# Geochemistry and microscopy from incubation experiments of Chesapeake Bay sediments conducted at Horn Point Laboratory

**Website**: https://www.bco-dmo.org/dataset/883171
**Data Type**: Other Field Results, experimental
**Version**: 1
**Version Date**: 2022-11-02

**Project**
» Collaborative Research: Probing the Metabolic and Electrical Interactions of Cable Bacteria in Anoxic Sediments (Anoxic Sediment Bacteria Interactions)

| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
These data were collected as part of a sediment incubation experiment, to investigate potential interactions between estuarine cable bacteria and their associated microbial community. Sediments were collected from the main channel of Chesapeake Bay at a mesohaline station that experiences seasonal oxygen depletion. The upper 10 centimeters (cm) of sediment was homogenized under anaerobic conditions, packed into polycarbonate core liners, and incubated in a dark climate-controlled room in aerated aquaria with artificial seawater (S=15.5; T=16 degrees Celsius). In a sub-set of core tubes, a 0.2 micron polycarbonate filter was held embedded at 0.5 cm depth, to block the downward growth of cable bacteria while allowing for porewater diffusion. The sediments (with and without embedded filters) were destructively sampled on 6 dates over 46 days, at which time nucleic acids, samples for microscopy, and samples for porewater geochemistry were collected. Microsensor profiling (oxygen (O2), pH, hydrogen sulfide (H2S)) was conducted alongside the destructive sampling. Associated nucleic acid data are available through NCBI Sequence Read Archive, under Bioproject PRJNA833464. Sediment porewaters were handled anaerobically, and were extracted by centrifugation followed by filtration (0.2 micron in-line filters). Geochemical data include porewater anions (sulfate and chloride) measured by ion chromatography, and ammonium and ferrous iron concentrations measured by standard colorimetric methods. Microscopy data include both counts of cable bacteria measured with FISH oligoprobe DSB706, and total single cell bacteria. The dataset was generated by Pinky Liau under the supervision of Dr. Sairah Malkin, Horn Point Laboratory, U. Maryland Center for Environmental Sciences.

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## Coverage

**Spatial Extent**: **Lat**:38.55505 **Lon**:-76.42794
**Temporal Extent**: 2018-12 - 2019-01

## Dataset Description

This is the first of 4 datasets associated with "Incubation Experiment (Liau) CB Sediments". There is additionally an amplicon dataset (16S rRNA gene, RNA and DNA) associated with this experiment: NCBI Sequence Read Archive, under BioProject PRJNA833464, accession numbers SAMN27993143 to SAMN27993196. The NCBI data will not be publicly available in May 2023.

### Methods & Sampling

The source material was collected from Chesapeake Bay (38.55505 N, 76.42794 W) (mesohaline), water column depth 26 meters, sediment horizon 0-10 centimeter below sea floor.

Incubation conditions: 16 degrees Celsius, S=15.5, dark, aerated.

**Incubation Set-up:** Sediments were homogenized and packed into polycarbonate core liners, sealed with a stopper at the bottom, and open to aerated aquarium water at the top. In a subset of cores, a polycarbonate filter (pore size 0.2 microns) was secured at 0.5 cm depth, to prevent downward growth of cable bacteria in these treatments. Sediments were incubated for up to 46 days. At 6 time points, microsensor profiles were measured, followed by destructive sampling.

**Porewater extraction**: Sediments were sectioned at 0.5 cm depth increments in an anaerobic glove bag under nitrogen atmosphere. Porewaters were separated by centrifugation (3500 rpm for 10 minutes), and filtered (0.2 micron) and aliquoted in the anaerobic glove bag. Samples for ferrous iron measurements were preserved with trace-metal grade nitric acid (final pH < 2).

**Geochemical Measurements**: Porewater sulfate and chloride were analyzed by suppressed ion chromatography following 100-fold dilutions (Dionex Integrion IC). Porewater ferrous iron (Fe2+) was measured colorimetrically using the Ferrozine assay. Ammonium was measured colorimetrically using the phenol hypochlorite method.

**Cell Enumeration:** Samples for microbial cell enumeration were preserved in 96% molecular grade ethanol (1:1 v/v), gently mixed with sterile autoclaved toothpicks, and stored at -20°C. Sediment-associated cells were separated from sediment grains using an acetate buffer to dissolve carbonates, followed by washing with NaCl solution, and then gentle vortexing with a detergent mixed with methanol (Lunau et al. 2005, Kallmeyer et al. 2008). Cells were recovered using density centrifugation with Nycodenz (50% w/v). Cable bacteria filaments were enumerated using fluorescence in situ hybridization (FISH) with the DSB706 oligoprobe (Malkin et al. 2022), and total cell counts were enumerated following staining with SyBR Green I.

### Data Processing Description

**Data Processing:**
Standard curves with a minimum of 5 concentrations were analyzed to calibrate the geochemistry data. Microscopy counts were computed based on minimum cell counts. For cable bacteria, a minimum of 200 fields or 30 filaments were counted (whichever came first). For single total cell counts, a minimum of 400 cells were counted, appropriately diluted to be captured in 10-20 fields. The proportion of the slide examined was computed based on the field size, confirmed with a stage micrometer.

**BCO-DMO Processing:**
- replaced "NA" with "nd" (no data value);
- renamed fields to comply with BCO-DMO naming conventions.

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## Data Files

| File |
| --- |
| **geochem.csv** (Comma Separated Values (.csv), 3.51 KB)<br>MD5:792d3f827a6a9580d7cc1cae63a8775b<br>Primary data file for dataset ID 883171 |

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## Related Publications

Kallmeyer, J., Smith, D. C., Spivack, A. J., & D'Hondt, S. (2008). New cell extraction procedure applied to deep subsurface sediments. Limnology and Oceanography: Methods, 6(6), 236–245. doi:10.4319/lom.2008.6.236
*Methods*

Liau, P., Kim, C., Saxton, M. A., & Malkin, S. Y. (2022). Microbial succession in a marine sediment: Inferring interspecific microbial interactions with marine cable bacteria. Environmental Microbiology. Portico. https://doi.org/10.1111/1462-2920.16230
*Results*

Lunau, M., Lemke, A., Walther, K., Martens-Habbena, W., & Simon, M. (2005). An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. Environmental Microbiology, 7(7), 961–968. https://doi.org/10.1111/j.1462-2920.2005.00767.x
*Methods*

Malkin, S. Y., Liau, P., Kim, C., Hantsoo, K. G., Gomes, M. L., & Song, B. (2022). Contrasting controls on seasonal and spatial distribution of marine cable bacteria (Candidatus Electrothrix) and Beggiatoaceae in seasonally hypoxic Chesapeake Bay. Limnology and Oceanography, 67(6), 1357–1373. Portico. https://doi.org/10.1002/lno.12087
*Methods*

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## Related Datasets

### IsRelatedTo

Malkin, S. (2022) **H2S microsensor profiling conducted alongside the destructive sampling of Chesapeake Bay sediments during incubation experiments at Horn Point Lab.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-09 doi:10.26008/1912/bco-dmo.883506.1 [view at BCO-DMO]

Malkin, S. (2022) **O2 microsensor profiling conducted alongside the destructive sampling of Chesapeake Bay sediments during incubation experiments at Horn Point Lab.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-08 doi:10.26008/1912/bco-dmo.883412.1 [view at BCO-DMO]

Malkin, S. (2022) **pH microsensor profiling conducted alongside the destructive sampling of Chesapeake Bay sediments during incubation experiments at Horn Point Lab.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-09 doi:10.26008/1912/bco-dmo.883488.1 [view at BCO-DMO]

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Sample_ID | A unique code for each sample | unitless |
| Treatment | The treatment of the sediments; values are “NoFilter” or “Filter”, as described in methods | unitless |
| Day | Time of sampling after cores were homogenized and incubated in days | days |
| TopDepth_cm | The upper depth of the sediment sample | centimeters below surface |
| BottomDepth_cm | The lower depth of the sediment sample | centimeters below surface |
| NH4_uM | Porewater ammonium concentration | micromolar (uM) |
| Fe2_uM | Porewater ferrous iron concentration | micromolar (uM) |
| Cl_mM | Porewater chloride concentration | millimolar (mM) |
| SO4_mM | Porewater sulfate concentration | millimolar (mM) |
| Microscopy_DSB706_cell_cm3 | Microscopy counts using DSB706 oligo probe (i.e., cable bacteria) | cells per cubic centimeter |
| Microscopy_AllSingleCells_cells_cm3 | Microscopy counts of all single cells (SYBR Green) | cells per cubic centimeter |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | |
| **Generic Instrument Name** | Aquarium |
| **Generic Instrument Description** | Aquarium - a vivarium consisting of at least one transparent side in which water-dwelling plants or animals are kept |

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|---|---|
| **Dataset-specific Instrument Name** | Zeiss AxioCam MR camera |
| **Generic Instrument Name** | Camera |
| **Dataset-specific Description** | Zeiss Axiophot fluorescent microscope equipped with a digital Zeiss AxioCam MR camera, and Zen Pro software |
| **Generic Instrument Description** | All types of photographic equipment including stills, video, film and digital systems. |

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|---|---|
| **Dataset-specific Instrument Name** | |
| **Generic Instrument Name** | Centrifuge |
| **Generic Instrument Description** | A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids. |

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|---|---|
| **Dataset-specific Instrument Name** | Zeiss Axiophot fluorescent microscope |
| **Generic Instrument Name** | Fluorescence Microscope |
| **Dataset-specific Description** | Zeiss Axiophot fluorescent microscope equipped with a digital Zeiss AxioCam MR camera, and Zen Pro software |
| **Generic Instrument Description** | Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments. |

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| **Dataset-specific Instrument Name** | Dionex Integrion IC with IonPac AS-19 analytical column |
| **Generic Instrument Name** | Ion Chromatograph |
| **Dataset-specific Description** | Porewater sulfate and chloride were analyzed by suppressed ion chromatography. |
| **Generic Instrument Description** | Ion chromatography is a form of liquid chromatography that measures concentrations of ionic species by separating them based on their interaction with a resin. Ionic species separate differently depending on species type and size. Ion chromatographs are able to measure concentrations of major anions, such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major cations such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. (from http://serc.carleton.edu/microbelife/research_methods/biogeochemical/ic....) |

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|---|---|
| **Dataset-specific Instrument Name** | ThermoScientific Genesis UV/Vis |
| **Generic Instrument Name** | Spectrophotometer |
| **Dataset-specific Description** | Used to determine NH4 and Fe |
| **Generic Instrument Description** | An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples. |

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## Project Information

**Collaborative Research: Probing the Metabolic and Electrical Interactions of Cable Bacteria in Anoxic Sediments (Anoxic Sediment Bacteria Interactions)**

**Coverage**: Chesapeake Bay sediments; Mid-Atlantic Coastal sediments

*NSF Award Abstract:*

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Marine sediments represent the world's largest repository of stored organic carbon, and understanding how microorganisms break down this carbon is an imperative for understanding global carbon cycling. Yet long-standing questions remain regarding how networks of microorganisms work together to accomplish the complete breakdown of organic carbon in marine sediments. Sediment microbes interact in a myriad of ways that couple their metabolism to the break down of organic carbon, including by sharing products of metabolism. Accumulating evidence further suggests that some microorganisms can interact by transferring electrons directly to other unrelated microorganisms. This ability occurs across diverse microorganisms and appears to be widespread in the biosphere, particularly in anaerobic environments such as marine sediments. This project addresses emerging questions about the identity and metabolic linkages between microorganisms that work together in natural anaerobic marine and estuarine sediments to break down organic carbon. The investigators approach these questions by focusing on the influence of a keystone bacterium on its surrounding microbial community. "Cable bacteria" are a recently discovered group of long filamentous bacteria that act as electrical conductors in aquatic sediments providing a conduit for electrons to commute from deeper sulfidic sediments up to the surface oxygen layer by the process of centimeter-scale electron transport. Since their discovery about 6 years ago, these bacteria have been observed in a wide range of depositional sedimentary environments, often at extremely high cell densities. Where these bacteria are abundant, such as in coastal marine muds, they drive intense localized changes in pH and strongly influence the mineral cycling. This research explores the direct and indirect influence of cable bacteria on the metabolic activity of associated microorganisms. This project also advance the education and training of two early-career investigators, two PhD students, and undergraduate students. The skills and expertise gained from these PhD research projects will enable the students to be competitive in academic pursuits and in bioinformatics and technology applications relevant to private industry. The scientific discoveries emerging from this work is being incorporated into undergraduate and graduate level courses in marine microbial ecology. The research team will reach out to the broader community by hosting public lectures promoting a better understanding of environmental microbial ecology.

The proposed work is to investigate the role of cable bacteria in structuring sediment microbial communities. Due to their growth strategy and morphology, cable bacteria are particularly amenable to experimental manipulation, providing an outstanding opportunity to better understand community interactions among microorganisms in a natural and complex anaerobic environment. The investigators will explore the interactions and relationships between cable bacteria and their associated microbial community by manipulating the growth and activity of cable bacteria and quantifying the resultant microbial community response. Specifically, this project aims to (1) identify microorganisms whose growth is enhanced by cable bacteria, (2) identify metabolic processes linked with cable bacteria activity using metatranscriptomics, (3) test specific metabolic links between sediment microorganisms and cable bacteria activity using a DNA-stable isotope probing (SIP) approach, and (4) visually confirm the identity and quantify key microorganisms associated with cable bacteria using microscopy. As more is learned about the identity and the mechanisms by which microorganisms are metabolically linked in anoxic sediments, we will be better able to understand and make predictions about how microorganisms function in their environment and how they can be utilized in bioengineered systems.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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## Funding


| Funding Source | Award |
|---|---|
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1756877 |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1756851 |


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