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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.

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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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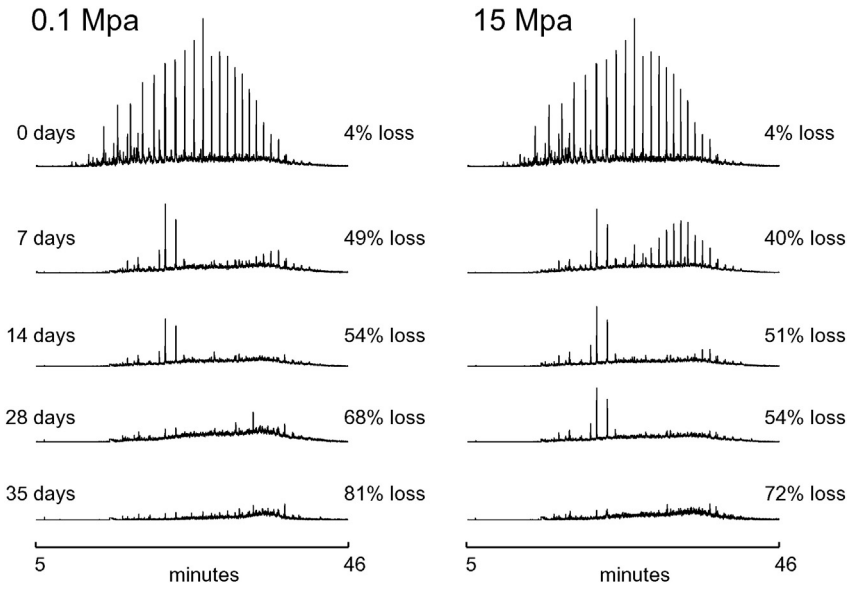

Fig. 1. Total ion chromatograms of oils extracted at various times after the initiation of the experiment, normalized to equivalent hopane concentrations. The 4% loss of the top panel indicates the evaporative loss of the extraction procedure.

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

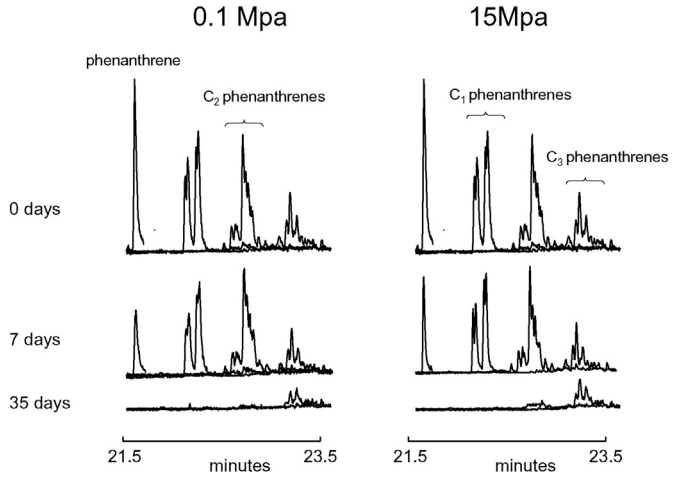

Fig. 2. Extracted ion chromatograms (m/z = 178, 192, 206 and 220) of phenanthrene, methyl (C1–), C2– and C3-phenanthrenes in oils after various incubation times, normalized to equivalent hopane concentrations. C2-phenanthrenes are the dimethyl and ethyl substituted forms, C-3 species include trimethyl-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

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Table 1
Concentrations of selected analytes in the incubations, together with estimates of the median and minimum half-lives measured here. ($n = 4$ for 0.1 MPa, $n = 8$ for 150 MPa). The concentrations assume full solubility – in fact the alkanes and the larger aromatics were likely mainly in small droplets at the beginning of the experiment. Realistic uncertainties in concentration are ±15%. C2-aromatics include dimethyl and ethyl substituted forms, C-3 aromatics include trimethyl- methyl-ethyl-, propyl- and isopropyl- forms, etc.

Compound	Concentration in seawater, ppb	Minimum half-life, days at 0.1 Mpa	Median half-life, days at 0.1 Mpa	Minimum half-life, days at 15 Mpa	Minimum half-life, days at 15 Mpa
Heptadecane	23.24	1.6	2.9	1.6	2.9
Pristane	21.88	5.3	11.0	5.3	21.1
Octadecane	23.77	1.4	2.7	1.4	2.0
Phytane	13.93	6.6	16.3	5.8	27.3
Hexacosane	14.53	2.0	3.5	3.3	4.9
Naphthalene	9.89	2.0	4.0	2.7	4.1
Methylnaphthalenes	44.32	1.7	3.7	3.0	5.0
C2-naphthalenes	54.62	2.6	3.8	2.9	5.8
C3-naphthalenes	36.52	2.8	4.3	3.1	7.2
C4-naphthalenes	5.79	3.3	5.3	3.4	10.3
Fluorene	2.22	2.4	6.0	4.2	8.5
Methylfluorenes	0.72	4.9	8.2	6.2	11.1
C2-fluorenes	1.69	6.8	8.4	8.3	14.8
C3-fluorenes	1.36	12.5	16.8	14.2	25.5
Phenanthrene	1.60	2.1	6.0	3.8	9.3
Methylphenanthrenes	2.40	1.9	6.8	3.4	12.9
C2-phenanthrenes	2.62	5.8	12.9	8.8	23.4
C3-phenanthrenes	1.62	19.1	35.6	29.8	45.2
C4-phenanthrenes	0.89	42.5	54.6	43.0	67.2
Benz[*a*]anthracene	0.04	5.7	23.7	20.0	37.1
Chrysene	0.16	41.0	72.0	25.8	96.2
Methylchrysenes	0.32	63.3	147.7	63.5	196.3

the indigenous microbes of near surface waters. The effect seems to be consistent for all the individual hydrocarbons measured here (Table 1 and Fig. 3), with biodegradation at a simulated depth of 1500 m being 33% slower than at surface pressure. The median apparent half-life of the total detectable hydrocarbons was 16 days at 1500 m compared to 13 days at the surface, both at 5 °C. Our experiments used pristine seawater (filtered through a 3 mm mesh to remove large eukaryotes and debris) and low concentrations of oil so that it is likely that there were enough nutrients and oxygen in the water for microbial growth, as there were in the dilute oil plume at 1200 m following the Macondo blowout (Hazen et al., 2010; Shiller and Joung, 2012). We note that our experiments, run at 3 ppm oil by volume because of the detection limits of our chromatography, were still at higher concentrations than the dispersed plume following the Macondo blowout – all measured values were below 1 ppm [e.g. Wade et al., 2016; Spier et al., 2013]. Our results are thus likely conservative estimates of the rates of biodegradation of dispersed spilled oil.

Discussions of the rate of biodegradation are complicated by uncertainties in how to discuss the phenomenon. At the biochemical level it would be appropriate to consider the rate in moles of substrate per mole of enzyme per unit time, but this is obviously impossible in the case of an oil spill in previously uncontaminated water. Since the seawater inoculum was from a relatively pristine environment, it is likely that a major part of the microbial response is the growth and increase in number of hydrocarbon-degrading organisms initially present at very low levels (Hazen et al., 2010), so the degradation includes growth kinetics of many different organisms, and likely their consumption by higher trophic levels. The simplest metric is the apparent half-life of the components analyzed, whether total detectable hydrocarbons or individual chemicals. But even this is complicated when samples with extents of degradation of other than near 50% are collected. We have chosen to calculate half-lives assuming simple first-order growth kinetics (Prince et al., 2007), recognizing that this is a gross approximation although widely used (French-McCay, 2004). The mean and median half-lives for some representative hydrocarbons are presented in Table 1; as discussed before (Prince et al., 2007), the median value is probably the more appropriate metric. While we collected no samples sooner than 7 days after the initiation of the experiment, we know from experience (Prince et al., 2007) that the calculated half-lives of 2–5 days should not be taken too literally because samples collected after 4 days of incubation in unacclimated seawater typically show only minimal degradation. Nevertheless, in many ways the half-life is a useful conservative estimate of the overall persistence of hydrocarbons because the substrates are usually not detectable after three or four 'half-lives'. Thus there were no detectable naphthalenes with up to four methyl substituents, fluorene, phenanthrene or methylphenanthrenes in any of the samples analyzed after 35 days, and no evaporation can have occurred in our sealed incubations.

We added dispersant (Prince, 2015) in our incubations in line with the strategy used in the Macondo response (Lubchenco et al., 2012). While this additional biodegradable organic matter put an additional 'load' on the oxygen and nutrients available at depth, it allowed the turbulence generated by the leaking oil to generate smaller oil droplets with increased surface area and decreased buoyancy (Brandvik et al., 2013), and the increased surface area stimulated biodegradation (Brakstad et al., 2015). In our case, the energy to disperse the oil came from an initial vigorous shaking before the bottles were completely filled and pressurized.

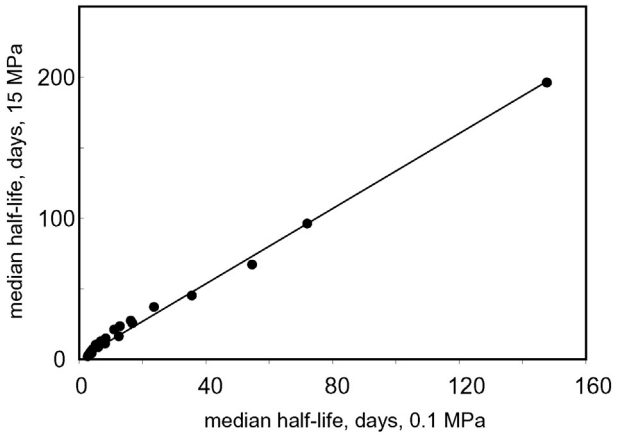

Fig. 3. The median half-life of the individual hydrocarbons of Table 1 at 0.1 and 15 MPa. The line is $y = 1.334\times$, with r^2 of 0.99.

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Contributors

RCP designed the experiment, did the GC/MS analyses, and initiated the manuscript. GWN and SJH operated and maintained the high-pressure apparatus. All authors have approved the final draft.

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