitrogen from soil organic matter was inhibited when CO_2 concentrations exceeded 4%.

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Degradation of hydrocarbons by anaerobic organisms is different from aerobic degradation, as they do not use the oxygenases used by aerobic organisms (Powell et al., 2006). Both aliphatic and aromatic hydrocarbon biodegradations are more rapid when adequate soil oxygen is present (Zhou and Crawford, 1995), although Hasinger et al. (2012) suggested that anaerobic degradation patterns may be different from those of aerobic degradation with respect to hydrocarbon compound structure.

In the absence of adequate levels of O_2, anaerobic degradation of alkanes and aromatics through respiratory denitrification proceeds using nitrate as the terminal electron acceptor (Ehrenreich et al., 2000; Hutchins, 1991). The nitrate is converted to N_2 or N_2O gases during this process (Burgin and Hamilton, 2007) and carbon is converted to CO_2 (So and Young, 2001). Anaerobic hydrocarbon degradation can also proceed using other terminal electron acceptors such as ferric iron or sulfate, although the energy return per unit of hydrocarbon degraded is greatest with O_2, followed by nitrate (Hasinger et al., 2012). Anaerobic pathways likely predominate deep in the soil profile, where oxygen diffusion is limited (Salminen et al., 2006). However, petroleum degradation through respiratory denitrification, stimulated by addition of nitrate fertilizer, may be as important as aerobic degradation (Powell et al., 2006).

The purpose of this study was to investigate the effects of varied O_2 levels on terminal electron acceptor consumption and hydrocarbon degradation in a petroleum contaminated sub-Antarctic soil. To that end, a series of respirometry chambers was set up with an array of headspace O_2 concentrations in support of in situ remediation studies conducted at Macquarie Island. To develop a better understanding of the potential for enhancement of in situ bioremediation via air sparging, a laboratory study was conducted to evaluate the relative efficiency of anoxic versus aerated remediation.

2. Methods

Soil was collected from a petroleum-contaminated site on sub-Antarctic Macquarie Island, Australia. Macquarie Island (54°29′59″S, 158°57′08″E) is located approximately 1500 km south-southeast of Tasmania. Mean daily temperatures range from 1.3 °C in September to 8.6 °C in January (Deprez et al., 1994). Macquarie Island has a moist climate and is ice-free. Annual rainfall is 952.6 mm.

The soil used in this study was collected from the Main Power House, where spills of Special Antarctic Blend fuel have occurred (Deprez et al., 1994; Rayner et al., 2007). The site from which soil was collected is waterlogged and contained approximately 12,500 mg kg^{-1} of diesel range organics (C_9 to C_{28}). Soil was spiked with ^{14}C hexadecane (1% by weight) in diesel fuel carrier, at 250 mg kg^{-1} soil. Soil was sieved (2 mm) and mixed in a 4 °C room. Each air-tight stainless steel 500 ml respirometer chamber was filled with 50 g (fresh weight) of soil. A solution of 1 g NH_4NO_3 dissolved in 10 ml H_2O was added at a rate of 175 mg N kg^{-1} dry soil. Final soil moisture content was 17.5% by weight. Mercury poisoned controls, treated with 10,000 mg kg^{-1} of $HgCl_2$ were used to evaluate abiotic processes.

Headspace oxygen contents of the respirometer chambers were adjusted by pressurizing chambers with 0.2 MPa N_2 gas into chambers, allowing chambers to equilibrate for 2 h, then relieving the pressure and allowing the chamber pressure to equilibrate with that of the atmosphere. This procedure was repeated to achieve chamber headspace compositions of approximately 0, 1, 2.6, 5.2, 10.5 and 20.9% O_2. Mercury-treated controls were included for the 0 and 20.9% O_2 treatments only.

Each chamber was connected to a NaOH trap to absorb CO_2 evolved during the microbial oxidation of contaminant and connected to a modified COMPUT-OX Respirometer (N-CON Systems, Crawford Ga). Oxygen consumption related to contaminant biodegradation resulted in a headspace pressure drop that triggered metered oxygen delivery into the vessels. CO_2 consumed by the NaOH trap was resupplied with pure O_2 in the 1, 3, 6, 10.4, and 20.9% treatments, and by N_2 in the 0% O_2 treatment. The respirometer recorded O_2 consumption every 30 min.

CO_2 traps were connected to the respirometer chambers with quick-connect fittings to allow them to be replaced periodically without opening the respirometer chamber. Chambers were filled with appropriate headspace gas (matching treatment O_2 levels) prior to re-attaching to the respirometer chambers. Headspace composition was adjusted by evacuating traps to 0.025 MPa and refilling with N_2 an appropriate number of times.

Chambers were periodically attached to O_2 sensors (KE series oxygen sensors, GS Yuasa Industry Ltd., Kyoto Japan) to monitor chamber O_2 levels. O_2 sensors were allowed to equilibrate for 24 h prior to reading. NaOH trap contents were collected periodically and analyzed for total and radiolabeled CO_2.

Total trapped CO_2 was assayed by titration. To measure the amounts of ^{14}C-hexadecane that were mineralized, and hence converted to $^{14}CO_2$, 1 ml of the liquid from CO_2 traps was mixed with 9 ml scintillant (Starscint, Packard) and allowed to equilibrate for at least 24 h. Radioactivity was measured on a 2250CA Tri-carb Packard Liquid Scintillation Analyzer. Quench was accounted for by the automatic efficiency control–quench curve, generated for quenched ^{14}C-standards (Amersham). The radioactivity in the trap at each time was calculated by multiplying the radioactivity in each 1 ml sample by the ratio of volume of sample removed to total trap volume.

Each treatment was replicated three times. Chambers were incubated at 6 °C for 12 weeks. Headspace gases were adjusted as necessary after 63 and 76 days, by pressurizing and equilibrating, as described in the experiment setup.

Post incubation soil contaminant and nitrogen levels were assayed. At the conclusion on the incubation, petroleum hydrocarbons were extracted from 10 g samples of soil with 10 mL of hexane, 10 mL of water, and 0.5 mL of an internal standard solution containing 1000 mg L^{-1} cyclo-octane, 100 mg L^{-1} D8-naphthalene, 100 mg L^{-1} p-terphenyl, and 1000 mg L^{-1} 1-bromoeicosane dissolved in hexane. Vials containing soil and extractant were tumbled end-over-end at 50 rpm overnight and allowed to settle. The clear hexane layer was analyzed via GC-FID with a 30 m, 0.32 mm i.d., 0.25 μm film thicknessBP-1 capillary column (SGE) and a split/splitless FocusLiner (SGE).

Hydrocarbon and O_2 consumption data were analyzed with SAS Proc ANOVA as a randomized complete block design (SAS Institute Inc., Cary, NC).

Inorganic nitrogen was extracted from the soil with 2 M KCl (10 g soil:30 ml KCl) and ammonium and nitrate nitrogen were assayed colorimetrically with a HACH DR 2700 spectrophotometer (HACH, Loveland, CO).

3. Results and analyses

Chamber O_2 levels were measured between day 57 and day 62. O_2 concentrations were 1.7% in the 0% treatment, 0.30 in the 2.6% treatment, 0.5% in the 5.2% treatment, 11.58 in the 10.5% treatment, and 16.6% in the 20.9% treatment. Headspace composition was adjusted on day 63. Measured again between days 70 and 75, the chamber O_2 levels were 1.4, 4.0, 4.9, and 15.6 for the 2.6, 5.2, 10.6 and 20.9% O_2 treatments, respectively. Following adjustment on day 76, O_2 levels were 2.8, 6.1, 8.5, and 19.5%, respectively.

Oxygen consumption, measured as O_2 metered in to the chambers via the N-CON respirometer in addition to O_2 added to adjust chamber headspace, is shown in Fig. 1. Vertical jumps represent addition of O_2 during chamber headspace adjustments. Total cumulative O_2 consumption was subject to ANOVA, results of which are shown in Fig. 2. Mercury poisoned controls consumed very little O_2. Oxygen consumption increased as headspace O_2 levels were increased, up to