Marine Pollution Bulletin xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Oil spill dispersants induce formation of marine snow by phytoplankton-associated bacteria

Justine S. van Eenennaam [a,*], Yuzhu Wei [a], Katja C.F. Grolle [a], Edwin M. Foekema [b], AlberTinka J. Murk [c]

[a] Sub-department of Environmental Technology, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands
[b] IMARES, Wageningen UR, P.O. Box 57, 1780 AB, Den Helder, The Netherlands
[c] Marine Animal Ecology Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 21 December 2015
Accepted 5 January 2016
Available online xxxx

Keywords:
Marine snow
Extracellular polymeric substances
Dispersants
Deepwater horizon
Marine phytoplankton
Symbiotic bacteria

ABSTRACT

Unusually large amounts of marine snow, including Extracellular Polymeric Substances (EPS), were formed during the 2010 Deepwater Horizon oil spill. The marine snow settled with oil and clay minerals as an oily sludge layer on the deep sea floor. This study tested the hypothesis that the unprecedented amount of chemical dispersants applied during high phytoplankton densities in the Gulf of Mexico induced high EPS formation. Two marine phytoplankton species (*Dunaliella tertiolecta* and *Phaeodactylum tricornutum*) produced EPS within days when exposed to the dispersant Corexit 9500. Phytoplankton-associated bacteria were shown to be responsible for the formation. The EPS consisted of proteins and to lesser extent polysaccharides. This study reveals an unexpected consequence of the presence of phytoplankton. This emphasizes the need to test the action of dispersants under realistic field conditions, which may seriously alter the fate of oil in the environment via increased marine snow formation.

1. Introduction

In April 2010, the drilling rig Deepwater Horizon exploded in the northeastern Gulf of Mexico, killing 11 people and causing crude oil to flow from the Macondo well at 1500 m depth (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011). When the well was finally capped in August, 780 million liters of crude oil had spilled into the Gulf of Mexico (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011). Spill response included skimming, in situ burning, and use of chemical dispersants. An unprecedented volume of dispersants, 6.8 million liters, was used, of which approximately 4 million liters at the sea surface and 2.8 million liters by subsurface injection at the wellhead (BP, 2014a, 2014b).

During the spill, an abnormally large amount of marine snow was observed at the sea surface (Dell'Amore, 2010; Passow et al., 2012). The marine snow event resulted in a depositional pulse of particulate matter to the sediment, during which the sediment mass accumulation rates were 10-fold higher than rates observed over the past ~100 years (Brooks et al., 2015). An estimated 1200 mile^2 of deep ocean sediment (NRDC, 2015) was covered with a thick layer of oily material including sea snow and particulates (Brooks et al., 2015), which smothered benthic macrofauna and meiofauna (Montagna et al., 2013) and corals (White et al., 2012). There was an 80–93% decline in density of benthic foraminifera (Schwing et al., 2015), which could be due to changes in sediment redox conditions and a reduction of pore-water oxygen concentration, or PAHs and other toxic compounds in oil and/or dispersants (Hastings et al., 2014). Vertical mixing in the top layers stopped, indicating a shutdown of bioturbation (Brooks et al., 2015). The flocculent material on the sediment after the depositional pulse contained crude oil and surface materials, including phototrophic organisms (Hollander et al., 2014) and particulate matter. The depositional pulse, mediated by marine snow, brought surface materials down through the water column to the sea floor. This process was named MOSSFA: marine oil-snow sedimentation and flocculent accumulation (MOSSFA Steering Committee, 2013). Through the MOSSFA process, marine snow influenced the fate of the dispersed oil by concentrating it on the sea floor (Kinner et al., 2014). In this way, marine snow provides an alternative route by which even the deep sea benthic organisms can be exposed to dispersed surface oil.

Marine snow generally consists of aggregates including fecal pellets, mineral particles, live and dead bacteria, phytoplankton, and zooplankton (Wotton, 2004a). The separate particles can be glued together into aggregates by Extracellular Polymeric Substances (EPS) from various sources because of the stickiness of EPS (Wotton, 2004a). EPS excretion is a naturally occurring process, which can be performed by a wide variety of microorganisms, ranging from (cyano)bacteria (Arnosti et al., 2015; Fu et al., 2014; Gutierrez et al., 2013; Han et al., 2014) and fungi (Metzger et al., 2009) to diatoms and microalgae (Corzo et al., 2000; Mishra and Jha, 2009; Raposo et al., 2013).

* Corresponding author.
E-mail address: Justine.vaneenennaam@wur.nl (J.S. van Eenennaam).

http://dx.doi.org/10.1016/j.marpolbul.2016.01.005
0025-326X/

The main constituents of EPS are carbohydrates (Myklestad, 1995; Wotton, 2004a) although other constituents can include proteins, uronic acid, lipids, and humic substances (Al-Halbouni et al., 2009; McSwain et al., 2005; Wingender et al., 1999). One specific type of EPS is the Transparent Exopolymer Particles (TEP). These are distinct particles that are formed from dissolved carbohydrate polymers excreted by bacteria and algae (Decho, 1990; Passow and Alldredge, 1994; Stoderegger and Herndl, 1999). TEP can also be formed when bacteria hydrolyze mucus on the surface of diatoms (Smith et al., 1995). TEP are very sticky (Passow, 2002), and thus greatly contribute to the formation of marine snow (Alldredge et al., 1993). Larger marine snow flocs can form when TEP stick together, aggregate with other material such as organic compounds and clay particles, and eventually become negatively buoyant and sink (Simon et al., 2002; Wotton, 2004a).

Wotton (2004a, 2004b) described several causes for EPS excretion, such as attachment of bacteria in biofilms and protection against stressors. Excreted EPS protects bacteria and phytoplankton against several stressors, including extremely high or low salinity (Liu and Buskey, 2000; Mishra and Jha, 2009), other changes in the physico-chemical environment such as desiccation and light availability (De Philippis and Vincenzini, 1998; Han et al., 2014; Hill et al., 1994; Wotton, 2004a), exposure to oil and other chemicals (Passow et al., 2012) and silver nanoparticles (Joshi et al., 2012). Excess photosynthesis products due to overflow metabolism or nutrient limitation can be released via EPS (Corzo et al., 2000; Staats et al., 2000). EPS and marine snow are food sources for many pelagic and deep sea organisms since they consist of proteins and easily degradable carbohydrates (Decho, 1990). In this way, EPS and marine snow form a link in mass and energy transfer towards the deep sea ecosystems (Beaulieu, 2002; Simon et al., 2002; Wotton, 2004a).

Marine snow formation is a natural process that occurs widely, but has never been reported as excessively as was seen during the Deepwater Horizon oil spill. The unexpected massive production during the spill raises questions about its cause. One suggested explanation is that oil-degrading bacteria excrete EPS as dispersant to better degrade weathered oil by emulsification (Passow et al., 2012). However, pilot experiments in our laboratory showed that the production of EPS seemed to be connected to the presence of phytoplankton, and also dispersant application without oil addition induced marine snow production by phytoplankton communities. This leads to the hypothesis that the unprecedented use of chemical dispersants in the presence of phytoplankton has induced the highly increased marine snow formation during the oil spill. The composition of the produced EPS will determine its nutritional value and physical characteristics, and may also influence the interaction with the oil, affecting its ultimate fate and degradation rate in the oily sludge layer that settled on the sediment. Also, nutrient flows and oxygen use in the marine system will be impacted by the massive production of EPS and marine snow.

In this study, we investigate the EPS formation by marine phytoplankton cultures upon exposure to dispersants. We tested the roles of phytoplankton and associated bacteria with two types of marine phytoplankton: the green algae *Dunaliella tertiolecta* and the diatom *Phaeodactylum tricornutum*. The phytoplankton cultures with and without sterilization by antibiotics were exposed to dispersant. In addition, the phytoplankton-associated bacteria alone were tested after filtering out the phytoplankton. This filtrate too was tested with and without subsequent filter-sterilization. The formation and appearance of dispersant-induced EPS was studied and the composition of the EPS was biochemically characterized.

2. Materials and methods

2.1. Phytoplankton culture and treatment

Non-sterile cultures of *D. tertiolecta* (green algae, *Chlorophyceae*; hereafter called '*Dunaliella*') and *P. tricornutum* (diatom, *Bacillariophyceae*; hereafter called '*Phaeodactylum*') were provided by IMARES, part of Wageningen UR, Den Helder, The Netherlands. The cultures were grown on f/2 medium (according to the recipe provided by Culture Collection of Algae and Protozoa, Argyll, Scotland, United Kingdom), which was made with autoclaved artificial seawater (32 g/L AquaHolland artificial sea salt in demi water). The cultures were kept at 20 °C on a shaker (New Brunswick Innova 44, Eppendorf AG, Germany) at 80 rpm under continuous light of nine 15 W F15T8 Plant & Aquarium Fluorescent tubes (GE, Cleveland, OH, United States). Once a week the phytoplankton cultures were refreshed by transferring 40 mL into 200 mL fresh f/2 medium.

In order to create axenic cultures, phytoplankton cultures were treated with antibiotics (20 mL/L PenStrep Glutamine, Gibco by Life Technologies, Paisley, United Kingdom) for at least 7 days. Cell density was measured with a CASY cell counter Model TT (Roche Innovatis AG, Reutlingen, Germany). After selecting optimal absorbance and validation (Supplementary Material S1), absorbance at 670 nm was used to determine initial cell densities, measured with a Tecan Infinite M200 PRO spectrophotometer (Tecan Trading AG, Männedorf, Switzerland).

2.2. EPS formation experiments

All experiments were performed in 20 mL glass tubes with phytoplankton in an exponential growth phase. Autoclaved fresh f/2 medium (5.5 mL) was combined with 4 mL of the phytoplankton cultures or filtrates in autoclaved glass tubes. A dispersant dilution of 10 mL/L Corexit (Corexit EC9500A, kindly provided by Nalco, Sugar Land, Texas, USA) was prepared in f/2 medium. This was then filtered-sterilized through a 0.2 μm filter to remove any bacteriological contamination that could be in the Corexit stock, and 0.5 mL of the dispersant filtrate was added to each tube to make an experimental dispersant concentration of 0.5 mL/L in each tube. In pilot experiments (data in Supplementary Material S2), it was determined that a concentration of 0.5 mL/L Corexit induced rapid and consistent EPS formation in phytoplankton cultures. Considering an application rate of 1870–9354 l per km^2 (2–10 US gallons per acre) (US EPA, 1995) and taking into account that application will not be homogeneous, this concentration could be reached in the upper centimeters of the water column after surface application of dispersants. Since we were primarily interested in the mechanism of EPS production and its composition, we have chosen a relatively high dispersant concentration of which we were confident that EPS was produced. The tubes were capped with an aluminum cap, mixed, and placed at 20 °C under continuous light of a 90 W red-orange–blue-purple LED lamp (LEDSPECTRUM, Drunen, The Netherlands). The tubes were manually shaken twice a day and every day the visible EPS formation was noted (starting time of EPS appearance).

The following experimental conditions were tested for both phytoplankton species (Fig. 1): non-sterile phytoplankton culture (containing phytoplankton and associated bacteria; hereafter called 'non-sterile phytoplankton'), antibiotic treated phytoplankton culture (containing only phytoplankton; hereafter called 'antibiotic treated phytoplankton'), filtrate from non-sterile cultures (containing only bacteria, made by filtering through 0.45 μm filter to separate water with bacteria from algal cells; hereafter called 'phytoplankton filtrate'), and phytoplankton filtrate which was then filter-sterilized (0.2 μm filtered; hereafter called 'filter-sterilized phytoplankton'). In addition to the EPS formation experiments with phytoplankton, a free-living n-alkane degrading bacteria (*Rhodococcus qingshengii TUHH-12*) was tested for Corexit-induced EPS formation. This was tested because oil-degrading bacteria have been suggested to produce EPS upon exposure to oil, but their response to Corexit exposure has not yet been tested. Bacterial suspension in the exponential growth phase (1 mL) was diluted to 10 mL total volume of f/2 medium with 0.5 mL/L Corexit.

Please cite this article as: van Eenennaam, J.S., et al., Oil spill dispersants induce formation of marine snow by phytoplankton-associated bacteria, Marine Pollution Bulletin (2015), http://dx.doi.org/10.1016/j.marpolbul.2016.01.005