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The primary biodegradation of dispersed crude oil in the sea

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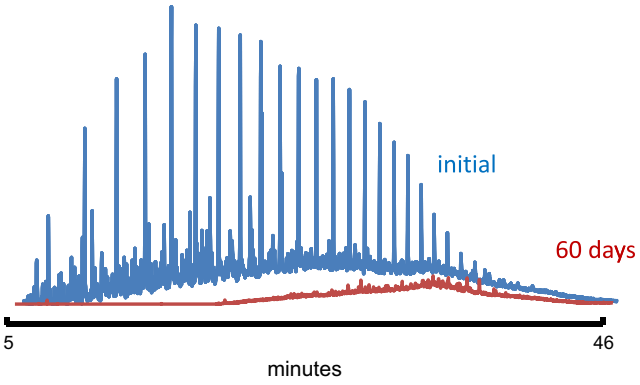
HIGHLIGHTS

- Dispersed crude oil is rapidly and extensively biodegraded in natural seawater.
- This occurs without the need for added nutrients or bacteria.
- This biodegradation extends to all resolvable classes of hydrocarbons except the hopanes.
- The approximate biodegradation half-life is 11–14 d.

GRAPHICAL ABSTRACT



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ABSTRACT

Dispersants are important tools for stimulating the biodegradation of large oil spills. They are essentially a bioremediation tool – aiming to stimulate the natural process of aerobic oil biodegradation by dispersing oil into micron-sized droplets that become so dilute in the water column that the natural levels of biologically available nitrogen, phosphorus and oxygen are sufficient for microbial growth. Many studies demonstrate the efficacy of dispersants in getting oil off the water surface. Here we show that biodegradation of dispersed oil is prompt and extensive when oil is present at the ppm levels expected from a successful application of dispersants – more than 80% of the hydrocarbons of lightly weathered Alaska North Slope crude oil were degraded in 60 d at 8 °C in unamended New Jersey (USA) seawater when the oil was present at 2.5 ppm by volume. The apparent halftime of the biodegradation of the hydrocarbons was 13.8 d in the absence of dispersant, and 11 d in the presence of Corexit 9500 – similar to rates extrapolated from the field in the Deepwater Horizon response.

1. Introduction

Large amounts of crude oil have been entering the world's oceans for millions of years, and a diverse group of microorganisms has evolved to take advantage of this rich source of reduced carbon (Prince, 2010; Prince et al., 2010). Much of the oil comes from natural seeps, and while some adheres to sediment particles and is degraded on the sea bottom, some rises to the sea surface to form slicks, and some disperses as small droplets in the water column (Farwell et al., 2009). Other oil comes from anthropogenic sources, both as runoff from land and from drilling and shipping accidents (National Research Council, 2003). Oil is an unusual substrate in two main regards. First, only a few molecules such as the smallest saturates (e.g. methane, ethane and propane) and aromatics (e.g. benzene, toluene, ethylbenzene and the xylenes) are significantly soluble – so biodegradation of most oil components must take place at the surface of the oil. Second, while oil provides a rich

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source of carbon and energy, it contains no significant amounts of biologically available nitrogen or phosphorus essential for microbial growth. Together these mean that the biodegradation of significant amounts of oil in aerobic environments is likely to be limited by the available surface area of the oil, and the presence of nutrients. The biodegradation of oil on shorelines has been enhanced by the judicious application of fertilizers to the oil (Bragg et al., 1994; Prince and Bragg, 1997), but adding nutrients to slicks on the open ocean is not usually appropriate in view of the likely competition for uptake by planktonic photoautotrophs. Instead the preferred approach for stimulating the biodegradation of oil slicks and subsea releases is to add chemical dispersants to substantially increase the surface to volume ratio of the oil, and allow the oil to disperse so that the natural background levels of biologically available nitrogen or phosphorus are adequate for microbial growth (National Research Council, 2005). For example, dispersants were used on a large scale following the spill from the *Sea Empress* in South Wales (Lunel et al., 1997), and more recently at the leaking wellhead of the *Deepwater Horizon* blowout in the Gulf of Mexico (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011).

Despite these large-scale uses, and many tests of the effectiveness of dispersants in dispersing crude oil, from laboratory through large tanks, field trials and actual use (e.g. Mukherjee and Wrenn, 2009; Li et al., 2009a,b; Belore et al., 2009; Lewis et al., 2010), few papers have addressed the question of the biodegradation of dispersed oil under environmentally relevant conditions. Oil slicks of light to medium oils spread rapidly until they are only approximately 100 μm or less in thickness (Lehr et al., 2002). Applying dispersant to such a slick in 1-m waves causes nearly immediate mixing of dispersed oil into the top 1–1.5 m of the water column (Delvigne and Sweeney, 1988), resulting in immediate dilution by a factor of about 10 000 to give an average oil concentration of ~100 ppm (Belore et al., 2009). The dispersed droplets are so small, typically 10–100 μm in diameter (Li et al., 2011), that they are entrained in the water column as discrete droplets that do not coalesce but diffuse to ever more dilute concentrations. It is technically challenging to do biodegradation experiments at such dilutions, and no published work that we have found (Bruheim et al., 1999; Lindstrom and Braddock, 2002; MacNaughton et al., 2003; Venosa and Holder, 2007; Zahed et al., 2010) comes very close.

We report here the biodegradation of fresh and lightly weathered crude oil (the latter mimicking exposure at sea for 24 h) with and without the dispersant Corexit 9500 (Nalco, 2011), at concentrations approaching those seen following a real spill in natural seawater with no added nutrients or bacteria. This mimics the conditions for natural biodegradation, but at the expense of demonstrating the physical effectiveness of the dispersant – at such concentrations, and in stirred laboratory experiments, Alaska North Slope crude oil disperses quite well with no additions. We show that oil is extensively degraded under such conditions, and that Corexit 9500 exerts no inhibitory effects.

2. Materials and methods

Seawater was collected from the New Jersey shore in April 2010 and January and April 2011 (winter conditions, salinity = ~28 ppt, temperature = ~8 °C). Nitrate and phosphate levels were below detection limits with simple laboratory colorimetric tests, but are likely to have been near 7 and 0.5 μM respectively (Louanchi and Najjar, 2001). The seawater was transferred to a cold room at 8 °C and aerated for 24 h prior to assembly of the experiments in 5 L carboys. Four liters of seawater were placed in each carboy, and were stirred with a large magnetic stirrer to generate a 2 cm vortex. We tried two methods of adding oil to these vessels, with indistinguishable biodegradation results. In the first, Alaska North Slope crude oil was weathered by evaporation at laboratory room temperature in a hood until it had lost 20% of its weight. Corexit 9500 was added at 5% to an aliquot, and 10 μL of the weathered oil or weathered oil plus dispersant was added to each carboy (both in triplicate) with a positive displacement pipette to yield 2.5 ppm oil by volume. As shown below, these experiments revealed no substantial difference in the extensive biodegradation seen after 60 d. In the second pair of experiments, 10 μL of fresh Alaska North Slope crude oil was added to the 4 L batches of seawater without dispersant: the dispersed oil was generated by floating 1 mL of oil on 1 L of seawater in an aspirator vessel. The water was stirred vigorously with a magnetic stir bar to generate a 2 cm vortex, and 67 μL of Corexit 9500 was dropped on the slick with a positive displacement pipette. The oil instantly dispersed throughout the water column, and after 2 min of stirring the stirrer was turned off and the contents allowed to settle for a few minutes. Dispersed oil was drawn off from the bottom of the aspirator bottle, and 10 mL of the dispersed oil/water mixture was added to 4 L of seawater. In this case enough replicate vessels were assembled, in two separate campaigns, that duplicate carboys of oil with and without Corexit could be sacrificed at 0, 4, 6, 11, 24 and 41 d.

At the designated time, oil was extracted from each carboy, three times, with methylene chloride, dried with sodium sulfate, and analyzed by gas chromatography coupled with mass spectrometry (Douglas et al., 1992). Care was taken to prevent complete evaporation of the methylene chloride so as not to lose volatile oil components during extraction. Oil biodegradation was followed with respect to 17α(H),21β(H)-hopane as a conserved internal marker within the oil (Prince et al., 1994). Apparent half-times of the loss of analytes were calculated as before (Prince et al., 2007). Two-dimensional GC followed earlier methods (Wang et al., 2003), but used a flame ionization detector; the first column separated the oil components by boiling point, the second by polarity. This approach separates the oil into eight major classes: 3-ring aromatics, 2-ring aromatic + 1-saturated ring or 5-saturated rings, 2-ring aromatics or 4-saturated rings, 1-ring aromatic + 1-saturated ring or 3-saturated rings, 1-ring aromatic or 2-saturated rings, 1-saturated ring, alkanes, and hopanes.

3. Results

Fig. 1 shows total ion chromatograms of the initial oils and oils extracted after biodegradation with the natural indigenous microbiota and nutrients at 8 °C. The top trace is the original oil before addition to the seawater; the sample with Corexit had this added to the neat oil, although the experiment used oil dispersed by addition of the dispersant to the oil on the water surface, as described above. It is noteworthy that the initial sample with dispersant shows prominent peaks near 28 min that are not seen in the samples extracted from the water phase – these are some of the surfactant molecules (Nalco, 2011) that are not extractable from water with methylene chloride. On the other hand, the features near 14 min can be attributed to the hydrocarbon solvent of the dispersant (Nalco, 2011) – these are extracted from water with our protocol, as shown in the samples labeled Day 0. These samples were extracted within 10 min of the initial assembly of the experimental systems, and it is noteworthy that in this time the samples without dispersant had lost most hydrocarbons smaller than naphthalene. The evaporative loss was much reduced in the dispersed sample, presumably because the hydrocarbons were in the bulk water phase.

The oils were extensively and rapidly degraded, with both the alkanes responsible for the sharp peaks and the unresolved material showing substantial losses. Very similar extensive biodegrada-