Marine Pollution Bulletin 130 (2018) 170–178

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Diagnostic tool to ascertain marine phytoplankton exposure to chemically enhanced water accommodated fraction of oil using Fourier Transform Infrared spectroscopy

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ARTICLE INFO

Keywords:
Macromolecules
FTIR
Phytoplankton
Oil
Dispersant

ABSTRACT

Phytoplankton alter their macromolecule composition in response to changing environmental conditions. Often these changes are consistent and can be used as indicators to predict their exposure to a given condition. FTIR-spectroscopy is a powerful tool that provides rapid snapshot of microbial samples. We used FTIR to develop signature macromolecular composition profiles of three cultures: *Skeletonema costatum*, *Emiliania huxleyi*, and *Navicula* sp., exposed to chemically enhanced water accommodated oil fraction (CEWAF) in artificial seawater and control. Using a multivariate model created with a Partial Least Square Discriminant Analysis of the FTIR-spectra, classification of CEWAF exposed versus control samples was possible. This model was validated using aggregate samples from a mesocosm study. Analysis of spectra and PCA-loadings plot showed changes to carbohydrates and proteins in response to CEWAF. Overall we developed a robust multivariate model that can be used to identify if a phytoplankton sample has been exposed to oil with dispersant.

1. Introduction

The 2010 Deepwater Horizon Oil Spill in the Gulf of Mexico was addressed by aerial and underwater application of the EPA-approved chemical dispersant Corexit (Kujawinski et al., 2011). After the spill, immense amounts of marine snow were visible both in surface waters and in sediment traps (Passow et al., 2012). Marine snow is characterized as visible aggregates formed as a result of the inter-cross-linking of exopolymeric substances (EPS) secreted by microbes, both phytoplankton and bacteria (Passow et al., 2012; Quigg et al., 2016). These microbes play an important role in dispersion and degradation of oil during a spill. As they constitute the bottom of the food chain, their association with oil and dispersant could lead to biomagnification and/or bioaccumulation of toxic components transferred to the food web (Torres et al., 2008; Quigg, 2008). The exact mechanism of how a dispersant affects growth and development across trophic levels still remains to be established (Lönning and Hagström, 1976; Hagström and Lönning, 1977; Lewis and Pryor, 2013; Almeda et al., 2014a, b; Lively and McKenzie, 2014). These effects can nonetheless be observed far from the site of an oil spill and dispersant (i.e., Corexit) application such that it is likely to obfuscate the source. Therefore, an easy mode of detection to predict the exposure of phytoplankton and bacteria to chemically enhanced (Corexit) water accommodated oil fraction (CEWAF) would be very beneficial.

Both phytoplankton and bacteria change their cellular macromolecular composition depending on their growth environment. For example, several reports have suggested an increase in lipid and a decrease in protein and chlorophyll concentrations in phytoplankton growing in nitrogen-limited conditions (Kamalanathan et al., 2016; Rodolfi et al., 2009; Griffiths and Harrison, 2009). Similarly, phosphate limitation has also been known to increase the lipid content in phytoplankton (Kamalanathan et al., 2015; Sharma et al., 2012; Liang et al., 2013). In diatoms, silicate limitation has been found to induce lipid production (Jiang et al., 2012). This sort of consistency allows for prediction of the consequences of environmental growth conditions on phytoplankton, as recently reviewed by Finkel et al. (2016). Such characteristic changes in response to oil and/or dispersant is as yet not available. Fourier Transform Infrared (FTIR) spectroscopy is a method

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https://doi.org/10.1016/j.marpolbul.2018.03.027
Received 7 November 2017; Received in revised form 15 March 2018; Accepted 16 March 2018

Table 1
Band assignments for Fourier transform infrared spectroscopy.

Wavenumber (cm^{-1})	Assignment	Reference and references therein
1070–1080	ν(Si−O) of silicate/silicic acid frustule	(Coates, 2000)
900–1200	ν(C−O−C) of polysaccharides	(Giordano et al., 2001)
1575–1705	νC═O of amide I	(Giordano et al., 2001)
1480–1575	δ(N−H) of amide II	(Coates, 2000)
1708–1780	νC═O of ester groups from lipids and fatty acids	(Giordano et al., 2001)
2800–3000	ν(C−H) of saturated CH from methyl groups and methylene groups of saturated fatty acids	(Coates, 2000)
~3015	(C═C−H) of unsaturated fatty acids	(Coates, 2000)

ν = stretch; δ = deformation (bend).

for rapid detection of the macromolecular chemical profile requiring very little sample, time and resources. Combined with multivariate statistics, FTIR has been proven to be an especially useful tool in understanding and prediction of the macromolecular profiles in phytoplankton (Sackett et al., 2013; Sackett et al., 2016; Finkel et al., 2016).

Here we apply FTIR spectroscopy in combination with multivariate statistics in an effort to develop a model that distinguishes the CEWAF-exposed samples (phytoplankton, natural aggregates) from controls. Such a model would help determine exposure to CEWAF rapidly in the event of a future oil-spill. We used cultures of *Skeletonema costatum* (diatom; UTEX LB2308), *Navicula* sp. (diatom; UTEX B SP11), and *Emiliania huxleyi* (coccolithophore; CCMP 1280) for building this model; these species are common in marine and coastal environments. The generated model was tested using aggregates naturally formed when exposed to CEWAF and control treatments harvested from a mesocosm study using Gulf of Mexico waters. In addition, we also characterized the macromolecular composition of the samples with the help of mean integrated peak areas and principal component analysis.

2. Materials and methods

2.1. Laboratory scale culture experiment

S. costatum, *E. huxleyi* and *Navicula* sp. were maintained in f/2 growth media at 19 °C, 40 μmol photons $m^{-2} s^{-1}$ in a 12:12 day-light cycle. The f/2 media was prepared by filtering (0.45 μm groundwater filter cartridge, Millipore, USA) seawater collected from the Gulf of Mexico (Galveston Bay, Texas), followed by further sterilization by autoclaving after the addition of sterile nutrients (N, P, Si, trace metals and vitamins). A mixture of 20:1 Oil: Corexit was added to the sterile f/2 growth media and stirred overnight in the dark. Before decanting into culture vessels, the solution was filtered through a 20 μm nylon sieve to ensure that the resulting CEWAF media was devoid of large oil droplets. Sandoval et al. (2017) showed that the average droplet size in CEWAF treatments is < 2 μm. The same study also reported that the oil composition of CEWAF was highly similar to the whole oil. Therefore, the impacts of oil droplets in our study should be minimal. The estimated oil equivalent concentration at the start of these experiments was 62.56 (± 4.61) $mg \cdot l^{-1}$ (determined according to Wade et al., 2011).

Cultures were inoculated into triplicate control (f/2 media) and CEWAF treatments with starting cell densities of: 3.3×10^4 cells ml^{-1}, 4.2×10^4 cells ml^{-1} and 7.2×10^4 cells ml^{-1} for *S. costatum*, *E. huxleyi* and *Navicula* sp. respectively. Glass bottles (1 l) which had been pre-acid washed and sterilized were used for cultivation with their caps tightly screwed. The cultures were stirred continuously and sampled every two days to measure growth rates and phytoplankton physiology. Cell counts were performed using a Neubauer heamocytometer and growth calculated as a change in cell density over time. The maximum quantum yield of photosystem II (F_v/F_m), a proxy of phytoplankton health, was monitored using Pulse Amplitude Modulated (PAM) fluorometry on dark-adapted (10 min) culture samples.

2.2. Mesocosm experiment

A mesocosm study was carried out by collecting water from the Gulf of Mexico (29°18 N, 94°49 W) in July 2016 (Salinity: 31.13 ppt, pH: 8.02, Temperature: 30.5 °C) and supplemented with nutrients (f/20). This amended seawater served as a control treatment; CEWAF (81.06 (± 20.50) $mg \cdot l^{-1}$) was prepared by mixing oil and Corexit as described above except there was no pre-filtration through the 20 μm mesh and the media was not sterilized. Specific details can be found in Wade et al. (2017). Each mesocosm treatment was prepared in triplicate 130 l tanks filled with 81 l of either control or CEWAF. Natural microbial populations in each tank were incubated at 22 °C at an average light intensity of 33 μmol photons $m^{-2} s^{-1}$ under a 12:12 day-light cycle. Aggregates appeared in the tanks within 24 h but they were not collected until after 4 days. They were collected with a stainless steel syringe needle from the bottom of the tanks and homogenized in a bottle before being harvested as described below for the FT-IR spectroscopy measurements.

2.3. FTIR spectroscopy

At the end of the laboratory (Day 14) and mesocosm experiment (Day 4), cultures and aggregates were harvested and washed twice in MilliQ water by centrifuging the suspension at 2000 × *g* for 10 min to remove the CEWAF and other media components that might interfere with infrared radiation absorbance. The samples were immediately stored at −4 °C in the dark. The pellets were dried using the commercial hand-held dryer (Heraud et al., 2007; Dao et al., 2017). Spectra were obtained on dried pellets using a Varian 3100 FTIR Excalibur series spectrometer controlled by Varian Resolutions-Pro 4.0 software. Absorbance spectra from 3650 to 600 cm^{-1} were collected at a spectral resolution of 8 cm^{-1} with 50 scans co-added (Giordano et al., 2001; Sackett et al., 2013; Dao et al., 2017). Background spectra were collected before each sample measurement. Three independent measurments were taken for each sample to reduce the influence of error. The band assignments for each functional group are provided in Table 1. ATR correction for the diamond crystal with a special crystal angle of incidence 38.7 and crystal and sample refractive index 2.4 and 1.5 was applied to the spectra and exported in GRAMS format for multivariate analysis using the software, The Unscrambler X v 10.4 (Camo Inc., Oslo, Norway).

2.4. Spectral pre-processing and multivariate and univariate analysis

Second derivatives of the spectra were determined using Savitzky-Golay algorithm with polynomial order 3 and 4 smoothing points. This was followed by Extended Multiplicative Signal Correction of the biological bands of interest in the spectra (800–1800 cm^{-1}, 2800–3050 cm^{-1}), which allowed for correction of variation due to any differences in the sample thickness and baseline variations (Sackett et al., 2013). Mean integrated areas were calculated for amide I and II, carbohydrates, silicate, and ester fatty acids at their respective wavenumbers (Table 1), and expressed as ratios to the amide I band, a commonly used normalization approach (Meng et al., 2014; Baker