Sulfate reduction rates at Main Endeavor grotto chimney from samples collected on RV Atlantis (AT18-08) during Jason II dives in the Juan de Fuca Ridge from July to August 2011

Website: https://www.bco-dmo.org/dataset/661557
Data Type: Cruise Results
Version: 1
Version Date: 2016-10-13

Project
» Characterizing the distribution and rates of microbial sulfate reduction at Middle Valley hydrothermal vents (Middle Valley Vents)

Program
» Center for Dark Energy Biosphere Investigations (C-DEBI)

Contributors	Affiliation	Role
Girguis, Peter	Harvard University	Principal Investigator
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Ake, Hannah	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Sulfate reduction rates at Main Endeavor grotto chimney from samples collected on RV Atlantis (AT18-08) during Jason II dives in the Juan de Fuca Ridge from July to August 2011

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Coverage

Spatial Extent: **Lat**:47.95 **Lon**:-129.1
Temporal Extent: 2011 - 2011

Dataset Description

The effects of key environmental variables (temperature, pH, H2S, SO42-, DOC) on sulfate reduction rates in material recovered from a hydrothermal flange from the Grotto edifice in the Main Endeavor Field, Juan de Fuca Ridge. Sulfate reduction was measured in batch reactions across a range of physico-chemical conditions. Temperature and pH were the strongest stimuli, and maximum sulfate reduction rates were observed at 50 degrees celsius and pH 6.

Information for this dataset was derived from single massive piece of hydrothermal deposit (approximately ~100 kg in weight) that was recovered from a flange on the Grotto vent (47.949, -129.098) at a depth of 2188.3 m (Dive J2-575, AT-18-08, *R/V Atlantis*) and brought up to the surface in the basket of the *ROV Jason*

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Analysis and write-up of these data are found in Frank *et al.*, 2015

Methods & Sampling

Tables and Figures referenced in the acquisition description are found in the paper Frank *et al.*, 2015

Once on board ship, tubeworms and other macrofauna were removed from the samples and the large pieces were broken into more manageable fragments (~10-20 cm3) with a flame-sterilized chisel and sledgehammer, with the user wearing sterile nitrile gloves. Samples were quickly transferred to 0.2 um-filtered anaerobic (nitrogen-sparged) seawater. Samples were further broken down into smaller sizes while in anaerobic water, and subsamples from the interior of the fragments were immediately transferred to gastight jars (Freund Container Inc.) filled with sterile anaerobic seawater containing 2 mM sodium sulfide at pH 6, and stored at 4 degrees celsius for incubations and analyses. The sterile sulfidic seawater in the gastight jars were refreshed periodically during storage at 4 degrees celsius. The majority of the rate experiments (80%) were set up immediately on the ship using freshly collected samples. In parallel, subsamples (~1 cm3) from each flange were preserved aboard ship in glutaraldehyde (2.5% in phosphate buffered saline, PBS, pH 7.0), then prepared for electron microscopy via ethanol dehydration and critical point drying before being sputtered with a thin layer of gold-palladium to improve image resolution. Samples were imaged with a Zeiss model EVO Scanning Electron Microscope (SEM).

Prior to incubation, each flange subsample was pulverized by hand for about one hour to minimize fine-scale geological and microbial heterogeneity and facilitate more accurate experimental replication (akin to slurry experiments in sediments; Fossing & Jørgensen 1989; Weber & Jørgensen 2002; Jørgensen et al. 1992). Specifically, each subsample was pulverized with a flame-sterilized sledgehammer in sterile seawater actively bubbled with nitrogen within an anaerobic chamber. For each independent treatment, aliquots of 7.5 mL flange slurry (approx. 29 g wet weight and 20 g dry weight) were transferred into Balch tubes in an anaerobic chamber, and supplemented with 15 mL of sterile artificial seawater media designed to mimic the geochemical conditions within a hydrothermal flange (400 mM NaCl, 25 mM KCl, 30 mM CaCl2, 2.3 mM NaHCO3, 14 mM NaSO42-, 1 mM H2S, and 50 uM dissolved organic carbon - consisting of equimolar proportions 10 uM of pyruvate, citrate, formate, acetate, lactate) under a pure nitrogen headspace.

Concentrations of sulfide, sulfate and dissolved organic carbon (DOC) were varied independently to investigate concentration dependent effects on the rates of SR. The range of experimental conditions tested was determined from previously published concentration profiles of aqueous species modeled as functions of temperature and position within the Grotto vent structure (Tivey, 2004). Concentrations were varied by orders of magnitude within the modeled ranges to simulate conditions representative of different mixing regimes between seawater and vent fluid (Table 1). The range of DOC (which we approximate as a mix of pyruvate, citrate, formate, acetate, lactate – most of which have been identified to varying degrees within vent fluid and are known carbon sources for heterotrophic SR in culture) concentrations tested were based on the average DOC concentrations measured within diffuse fluids at the Main Endeavor Field (Lang et al., 2006; Lang et al., 2010). Hydrogen sulfide was present as H2S (pKa in seawater of 6.60) across all the conditions tested (Amend & Shock, 2001). Incubations were carried out at pH 4 (to simulate the pH of end-member Grotto vent fluid and the average calculated pH of mixed fluids in highly reduced zones within the flange; Tivey 2004) as well as pH 6 (representative of the calculated pH in fluid mixing zones; Tivey 2004). All the results are presented and discussed in the context of the initial measured media conditions.

Sufficient 35SO42- was added to achieve 15 uCi of activity. Samples were incubated anaerobically for 1, 3 or 7 days at ambient seawater (4 degrees celsius), thermophilic (50 degrees celsius) and hyperthermophilic (90 degrees celsius) temperatures. The range of temperatures considered was representative of different thermal regimes associated with the surface, outer layer and middle regions of hydrothermal chimneys (Tivey 2004; Kormas et al. 2006; Schrenk et al. 2003). Negative controls consisted of samples amended with 28 mM molybdate to inhibit SR (Newport & Nedwell, 1988; Saleh et al., 1964). Three biological replicates were run for each treatment, and two biological replicates for each control.

Upon completion, reactions were quenched with the injection of 5 mL 25% zinc acetate, at pH 8 (i.e. 20-fold excess Zn), and all samples were frozen at -20 degrees celsius for further analysis. 80% of incubations were performed shipboard with freshly collected samples and the remaining 20% of incubations were completed within one year of collection.

To determine SR rates, samples were thawed and the supernatant was removed and filtered through a 0.2 um syringe filter. The homogenized flange that remained in the tube was washed three times with deionized water to remove any remaining sulfate. One gram (wet weight) of flange material was added to 10 mL of a 1:1 ethanol to water solution in the chromium distillation apparatus, and then degassed with nitrogen for 15 minutes to drive the environment anoxic. Hydrogen sulfide gas was evolved after the anaerobic addition of 8 mL of 12 N HCl and 10 mL of 1 M reduced chromium chloride, followed by 3 hours of heating. The resulting hydrogen sulfide gas was carried via nitrogen gas through a condenser to remove HCl, and was then trapped as zinc sulfide in a 25% zinc acetate solution. To moderate potential artifacts of hot distillation methods including elevated rates in control samples, experiments were analyzed in triplicate, on different days and with different glassware to minimize cross-contamination, and any activity observed in “control” samples was deleted from the treatments. The radioactivity of the resulting sulfide (Zn35S) and the remaining sulfate from the supernatant (35SO42-) were measured via liquid scintillation counter in Ultima Gold scintillation cocktail (ThermoFisher Inc., Waltham, MA).

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Data Processing Description

Rates were determined using the following calculation as in (Fossing & Jorgensen, 1989).

$$\frac{\mathrm{nSO_4^{2-}} \cdot a \cdot 1.06}{(a + A) \cdot t}$$

Where nSO42- is the quantity (in moles) of sulfate added to each incubation (14 mM * 15 mL = 210 umol), a is the activity (dpm) of the trapped sulfide, 1.06 is the fractionation factor between the hydrogen sulfide and sulfate pools (Jørgensen & Fenchel 1974), A is the activity of the sulfate pool at the completion of the incubation and t is the incubation time (days). The rates are presented in units of nmol S g-1 day-1. As previously mentioned, SR rates are numerically presented as the difference in rates between experimental and the molybdate inhibited controls, further mitigating any potential artifacts caused by hot distillation methods.

BCO-DMO Data Processing Notes:

-reformatted column names to comply with BCO-DMO standards
-filled in all blank cells with nd
-removed spaces and replaced with underscores

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Data Files

File
SRR.csv (Comma Separated Values (.csv), 4.24 KB) MD5:9c8f3b1582f168e11d913f1bc691bbc9 Primary data file for dataset ID 661557

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Related Publications

Frank, K. L., Rogers, K. L., Rogers, D. R., Johnston, D. T., & Girguis, P. R. (2015). Key Factors Influencing Rates of Heterotrophic Sulfate Reduction in Active Seafloor Hydrothermal Massive Sulfide Deposits. Frontiers in Microbiology, 6. doi:10.3389/fmicb.2015.01449

General

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Parameters

Parameter	Description	Units
experiment_num	PI issued experiment ID number	unitless
sulfide	Independently varied concentration of sulfide	molar (M)
DOC	Independently varied dissolved oxygen concentration	micromoles (uM)
sulfate	Independently varied concentration of sulfate	millimoles (mM)
pH	pH of media added to incubations; Incubations were carried out at either pH 4 or 6; 4: simulates the pH of end-member Grotto vent fluid and the average calculated pH of mixed fluids in highly reduced zones within flange (Tivey 2004). 6: represents the calculated pH in fluid mixing zones (Tivey 2004).	pH
temperature	Temperatures at which samples were incubated anaerobically for 1, 3, or 7 days. 4 C: ambient seawater; 50 C: thermophilic; 90 C: hyperthermophilic.	celsius (C)
inoculum	Fresh: incubation performed on shipboard with freshly collected samples; Stored: incubation completed within one year of collection.	unitless
SRrate_3day	Sulfate reduction rate after 3 day incubation	nmol/gday
SRrate_3day_replicates	Number of replicates used for 3 day incubation	count
SRrate_7day	Sulfate reduction rate after 7 day incubation	nmol/gday
SRrate_7day_replicates	Number of replicates used for 7 day incubation	count
SRrate_inhibited	Sulfate reduction rate molybdate inhibited controls	nmol/gday
SRrate_inhibited_replicates	Number of replicates used for molybdate inhibited controls	count
lat	Latitude	decimal degrees
lon	Longitude	decimal degrees

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Instruments

Dataset-specific Instrument Name	Zeiss model EVO Scanning Electron Microscope
Generic Instrument Name	Electron Microscope
Dataset-specific Description	Tubeworm and macrofauna subsamples were imaged.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of electrons behaving as waves.

Dataset-specific Instrument Name	Incubator
Generic Instrument Name	In-situ incubator
Dataset-specific Description	Used aboard ship and in lab
Generic Instrument Description	A device on a ship or in the laboratory that holds water samples under controlled conditions of temperature and possibly illumination.

Dataset-specific Instrument Name	Liquid scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Used to quantify activity
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

Dataset-specific Instrument Name	DO sensor
Generic Instrument Name	Oxygen Sensor
Dataset-specific Description	DOC was measured
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	pH sensor
Generic Instrument Name	pH Sensor
Dataset-specific Description	pH sensor
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

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Deployments

AT18-08

Website	https://www.bco-dmo.org/deployment/568087
Platform	R/V Atlantis
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/AT18-08_nemo11-cruise-report.pdf
Start Date	2011-07-19
End Date	2011-08-01
Description	Data expected from C-DEBI investigator, Julie Huber. Additional cruise information and original data are available from the NSF R2R data catalog.

AT18-08_Jason_Dives

Website	https://www.bco-dmo.org/deployment/661574
Platform	R/V Atlantis
Start Date	2011-07-21
End Date	2011-07-31

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Project Information

Characterizing the distribution and rates of microbial sulfate reduction at Middle Valley hydrothermal vents (Middle Valley Vents)

Coverage: Middle Valley vent field along the Juan de Fuca Ridge

This project characterizes rates of microbially mediated sulfate reduction from three distinct hydrothermal vents in the Middle Valley vent field along the Juan de Fuca Ridge, as well as assessments of bacterial and archaeal diversity, estimates of total biomass and the abundance of functional genes related to sulfate reduction, and in situ geochemistry. Maximum rates of sulfate reduction occurred at 90°C in all three deposits. Pyrosequencing and functional gene abundance data reveal differences in both biomass and community composition among sites, including differences in the abundance of known sulfate reducing bacteria. The abundance of sequences for Thermodesulfovibro-like organisms and higher sulfate reduction rates at elevated temperatures, suggests that Thermodesulfovibro-like organisms may play a role in sulfate reduction in warmer environments. The rates of sulfate reduction observed suggest that - within anaerobic niches of hydrothermal deposits - heterotrophic sulfate reduction may be quite common and might contribute substantially to secondary productivity, underscoring the potential role of this process in both sulfur and carbon cycling at vents.

This project was funded, in part, by a C-DEBI Graduate Student Fellowship.

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Program Information

Center for Dark Energy Biosphere Investigations (C-DEBI)

Website: http://www.darkenergybiosphere.org

Coverage: Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:
(1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
(2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep subseafloor ecosystems;
(3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
(4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

Data Management:
C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their Data Management Plan (PDF) and in compliance with the NSF Ocean Sciences Sample and Data Policy. The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1061934
NSF Division of Ocean Sciences (NSF OCE)	OCE-0838107
NASA Astrobiology Science & Technology for Exploring Planets (NASA-ASTEP)	NNX09AB78G
NASA Astrobiology Science & Technology for Exploring Planets (NASA-ASTEP)	NNX07AV51G

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