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Biotransformation of petroleum hydrocarbons and microbial communities in seawater with oil dispersions and copepod feces

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ABSTRACT

To determine biotransformation of components in crude oil dispersions in the presence of feces from marine copepods, dispersed oil was incubated alone, with the addition of clean or oil-containing feces. We hypothesized that the feces would contribute with nutrients to bacteria, and higher concentrations of oil-degrading bacteria, respectively. Presence of clean feces resulted in higher degradation of aromatic oil compounds, but lower degradation of n-alkanes. Presence of oil-containing feces resulted in higher degradation of n-alkanes. The effect of clean feces on aromatic compounds are suggested to be due to higher concentrations of nutrients in the seawater where aromatic degradation takes place, while the lower degradation of n-alkanes are suggested to be due to a preference by bacteria for feces over these compounds. Large aggregates were observed in oil dispersions with clean feces, which may cause sedimentation of un-weathered lipophilic oil compounds towards the seafloor if formed during oil spills.

1. Introduction

Dispersion of oil into the water column is one of several weathering processes an oil spill in marine environments is subjected to. This process is driven by mechanical action on surface oil slicks, and the concentration and size of the oil droplets therefore depends on sea state, in addition to properties of the oil (Daling et al., 1990; Delvigne, 1993). During oil spills, chemical dispersants can be used to reduce the surface tension between the oil and the water, to enhance the formation of small oil droplets in the water (Brandvik, 1997). The small oil droplets (diameter < 100 μm) have slow surfacing velocity in seawater and accumulate in the water column (Lee et al., 2013). These droplets may drift passively with underwater ocean currents (Camilli et al., 2010). Suspended oil droplets can interact with organic particles, and may also interact with copepod feces (Muschenheim and Lee, 2002). The interaction between oil, and oil and organic particles in the water column has been shown to cause formation of large oil-containing aggregates, classified as marine snow (Fu et al., 2014; Passow et al., 2012). Marine snow is a large aggregate (≥0.5 mm) of organic particles which contribute substantially to the transport of organic materials towards the ocean seafloor (Alldredge and Silver, 1988).

The dispersion of oil into the water alters the location of the oil from the surface to the water column. However, for ultimate removal of petroleum hydrocarbon (HC) compounds from marine environments, biological weathering of oil is fundamental. Biological weathering includes biotransformation and mineralization of HC by microorganisms (Head et al., 2006). Oil-degrading bacteria are ubiquitous in marine environments, and these bacteria have been identified within several phyla (Alpha- and Gammaproteobacteria, Actinobacter, Flexibacter-Cytophaga-Bacteroides) (Harwati et al., 2007; Prince, 2005). Oil-degrading bacteria may be obligate hydrocarbonoclastic bacteria or capable of utilizing a range of organic compounds (Ward and Brock, 1976; Yakimov et al., 2007). For the utilization of bioavailable HC, bacteria need an electron acceptor and inorganic nutrients. Since oil has a high content of carbon, but contains low or no inorganic nutrients, biodegradation of oil spills by bacteria can be enhanced by the addition of nutrients to areas affected by oil spills (Atlas, 1995; Röling et al., 2002). Especially the ratios of carbon to nitrogen (C:N) and carbon to phosphorus (C:P) are of importance for bacterial oil degradation (Atlas and Bartha, 1972). A ratio of C:N:P of 100:10:1 has generally been regarded as optimal for bacterial activity (e.g. Bouchez et al., 1995; Obbard et al., 2004). Feces from copepods have higher nitrogen concentrations relative to carbon concentrations when compared to other organic particles in the water (Bathmann et al., 1987; Paffenhöfer and Köster, 2005). Copepod fecal pellets are in addition abundant, and constitute up to 90% of the total suspended material in

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the surface waters of the Norwegian Sea (Bathmann et al., 1987). The presence of copepod feces during an oil spill incident may thus increase microbial biotransformation of HC by supplying oil-degrading microorganisms with inorganic nutrients. During oil spills, this effect may be reduced, as oil-exposed *Calanus finmarchicus* defecate less than control copepods (Spooner and Corkett, 1979; Størdal et al., 2015). However, oil-containing feces from oil-exposed copepods have been shown to contain high concentrations of viable oil-degrading bacteria (Størdal et al., 2015). The presence of oil-containing feces may thus also enhance the degradation of oil compounds.

The purpose of this study was to investigate the influence of copepod feces on biodegradation of oil spills, and microbial communities in oil dispersions in a laboratory set-up. The two main aims were to investigate if biotransformation of target oil compounds was increased by the presence of copepod feces, which were assumed to contribute with nutrients, and if biotransformation also was increased by the presence of oil-containing feces, which have increased concentrations of viable oil-degrading microorganisms (Størdal et al., 2015). In this experiment, feces from the ubiquitous calanoid copepod *C. finmarchicus*, was used as a model matrix for copepod feces. This copepod is found at high densities in the North Sea, the Norwegian Sea, and the Barents Sea (Helle, 2000). Three different oil dispersions were included: I) Dispersed oil in seawater (no feces, oil dispersion control); II) Dispersed oil with the addition of clean copepod feces; and III) Dispersed oil with the addition of oil-containing feces. Copepod feces were collected from two groups of *C. finmarchicus*; one group that was kept in clean seawater and one group that was kept in seawater with dispersed oil. To evaluate reduction in feces production due to oil exposure, feces quantity from oil-exposed copepods was related to feces quantity from control copepods.

2. Materials and methods

2.1. Experimental design

Fig. 1 shows a schematic overview of the experiment. The laboratory set-up included: 1) collection of feces after 48 h of feeding of control copepods in clean seawater (clean feces) and from oil-exposed copepods feeding in oil dispersions (oil-containing feces), 2) incubation of the copepod feces from the two exposure separately in oil dispersions (nominal oil concentration 2 μL L^{-1}). To evaluate the extent of biotransformation in the oil dispersions, petroleum HC were quantified using gas chromatography-mass spectrometry (GC-MS) in the crude source oil before the experiment and after 14 days of incubation of the dispersion. Concentrations of viable microorganisms (heterotrophic microorganisms [HM] and oil-degrading microorganisms [ODM]) were determined in the samples by cultivation, using most probable number method (MPN; Brown and Braddock, 1990). The total numbers of microorganisms were also determined, by enumeration using 4′, 6′-diamidino-2-phenylindole (DAPI) staining and oil immersion fluorescence microscopy. The relative abundances of bacteria in the total microbial communities were determined using 16S rRNA gene amplicon library analysis.

2.1.1. Oil droplet dispersions and seawater

The petroleum oil used in this experiment was a naphthenic North Sea crude oil from the Troll exploration field. The oil was artificially weathered to 200 °C, which corresponds to approximately 24 h of weathering at sea as described by Stiver and Mackay (1984). The weathered oil was filtered (VWR, polytetrafluoreten filter, 0.2 μm pore size), and used for producing oil dispersions as described by Nordtug et al. (2011). This method includes forcing a water/oil-mixture through several nozzles with diameter of 0.5 mm, and enable tight control of the size distribution of the droplets and the concentration of the oil in seawater by adjusting the flows of water and oil, respectively. The oil dispersions produced for exposure of copepods and the oil droplet dispersions incubated with clean and oil-containing copepod feces were generated under identical conditions. The flow of water was 160 mL min^{-1} and the flow of oil was 0.35 μL min^{-1}. This produced oil dispersions with nominal concentration of 2 μL L^{-1}, and oil droplets with diameter < 40 μm.

Seawater used in the experiment is continuousl y supplied to our laboratory facilities from 80 m depth in Trondheimsfjorden (63°26′N, 10°23′E), sand filtered to remove coarse particles, and filtered (5 μm exclusion limit) prior to use.

2.1.2. Copepod husbandry and exposure of copepods

To obtain copepod feces to incubate with the oil dispersions, feces were collected from the copepod *C. finmarchicus* (Gunnerus) feeding in clean seawater and in oil dispersions in seawater. *C. finmarchicus* was obtained from the culture running at NTNU/SINTEF Sealab (Trondheim, Norway). The culture copepods were kept in continuously running seawater at 8–10 °C and were regularly fed a diet of microalgae (*Rhodomonas baltica* Karsten, *Dunaliella tertiolecta* Butcher and *Isochrysis galbana* Parke). Further details on the culture have been described by Hansen et al. (2007).

The copepods were exposed in glass exposure tanks with round bottoms and detachable lids. Clean seawater or oil dispersions were supplied to the exposure tanks through teflon capillary tubing at an average flow rate of 27 mL min^{-1}. The copepod exposure solution was supplied at the bottom of the exposure tanks, while the outlet was positioned close to the surface. This generated a gentle mixing of the exposure solution. *C. finmarchicus*, 400 copepodite stage 5 (C 5) were carefully introduced to the exposure tanks using a ladle, and exposed to clean seawater or oil dispersions under dim light conditions for 48 h in a climate controlled room (10 °C). Copepods were fed with live *R. baltica*. (nominal concentration 400 μg C L^{-1}).

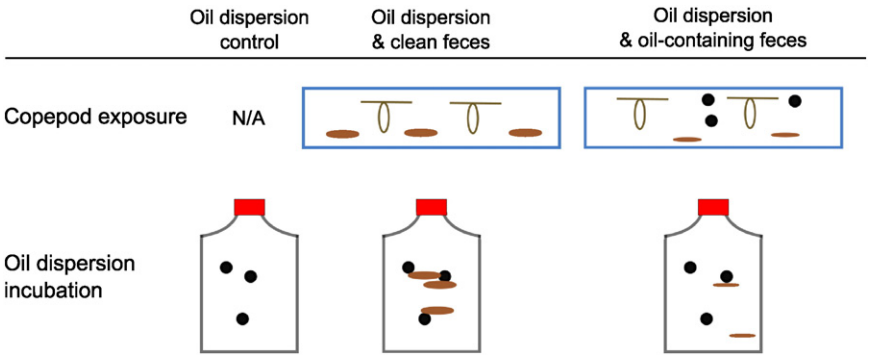

Fig. 1. Schematic layout of the experiment. Oil dispersions were incubated (14 days, 10 °C, dark) with clean copepod feces collected from copepods feeding in clean seawater and with oil-containing copepod feces collected from copepods feeding in oil dispersions. After the 14 day incubation, biodegradation of oil compounds and microbial communities in the oil dispersions was examined.