Tracing Microbial Transformation of Crude Oil Complex Matrix in Seawater

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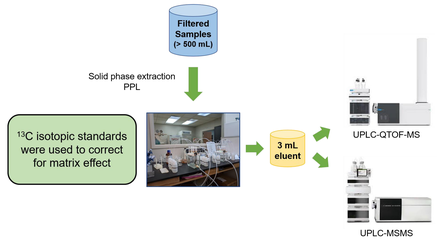
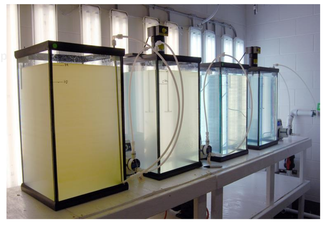
Experimental Method

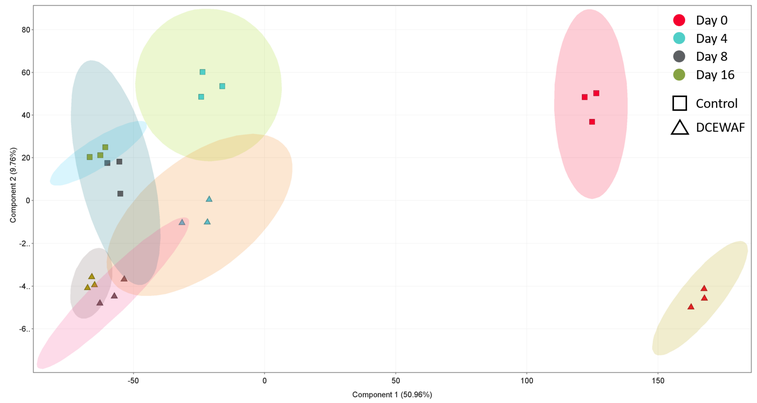
To understand the fate and residence time of oil hydrocarbons and their microbial degradation products, we conducted a 16-day mesocosm experiment comparing two treatments: (1) seawater only **controls** and (2) seawater amended with chemically dispersed oil (1:20 v:v dispersant:oil, ~0.5 mg/L oil; **DCEWAF**). At each time point, samples were fractionated into particulate (>2.7um) and dissolved phase (<0.2 um), and individual aliphatic and PAH concentrations were monitored. Petroleum hydrocarbons were extracted with dichloromethane and analyzed with gas-chromatography mass spectrometry (GC-MS).





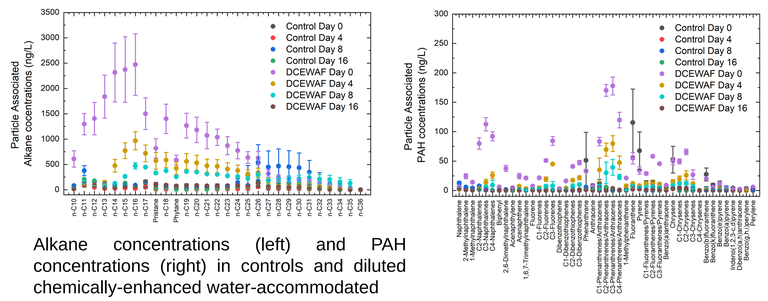
In parallel to this effort, we performed targeted and untargeted analyses of the hydrocarbon metabolites released into the seawater due to microbial oxidation. The targeted hydrocarbon metabolites were selected based on previous untargeted metabolomics and metagenomics screening (Liu et al., 2020). Dissolved metabolites samples were extracted with PPL solid phase extraction (Dittmar et al., 2008) and analyzed with ultrahigh-performance liquid chromatography mass spectrometry (UPLC-MS/MS) and UPLC Quadrupole Time-of-Flight MS (QTOF-MS).

Evolution of Chemical Features Over Time



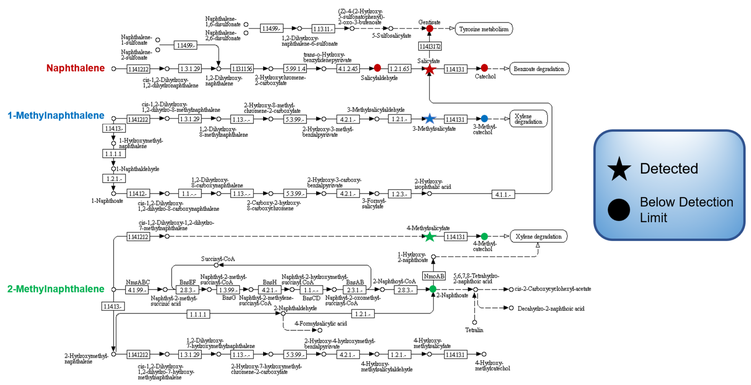
Principal component analysis (PCA) of chemical features in the dissolved phase, analyzed by UPLC-QTOF-MS.

Particle Associate Hydrocarbons



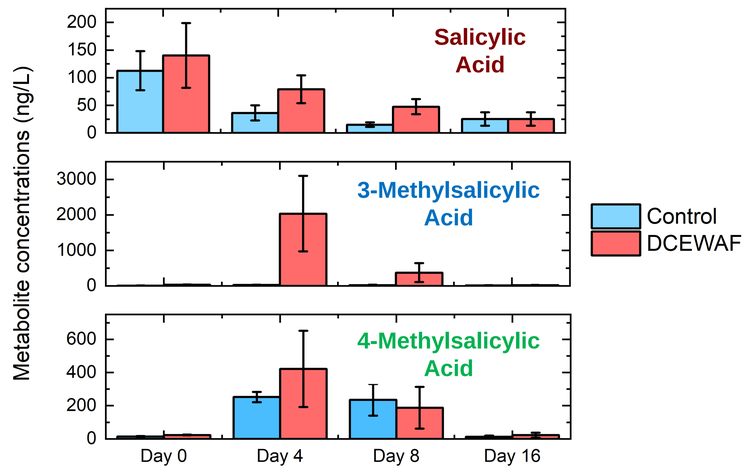
Alkane concentrations (left) and PAH concentrations (right) in controls and diluted chemically-enhanced water-accommodated fraction (DCEWAF) on particle phase, i.e. materials collected on filters. GF/F and Teflon filters are combined for particle phase analyses. Here, we operationally defined this fraction as particle associated fraction (PAF). PAF hydrocarbon concentrations were normalized based on the volume of water filtered. Petroleum alkanes are preferentially partitioned to PAF as expected, due to their lower water solubility. Similarly, higher molecular weight PAHs are preferentially partitioned to PAF.

Metabolic Pathways

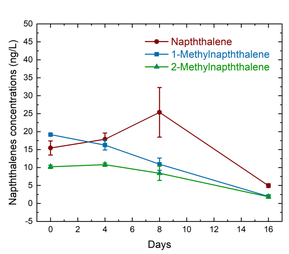


Degradation pathways of Naphthalenes. Target analytes are highlighted. Stars highlighted detected metabolites. Cirles highlighted analytes below detection limits.

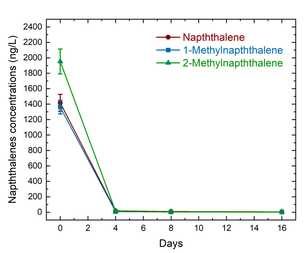
Targeted Metabolites



Concentrations of naphthalenes metabolites. Salicylic acid is one of the metabolic products of naphthalene, 3-methylsalicylic acid is one of the metabolic products of 1-methylnaphthalene, and 4-methylsalicylic acid is one of the metabolic products of 2-methylnaphthalene.

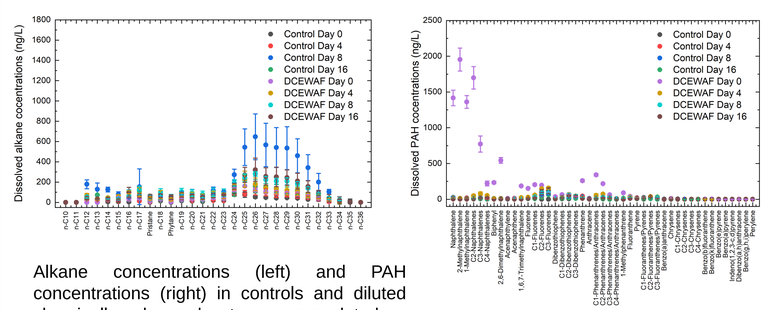


Naphthalene concentrations in controls.



Naphthalene concentrations in DCEWAF.

Hydrocarbons in Dissolved Phase



Alkane concentrations (left) and PAH concentrations (right) in controls and diluted chemically-enhanced water-accommodated fraction (DCEWAF) in dissolved phase, i.e. materials passed through the 0.2 µm Teflon filter. Dissolved hydrocarbon concentrations were normalized based on the volume of water filtered. We observed low concentrations of petroleum alkanes, as expected, due to their lower water solubility. Lower molecular weight PAHs are enriched in the dissolved phase.

References

1. Liu, Y, H.K. White, R.L. Simister, D. Waite, S.L. Lyons, E.B. Kujawinski: Probing the chemical transformation of seawater soluble crude oil components during microbial oxidation (Eartharxiv 10.31223/osf.io/xmv7e)
2. Dittmar, T.; Koch, B.; Hertkorn, N.; Kattner, G., A Simple and Efficient Method for the Solid-Phase Extraction of Dissolved Organic Matter (SPE-DOM) from Seawater. Limnol. Oceanogr. Methods 2008, 6, 230-235.

Acknowledgement

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Summary

- Both alkanes and PAHs were degraded substantially over the course of our experiment.
- Rapid degradation of PAHs were observed. Naphthalenes concentrations have reached to the baseline after day 4.
- Naphthalene degradation products such as the salicylic acids were observed in our Controls (Galveston Bay seawater), suggesting the presence of naphthalenes in the background.
- Elevated naphthalene degradations products relative to the Controls were observed in the dissolved phase at day 4 and day 8.
- Our results suggested that while low molecular weight PAHs such as naphthalenes are rapidly oxidized by microbes, their degradation products can be observed days after. The obviation of such extracellular metabolites in background seawater may suggest these metabolites can be served as markers for the fate of PAH (e.g., naphthelenes) in the environment.
- Untargeted analysis shows a clear distinction in the chemical fingerprints between control and DCEWAF. Although less significant, the chemical signatures of DCEWAF remain distinct after 16 days.