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Petroleum hydrocarbon and microbial community structure successions in marine oil-related aggregates associated with diatoms relevant for Arctic conditions

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ABSTRACT

Oil-related aggregates (ORAs) may contribute to the fate of oil spilled offshore. However, our understanding about the impact of diatoms and associated bacteria involved in the formation of ORAs and the fate of oil compounds in these aggregates is still limited. We investigated these processes in microcosm experiments with defined oil dispersions in seawater at 5 °C, employing the Arctic diatom *Fragilariopsis cylindrus* and its associated bacterial assemblage to promote ORA formation. Accumulation of oil compounds, as well as biodegradation of naphthalenes in ORAs and corresponding water phases, was enhanced in the presence of diatoms. Interestingly, the genus *Nonlabens* was predominating the bacterial communities in diatom-supplemented microcosms, while this genus was not abundant in other samples. This work elucidates the relevance of diatom biomass for the formation of ORAs, microbial community structures and biodegradation processes in chemically dispersed oil at low temperatures relevant for Arctic conditions.

1. Introduction

Marine snow (MS) is formed by natural processes in the oceans and plays a key role in the vertical flux and recycling of particulate and dissolved organic matter (DOM) in the water column (Lombard et al., 2013; Turner, 2002). MS is defined as aggregates ≥0.5 mm, composed of organic and inorganic particles such as minerals, detritus, bacteria, mucus, phytoplankton, or zooplankton faeces. The processes involved in MS formation are complex, but both physicochemical mechanisms (e.g. aggregation, coagulation, collision and break-up) and biological actions are suggested to be major contributors (Alldredge and Silver, 1988). Mucus material from various sources, including phyto- and zooplankton, can act as "glue" in the development of MS and bind together separate organic and inorganic constituents into aggregates (Wotton, 2004). This mucus is often termed transparent exopolymer particles (TEP) or extracellular polymeric substances (EPS). Phytoplankton biomass is often a main component of MS, typically dominated by diatoms and coccolithophores (Green et al., 2004; Gutierrez et al., 2013; Gutierrez et al., 2012a; Gutierrez et al., 2012b; Gutierrez et al., 2014). Phytoplankton provides organic material, but also inorganic material, such as calcite (coccolithophores) or silica (diatoms), acting as ballast material and increasing sinking velocities of aggregates due to the high density (Biermann and Engel, 2010; Lombard et al., 2013).

Most hydrocarbon biodegradation experiments in the marine environment have been performed with the free-living bacteria in the seawater, disregarding bacteria adhering to particulate matter and aggregates such as MS (Mishamandani et al., 2016). However, recent studies have revealed that bacteria associated with oil biodegradation are common members of microbial communities in natural MS aggregates, where phytoplankton and prokaryotic microorganisms are closely coexisting in mutually beneficial partnerships (Gutierrez and Aitken, 2014; Gutierrez et al., 2014; Kazamia et al., 2012; Thompson et al., 2017). In addition, oil droplets ingested by zooplankton may generate faecal aggregates, containing bacterial communities able to biodegrade oil (Størdal et al., 2015a; Størdal et al., 2015b). Aggregates of oil, bacteria, EPS and oil degradation products may also be formed during oil biodegradation, in the absence of phytoplankton or zooplankton (Bælum et al., 2012; Hazen et al., 2010). Also, it has been shown that non-polar substances are able to accumulate in the EPS matrix of biofilms (Martirani-Von Abercron et al., 2017). Inorganic material can interact with dispersed oil as oil-mineral aggregates

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(OMAs), known to cause oil sedimentation (Gong et al., 2014; Lee, 2002; Payne et al., 2003). OMAs form primarily close to riverine outflows, melting glaciers or sea ice, and in semi-enclosed bays, where suspended lithogenic particle concentrations are relatively high (Lee and Page, 1997; Payne et al., 2003).

The formation and fate of MS related to oil spills gained significant attention after the Deepwater Horizon (DwH) accident in 2010. During the oil spill, ~4.1 million barrels of light crude oil and gas were discharged from the Macondo well (MC252) over a period of 87 days. In addition, about 37,000 barrels of the chemical dispersant Corexit were applied on the sea surface and at the well at 1500 m depth, primarily in order to reduce oil surfacing, improve safety for operating vehicles, and reduce stranding along the shorelines of the Gulf of Mexico (Zukunft, 2010). In oil spill response operations, chemical dispersants can be used to break-up oil-slicks into micron-sized droplets, thereby leading to i) rapid dilution in the water column, that in turn ii) improves biodegradability by providing a readily accessible food source for indigenous oil degrading bacteria without exhausting natural nutrient levels (Lee et al., 2013). The subsurface application of dispersants directly at the well resulted in the formation of a deep-sea plume of small oil droplets. Aggregates of bacteria, polymeric material, oil and oil compound degradation compounds were detected in this plume (Hazen et al., 2010). It was suggested that bacterial blooms driven primarily by consumption of soluble hydrocarbons produced biomass that acted as flocculant to capture suspended hydrocarbon particles and promoted the formation of oily bacterial flocs (Valentine et al., 2014). In addition, surfaced oil was suggested to contribute to MS formation by processes like EPS produced by oil–degrading bacteria (floating biofilms), production of oil particulate matter through interactions of oil components with suspended matter and their coagulation, and coagulation of phytoplankton with oil droplets incorporated into the aggregates (Passow et al., 2012). Different bacteria associated with oil degradation (*Cycloclasticus*, *Thalassolituus*, *Marinobacter*) and EPS production (*Halomonas*, *Pseudoalteromonas*, *Colwellia*, *Alteromonas*) have been identified in MS particles (Gutierrez et al., 2013; Suja et al., 2017).

While considerable efforts have been made to investigate MS processes related to the DwH oil spill in the Gulf of Mexico (GoM), only a few studies have focused on oil releases relevant for other areas (Suja et al., 2017). In this study, we investigated the formation of oil-related aggregates as a site for oil biodegradation and microbial community successions at conditions relevant for cold seawater, employing an obligate psychrophilic diatom species, chemically dispersed oil, and natural seawater at low temperature.

2. Materials and methods

2.1. Cultivation of Fragilariopsis cylindrus

The obligate psychrophilic diatom *F. cylindrus* RCC 4289 (Roscoff Culture Collection; Station Biologique de Roscoff, Place Georges Teissier, 29,680 ROSCOFF Cedex, France) was selected to resemble Arctic algae-bloom conditions. The diatom was grown in L1 medium (nitrate concentration 0.9 mmol/L) with additional silicate (Guillard and Hargraves, 1993). The medium was prepared in natural local seawater and filter sterilized (0.22 μm Millipore filter; Millipore Corporation, Billerica, MA, USA) prior to use. The cultures were grown at 5 °C in 250 mL borosilicate flasks (Schott), capped with aluminum foil and random manual agitation, under a 16:8 h light:dark cycle regime (light intensity of 50 μmol photons $m^{-2} s^{-1}$). Growth was monitored by cell counting using light microscopy at 1250 times magnification. Cells from the stationary-state phase were used for experimentation.

2.2. Microcosm set-up

Pyrex flasks (2 L; Schott) were used in the experiments. The flasks were pre-treated as previously described (Brakstad et al., 2015), and then filled with natural seawater (acclimatized for 48 h at 5 °C), leaving about 50 mL space for adding oil dispersion stock solution (described in Section 2.3), diatoms and $HgCl_2$, respectively. Natural seawater was collected from a depth of 80 m (below thermocline) in the Trondheimsfjord (63°26′N, 10°23′E), outside the harbour area of Trondheim. The water is supplied via a pipeline system to our laboratories after passing through a sand filter. Samples amended with oil and diatoms (O + D-samples) contained oil dispersions adjusted to a nominal concentration of 30 mg/L oil droplets (median droplet diameter 9 μm) based on Coulter Counter measurements (see below), while diatoms were added to a final concentration of ~10,000 cells/mL, based on microscopic counting. Oil-amended samples (O-samples) and diatom-amended samples (D-samples) were treated accordingly without diatoms or oil, respectively. Sterilized controls were prepared like O + D-samples and supplemented with 100 mg/L $HgCl_2$. Finally, flasks were filled completely with acclimatized natural seawater to avoid any headspace, sealed tightly and mounted onto a slowly rotating (0.75 rpm) carousel (Brakstad et al., 2015). O + D-, O- and D-samples were prepared in triplicates and incubated in the dark in a temperature-controlled climate room at 4–5 °C over a period of 64 days. This temperature is relevant for Arctic surface seawater temperature in the summer season.

2.3. Oil dispersion stock solution

All flocculation experiments were conducted using dispersed fresh Troll C oil (batch 2007–0087) and Corexit 9500A (Nalco). The SINTEF oil droplet generator was used for generating oil dispersion stock solutions with defined droplet size distributions (Nordtug et al., 2015). Oil and dispersant were premixed at room temperature in a dispersant-to-oil ratio (DOR) of 1:100 and injected into a constant flow of filtered (1 μm) and acclimatized natural seawater which moves through a nozzle system, as described elsewhere (Nordtug et al., 2015). A Multisizer 4 Coulter Counter (Beckman Coulter Inc., Brea, CA, USA) fitted with a 100 μm aperture was used to determine oil droplet concentration and size distribution within a diameter range of 2.6–60 μm. Filtered (0.22 μm) seawater was used as electrolyte. Median droplet sizes reported here are expressed as median droplet diameter of droplet volume.

2.4. Sampling

Triplicate samples were sacrificed for analysis after 0 (30 min on carousel), 5, 21 and 64 days of incubation. Sterilized controls (one replicate each) were sampled at day 0 and 64. Particles in oil-amended samples with a diameter > 20 μm were defined as oil-related aggregates (ORAs) in the experiments reported here.

Sampling was performed by sacrificing entire bottles at the corresponding sampling time point. Aliquots were taken and subjected to Coulter Counter and dissolved oxygen (DO) analyses (Model 59 Dissolved Oxygen Meter, YSI Inc., Yellow Springs, Ohio, USA). The rest of the sample volume (2.2 L) was filtered through a 20 μm steel filter mesh (Teichhansel Teichshop/Siebgewebeshop; Bockhorn, Germany) using gravimetric force to capture ORAs. Biofilm attached to the glass wall was released by careful shaking prior to filtration. The steel filter was then divided using sterilized scissors and one half of each filter was extracted in 20 mL dichlormethane (DCM) for chemical analyses, while the other half was frozen for subsequent DNA extraction from the ORAs. Planktonic bacteria were collected by filtering 500 mL of the flow-through from the first filtration-step through a 0.22 μm membrane filter by using a vacuum pump. The membrane filter was frozen at −20 °C for subsequent DNA extraction. The rest of the flow-through (approximately 1.7 L) was acidified with 15% HCl to pH < 2 and subjected to solvent-solvent extraction with DCM.