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Assessing weathered Endicott oil biodegradation in brackish water

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ABSTRACT

We evaluated the biodegradability of physically (WAF) and chemically (CEWAF) dispersed oil in brackish water (salinity ~6.5 g/L), and the influence of nutrient availability (low nutrient-LN: background water vs. high nutrient-HN: addition of 100 mg NO_3-N/L and 10 mg PO_4-P/L to background water) on oil biodegradation rates at 15 ± 0.5 °C for 42 days. No oil removal occurred in WAF compared with CEWAF: 24% in HN and 14% in LN within two weeks. The oil biodegradation concerned mainly alkanes as confirmed by GC/MS analyses. Higher O_2 consumption (10.30 mg L^{-1} day^{-1}) and CO_2 production (3.89 mg C L^{-1} day^{-1}) were measured in HN compared with LN (O_2: 2.79 mg L^{-1} day^{-1}, CO_2:0.18 mg C L^{-1} day^{-1}). Estimated biomass of hydrocarbon degraders and heterotrophic bacteria was at least an order of magnitude larger in HN than in LN. Combining dispersants with nutrients could enhance oil biodegradation and help improve oil spill mitigation responses.

1. Introduction

Marine pollution due to frequent large amount of crude oil releases poses substantial threats to ecosystems of important environmental and socio-economic values (McGenity, 2014). The application of chemical dispersants on oil spills could be an effective mitigation response, particularly when large spills occur in ocean and mechanical recovery is not readily feasible. Recently, chemical dispersants have been widely applied following the blow-out of Deepwater Horizon (DWH) rig releasing 636 million liters of Macondo crude oil into the Gulf of Mexico over 3 months (Camilli et al., 2012; McGenity, 2014). While mechanical recovery directly removes spilled oil from the impacted environment, chemical dispersion facilitates oil removal by dispersing the oil as discrete small droplets into a large water column volume. The dispersed oil is primarily removed by microbial degradation or biodegradation (Head et al., 2006; McGenity, 2014; Prince et al., 2013; Venosa and Holder, 2013; Yakimov et al., 2007). The effectiveness of chemical dispersants in mitigating marine oil spills depends on the extent to which the dispersants contribute to enhance oil's dispersion, bioavailability and biodegradation in comparison with naturally occurring physical dispersion. A dispersant is more effective when it increases the mass fraction of small droplets resisting coalescence and remaining stable enough in the column for not resurfacing. These factors would likely increase oil biodegradation.

Oil biodegradation occurs mostly at the oil–water interface since many oil components are not readily soluble in water (Prince et al., 2013). Among the factors that could increase oil biodegradation include an increase in surface area to volume ratio of the dispersed oil, the abundance of microorganisms at the interface oil–water and the biodegradability of hydrocarbons in the dispersed droplets. The physical properties of oil (e.g. interfacial tension with water and oil viscosity) and specific oil-dispersant combinations could also influence largely oil dispersion in seawater. Thus, it is rather challenging to predict the dispersion effectiveness for a range of crude oils and refined petroleum products with specific dispersants (Canevari et al., 2001; Fiocco et al., 1999; Mukherjee et al., 2011; Mukherjee and Wrenn, 2011). Nonetheless, alkane biodegradation could potentially be enhanced by bacterial attachment on oil droplets (Bruheim et al., 1997; Van Hamme and Ward, 2001; Zhang and Miller, 1992, 1994). The oil biodegradation rate has also been suggested to correlate with oil droplet-size distribution (Venosa and Holder, 2007).

The stimulation of microbial activity and oil biodegradation can also be achieved by supplementing oxygen and/or nutrients (nitrogen and phosphorus) in sufficient concentrations (Boufadel et al., 2010; Bragg et al., 1994; Sharifi et al., 2011). A large range of NO_3-N (~2–10 mg/L) concentrations can support a maximum oil biodegradation. For instance, it has been reported that about

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1–2 mg N/L could support near optimum biodegradation activity (Venosa et al., 1996), and 2.5 mg NO_3-N/L was sufficient to engender the maximum rate of heptadecane biodegradation (Boufadel et al., 1999). For a maximum oil biodegradation, a N:P ratio of about 10:1 on a mass basis is generally recommended (Atlas and Bartha, 1973; Oh et al., 2003; Smith et al., 1998; Zahed et al., 2010).

This study investigated aerobic degradation of Endicott oil, Alaska, in controlled laboratory conditions and aimed at (1) comparing the effects of chemical dispersion and physical dispersion on oil biodegradation rate, and (2) evaluating the influence of nutrient availability on the biodegradation rate of dispersed oil. The results indicated that chemical dispersion coupled with nutrient supplementation contributed to enhance oil biodegradation.

2. Materials and methods

The materials and methods are summarized in Table 1 and further described in Sections 2.1–2.7.

2.1. Water sample collection and filtration

An expedition was conducted at Prince William Sound (PWS) south of the Valdez Narrows (61.04533 N, 146.70190 W), Alaska to collect brackish water (salinity ~ 6.5 g/L) samples using certified-clean amber-glass jugs. These jugs were individually filled by (1) holding them under the water surface (10–15 cm below), (2) removing the cap until fill, and (3) closing the cap at the specified depth before returning to the surface. The jugs were preserved on ice in coolers and shipped to the laboratory at New Jersey Institute of Technology (NJIT) within 2 days. At the laboratory, they were stores at 4° Celsius for ~1 week. Prior to oil–water mixing at the onset of the experiments, the water samples were filtered through a 10 μm Whatman paper filter using a filtering flask and a Buchner funnel. The filtration allowed for removing flagellates, ciliates, and other bacterial grazers; thus reducing the potential variability among the triplicate microcosms.

2.2. Oil–water mixtures

A volume of 4 L of the filtered brackish water was either physically or chemically mixed with weathered Endicott oil, Alaska in an aspirator bottle on magnetic stirrer as follows (1) add 4 L of water, (2) adjust the stirring speed to create a small vortex (~1/4 of water depth), (3) add gently 20 mL of Endicott oil (i.e., oil-to-water ratio (OWR) = 1:200 (v/v)) into the vortex, and (4) increase the mixing speed to draw the vortex all the way to the bottom of the bottle, such that air bubbles became entrained in the water column. For the chemical dispersion, 2 mL of a dispersant (Corexit 9500) were gently added to the oil in the vortex (dispersant-to-oil ratio (DOR) = 1:10 (v/v)) before increasing the mixing speed. A range of dispersant-to-oil ratio from 1:10 to 1:25 is reported in the literature (McFarlin et al., 2014; NCR, 2005). As Corexit 9500 (DOR = 1:10) has been shown to have no effect on the growth rate of hydrocarbon degraders on Alaska North Slope crude (NCR, 2005), a DOR 1:10 was selected for our experiments on Endicott oil. The oil–water mixing was performed for 16 h, followed by a settling time of 30 min. To limit potential oil biodegradation during the 16 h of mixing the oil with water, packs of ice were placed around the bottle to keep the mixtures at low temperature. To account for potential evaporative losses, the dispersed oil concentration and biomass were measured immediately after the mixing time. These measurements are reported as time zero. Two types of mixtures were generated and referred herein as (1) water accommodated fraction (WAF) i.e. brackish water with oil (no dispersant), and (2) chemically enhanced water accommodated fraction (CEWAF) i.e. brackish water with oil and a dispersant (Corexit 9500).

2.3. Oil biodegradation in sealed respirometric-microcosms

Key parameters (oil removal, biomass production, O_2 consumption, nutrient consumption and CO_2 production) of aerobic Endicott oil (Alaska) biodegradation was evaluated using sealed respirometric-microcosms (Fisher, 2005) containing either 100 mL of WAF or CEWAF. The microcosms used in our experiments are suitable for studying microbial respiration in terms of efficiency (Reid et al., 2001) and similar to common respirometer system reported in the literature (Balba et al., 1998; Lamy et al., 2013; Reid et al., 2001). They were constructed using modified 250-mL wide-mouth, screw-cap Erlenmeyer flasks (Fig. 1). They consisted of (1) a CO_2 trap tubes filled with a CO_2 trapping solution (sodium hydroxide: NaOH), (2) a sample port in the cap of the trap tubes for periodically removing or replacing the trapping solution,

Table 1
Summary of materials and methods.

Methods	Description
Seawater collection/ preservation	Collected at Prince William Sound/Alaska (Salinity: 6.5 g/L) using Certified-clean amber glass bottles, kept at ~4 °C
Sample filtration	Vacuum filtration (10 μm Whatman paper filter)
Dispersed oil	Chemically (Corexit 9500) enhanced water accommodated fraction (CEWAF) Physically water accommodated fraction (WAF) Oil-to-water ratio (OWR) = 1:200 (v/v) Dispersant-to-oil ratio (DOR) = 1:10 (v/v)
Nutrient	Low (LN) :(CEWAF or WAF only) High (HN): (CEWAF or WAF + 100 mg NO_3-N/L and 10 mg PO_4-P/L)
Active/control microcosms	Active :No addition of bactericide Control: Addition of bactericide (0.5% w/v of sodium azide)
Oil measurements	Gravimetric methods/total Petroleum hydrocarbon (TPH) Gas chromatography/mass spectrometry (GC/MS)/Oil components (Alkanes, PAHs, alkylated PAHs) Thin layer chromatography/Flame ionization detector (TLC-FID)/Relative fraction of total alkanes, aromatics, resins and asphaltenes
Biomass estimation	Most probable number (MPN):alkanes, PAHs, and heterotrophic bacteria
O_2 consumption	Measured of volume; Theoretical calculations based on biomass formula
CO_2 production	Titration of CO_2 trapping solution; Theoretical calculations based on mass balance
Nutrient consumption	Theoretical calculations of nitrogen and phosphorus consumption based on biomass formula ($C_5H_7O_2NP_{0.1}$) Rittman and McCarty (2001)