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The effects of sunlight on the composition of exopolymeric substances and subsequent aggregate formation during oil spills

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ABSTRACT

During the Deepwater Horizon oil spill, a large amount of marine oil snow (MOS) was formed in surface waters of the Gulf of Mexico (GOM) that eventually settled to the bottom. MOS consists of a microbially colonized matrix of extracellular polymeric substances (EPS), to which cells, and both ionic (e.g., trace nutrients, Ca^{2+}) and non-ionic (e.g., toxic oil) substances can attach. The secretion of EPS is one of the microbial defense strategies against harmful or stressful environmental situations. In the surface waters, the toxicity of oil can be enhanced by elevated oxidative stress through UV radiation. To test the effects of sunlight on the composition and secretion of EPS and the subsequent aggregation process, we conducted short-term irradiation experiments in three treatments, i.e., control (GOM coastal seawater), water accommodated fraction of oil (WAF), and chemically-enhanced WAF (CEWAF). EPS composition (mainly carbohydrates and proteins) was quantified in the colloidal and aggregate fractions. In addition, bacterial abundance, live/dead cell ratio, particle size distribution, and the ambient hydroxyl radical (·OH) formation rate were measured under these conditions. We found that in the presence of oil, natural sunlight stimulated polysaccharide secretion, coinciding with increased reactive oxygen species (ROS; i.e. ·OH) production. Moreover, formation of larger sized aggregates ($> 10\,\mu m$) was observed in the irradiated WAF treatments. The results support the hypothesis that sunlight plays an important role in MOS formation during an oil spill.

1. Introduction

During the Deepwater Horizon (DwH) oil spill in the Northern Gulf of Mexico (GOM) on April 2010, 210 million gallons of oil were released, and a large amount of dispersant (i.e., Corexit) was applied to solubilize the oil into small droplets (Chanton et al., 2014). It was observed that a large amount of marine oil snow (MOS), which consists of a matrix of microbial colonized exopolymeric substances (EPS), was formed and resulted in sedimentation of oil to the seafloor (Passow et al., 2012). EPS are generally high-molecular-weight substances with a distinct composition and serve complex roles (Quigg et al., 2016). The major components include polysaccharides, which are hydrophilic, and proteins, which are amphiphilic, whereby hydrophobic interactions can significantly impact gel assembly (Ding et al., 2008; Chin et al., 1998). In addition, the anionic moieties of the EPS (including acid polysaccharides such as uronic acids) can form crystalized aggregate networks through "bridging" with divalent cations (e.g., Ca^{2+}, Mg^{2+}) in seawater (Chin et al., 1998). In the presence of oil, the hydrophobic portions of EPS are able to absorb the non-polar organic components and thus trap the oil into networks of gels (Liu et al., 2001; Quigg et al., 2016). Thus, EPS can partially regulate the partitioning of oil within the sinking marine snow (Santschi, 2017).

The synthesis and secretion of EPS is thought to be one of the defensive strategies used by phytoplankton and bacteria against harmful or stressful environmental conditions. In the case of the DwH oil spill, the polycyclic aromatic hydrocarbons (PAHs) associated with the plume proved to be toxic and mutagenic to bacteria and phytoplankton (Paul et al., 2013). It has been shown that EPS was over-produced in the presence of diesel oil and *n*-hexadecane (Kang and Park, 2010). In a recent mesocosm experiment simulating the DwH oil spill, the water accommodated fraction of oil (WAF) and chemical enhanced WAF (CEWAF) were shown to promote EPS production (Hatcher et al., 2018). In the surface waters, the toxicity of oil can be enhanced by UV radiation (Okay and Karacik, 2007; Steevens et al., 1999). In previous

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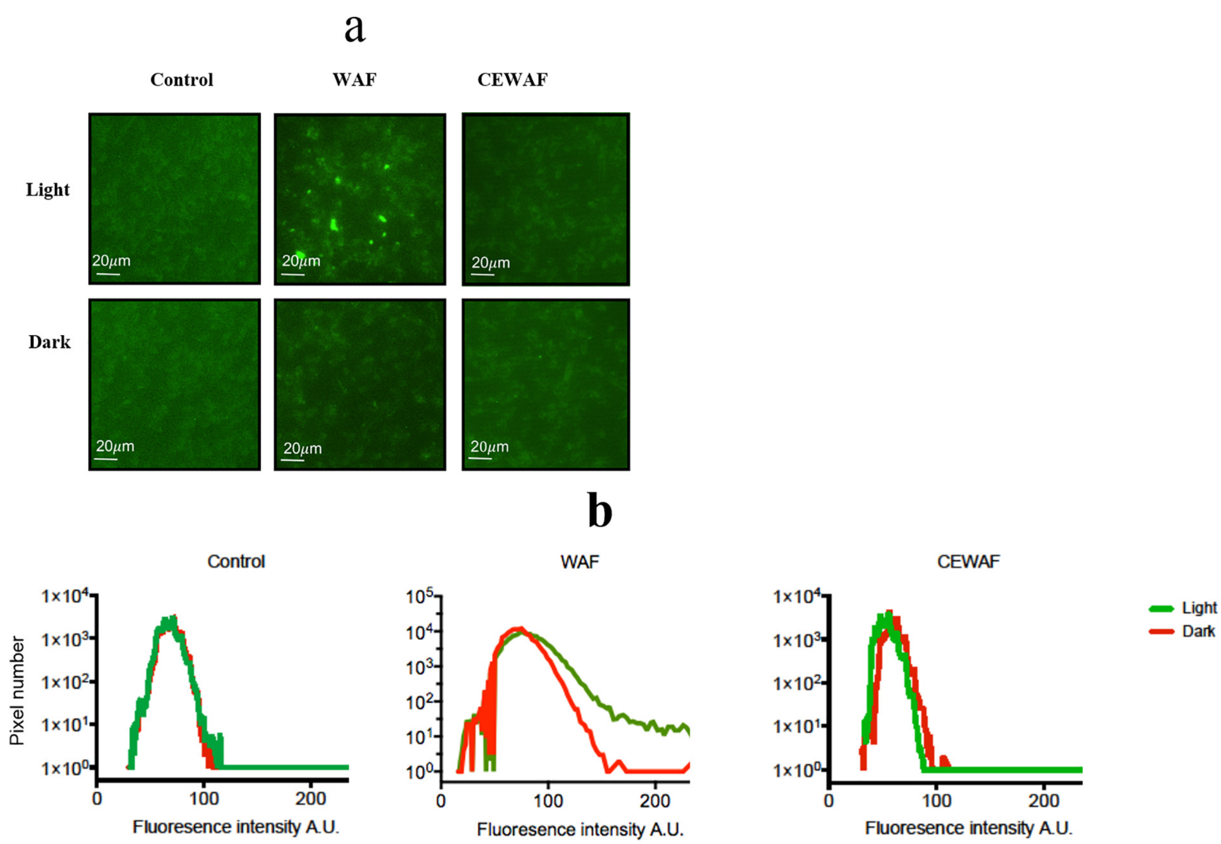

Fig. 1. Aggregates in control, water accommodated fraction (WAF), and chemically-enhanced WAF (CEWAF). a) Images of chlortetracycline hydrochloride (CTC) stained aggregates on 0.04 mm^2 square area of the filter; b) The pixel number in each image at different fluorescence intensity value analyzed by Image J and displayed as a histogram. Pixel number at high fluorescence intensity (pixels distribute in the "bright" area) reflects size and/or amount of aggregates.

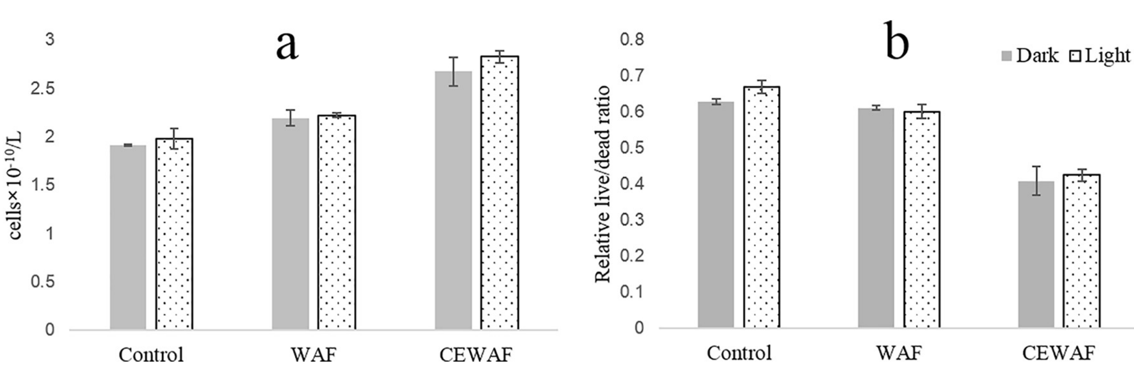

Fig. 2. Microbial growth and death in control, WAF, and CEWAF. a) Microbial abundance, counted for both live and dead cells; b) Relative live/dead cell ratios.

studies, the sensitivity of *Phaeodactylum tricornutum* in the presence of PAHs was 2–3 times greater under UV exposure than non-exposure (Okay and Karacik, 2007). The smallest picocyanobacteria species were the most sensitive to PAHs under UV exposure (Echeveste et al., 2011), possibly due to their large surface area to volume ratios. UV exposure can damage biomolecules such as DNA, proteins and lipids, either directly through direct photo-oxidation of the biomolecules, or indirectly, through oxidation by reactive oxygen species (ROS) that are generated in the water, such as singlet oxygen, superoxide, hydroxyl radical (·OH), and hydrogen peroxide (Agogué et al., 2005; Rastogi et al., 2017). Many cyanobacteria (Rastogi et al., 2014) e.g., *Nostoc commune* (Ehling-Schulz et al., 1997; Li et al., 2011), *Microcoleus vaginatus* (Chen et al., 2009) produce EPS to generate a buffer zone between the living cell and the ambient environment to chemically quench the hazardous ROS (Wotton, 2004). Oil could also be a source of ROS (Ray and Tarr, 2014a,b), which contributes partially to its photo-toxicity. It is thus an important question whether the microbes released more EPS or altered the EPS composition in response to elevated ROS in the presence of oil under sunlight conditions.

From the reasoning above, we hypothesize that UV irradiation can influence EPS production and composition, specifically in the presence of oil, and subsequently influence the aggregation process. To test this hypothesis, we conducted solar irradiation experiments using GOM natural bacterial consortia. Aggregates size was measured in three treatments: control, WAF, and CEWAF after irradiation. The EPS composition (mainly polysaccharides and proteins, with minor amounts of acid polysaccharides) were quantified in the colloidal fractions ($>$ 3 kDa and $<$ 0.4 μm), and aggregates (size $>$ 3 μm or $>$ 0.4 μm). In addition, bacterial abundance, live/dead cell ratio, and ambient ·OH formation rate were measured to investigate the factors affecting aggregation.