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The biodegradation of crude oil in the deep ocean

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ABSTRACT

Oil biodegradation at a simulated depth of 1500 m was studied in a high-pressure apparatus at 5 °C, using natural seawater with its indigenous microbes, and 3 ppm of an oil with dispersant added at a dispersant:oil ratio of 1:15. Biodegradation of the detectable hydrocarbons was prompt and extensive (>70% in 35 days), although slower by about a third than under otherwise identical conditions equivalent to the surface. The apparent half-life of biodegradation of the total detectable hydrocarbons at 15 MPa was 16 days (compared to 13 days at atmospheric pressure), although some compounds, such as the four-ring aromatic chrysene, were degraded rather more slowly.

1. Introduction

The 2010 blowout of the Macondo oil well at 1544 m in the Gulf of Mexico (Lubchenco et al., 2012) renewed interest in the effects of pressure on oil biodegradation. Early work by Schwarz et al. (1974, 1975) used microbes collected at the water-sediment interface at 4940 m, 240 km east of Cape Canavaral, FL. Cultures were grown on hexadecane at 0.1 and 50 MPa (equivalent to surface and 5000 m conditions) at both 4 and 20 °C, under aerobic conditions, and growth was substantial at both temperatures and pressures, although somewhat slower at high pressure and low temperature. Bazylinski et al. (1989) isolated aerobic organisms able to grow on hexadecane and naphthalene from sediment cores collected at the Guaymas hydrothermal vent site in 2000 m of water, Cui et al. (2008) isolated aromatic degraders from the Middle Atlantic Ridge at 3542 m, and Tapilatu et al. (2010) isolated alkane degraders from 2400 m in the Mediterranean, although none were tested under high pressure conditions. Grossi et al. (2010) isolated a piezotolerant *Marinobacter* from deep (3475 m) Mediterranean water that grew aerobically at essentially similar rates on hexadecane at 0.1 and 35 MPa. Thus there was good precedence that oil degradation was likely to occur at depth following the Macondo blowout (Lubchenco et al., 2012), although only two or three substrates had been tested, and the growth media had been dramatically high in inorganic nutrients (up to 12.5 mM NH_4NO_3 and 6.2 mM phosphate (Schwarz et al., 1974, 1975; Bazylinski et al., 1989; Cui et al., 2008; Tapilatu et al., 2010; Grossi et al., 2010)), far higher than natural levels in the ocean (Garcia et al., 2009).

The expectation that biodegradation would proceed apace at depth following the Macondo blowout was borne out by Hazen et al. (2010). They found that some hydrocarbon components of the dilute 'plume' of oil entrained in the Gulf of Mexico at 1200 m (<1 ppm oil (Wade et al., 2016; Spier et al., 2013)) were undergoing rapid biodegradation (half-life of days!) despite only background levels of nutrients (Shiller and Joung, 2012).

Nevertheless, the majority of high pressure work has focused on very few hydrocarbons, usually hexadecane and naphthalene. These typically make up much <1% of crude oil hydrocarbons in weathered crude oil, so in this work we have examined the biodegradation of the total GC-detectable hydrocarbons in an artificially weathered oil, and many individual chemical species including those on the USEPA priority pollutant list (Keith and Telliard, 1979). We used environmentally relevant concentrations (Lee et al., 2013) of a dispersed lightly weathered crude oil at high pressure (15 MPa, equivalent to 1500 m) in natural seawater collected near Newfoundland, and followed the biodegradation of the oil by the indigenous microbes at a relevant temperature. As a reference, we compare this process at high pressure to the biodegradation by the same organisms at atmospheric pressure. We find that biodegradation at depth extends to all the saturated and aromatic hydrocarbons that are degraded under surface conditions, and that biodegradation is only mildly slowed by high pressure.

2. Methods

Experiments used unamended seawater collected in Logy Bay from a depth of 8 m and piped into the Cold-Ocean Deep-Sea Research Facility at Memorial University. Incubations were performed in 1 l Teflon (polytetrafluoroethylene) bottles (VWR) with 3 μl (3 ppm by volume) of an artificially weathered (20% loss by evaporation) European crude

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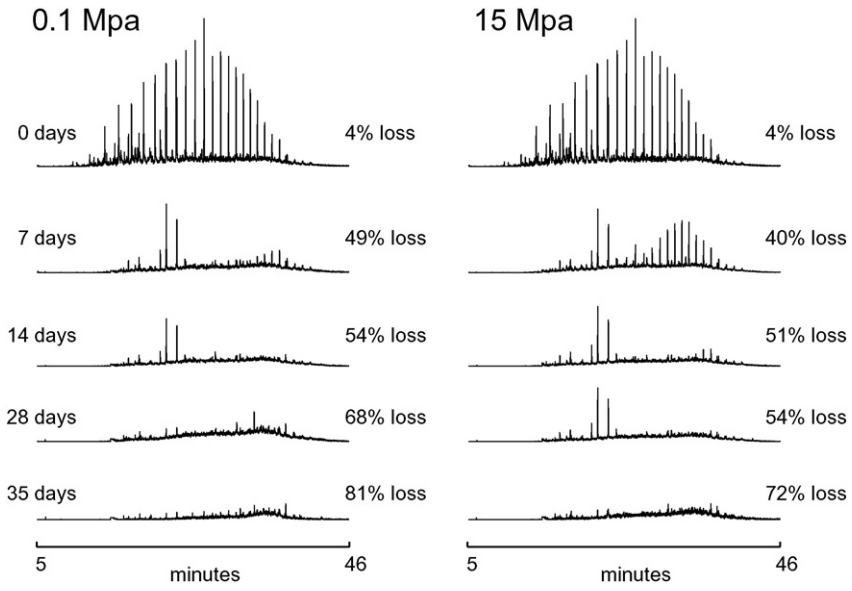

Fig. 1. Total ion chromatograms of oils extracted at various times after the initiation of the experiment, normalized to equivalent hopane concentrations. The 4% loss of the top panel indicates the evaporative loss of the extraction procedure.

oil that was itself amended with Corexit 9500 (Nalco, 2014) at a dispersant:oil ratio of 1:15. We used a partially evaporated oil because the volatile components of a fresh oil would likely be lost during the subsequent extraction and concentration of the oil.

The Teflon bottles were filled to 75% with fresh seawater, the oil added with a positive displacement pipette, and the bottles capped and shaken vigorously for 30 s to disperse the oil. The bottles were then filled to the brim with minimal air space, sealed, loaded into the high-pressure vessels (Memorial University Deep Sea Lab, 2016; Pradillon et al., 2004), and pressurized to 15 MPa. Samples destined to be harvested after one week were incubated in their own pressure vessel; the others shared a pressure vessel, and were all depressurized at 14, 28 and 35 days, the latter two re-pressurized after earlier samples were collected. After depressurization, the bottles were allowed to sit for 30 min upside down in case oil was lightly adhering to the lids, righted, opened, 50 ml of seawater removed (to allow for expansion upon freezing), and frozen to −20 °C. The pressurized vessels were maintained at 5 °C as representative of deep sea conditions such as those following the Macondo blowout (Redmond and Valentine, 2012), as were control incubations at atmospheric pressure, also kept in the dark. The pressurized samples were run in duplicate, the atmospheric pressure ones as single samples. None were stirred or otherwise agitated during the experiment.

The frozen bottles were shipped to New Jersey, where they were thawed and the oil extracted with methylene chloride (Prince et al., 2013) within 30 min of thawing. The extracts were dried by passing through a column of anhydrous sodium sulfate, and adjusted to approximately 300 μl with care not to allow evaporation of solvent to dryness. GC/MS analysis followed our earlier work (Prince et al., 2013), and biodegradation was assessed using hopane as a conserved internal marker (Prince et al., 1994).

3. Results

In complete accord with earlier work at atmospheric pressure at 5 °C (Brakstad et al., 2015), oil biodegradation was prompt and extensive at 5 °C (Fig. 1), and only slightly slower at high pressure (15 MPa, equivalent to a depth of 1500 m). At the oil concentration used here (3 ppm), the *n*-alkanes were almost completely consumed within 7 days, although the biodegradation of those with carbon number > 23 was visibly slower at high pressure; all *n*-alkanes were essentially completed consumed by 14 days. As expected (Pirnik et al., 1974; Prince et al., 2007), the biodegradation of the branched alkanes pristane and phytane was somewhat slower; they are the two prominent peaks in the day 7 and 14 samples at 0.1 MPa and day 7, 14 and 28 at 15 MPa, but they were completely consumed within 35 days at 15 MPa. The biodegradation of phenanthrene and its alkylated congeners up to those with the equivalent of three methyl substituents was also essentially complete within 35 days (Fig. 2). Chrysene, the most abundant 4-ring aromatic in crude oils, was also partially degraded by the end of the experiment, with an apparent median half-life of <100 days. The slowest biodegradation recorded here was of the methylchrysenes (Table 1).

4. Discussion

Our results extend and confirm the earlier work of Schwarz et al. (1974, 1975), demonstrating that pressures equivalent to a depth of 1500 m have a small inhibitory effect on hydrocarbon degradation by

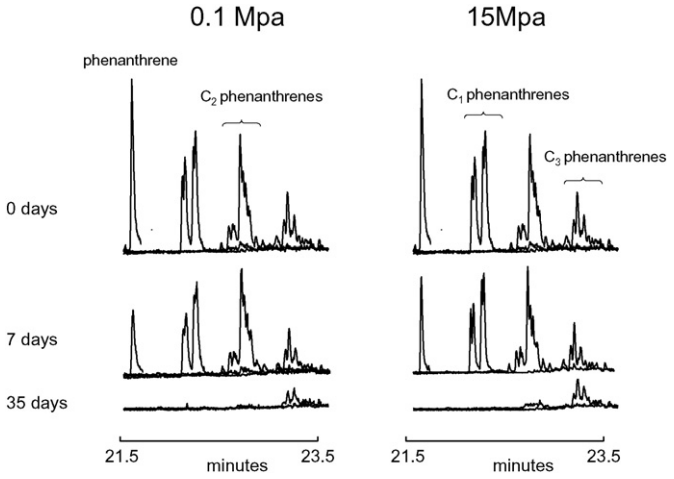

Fig. 2. Extracted ion chromatograms (m/z = 178, 192, 206 and 220) of phenanthrene, methyl (C1–), C2– and C3-phenanthrenes in oils after various incubation times, normalized to equivalent hopane concentrations. C2-phenanthrenes are the dimethyl and ethyl substituted forms, C-3 species include trimethyl- methyl-ethyl-, propyl- and *iso*propyl- forms, etc.