NH4Cl effect on growth, yield, and methane production of thermophyles, Table 3 from Topcuoglu et al (2016)

Website: https://www.bco-dmo.org/dataset/664306
Data Type: experimental
Version:
Version Date: 2016-11-08

Project
» Event response to an eruption at Axial Seamount (NeMO2015)

Program
» Ocean Observatories Initiative (OOI)

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Dataset Description

This dataset includes growth rate, cell yield based on methane production and cell-specific CH4 production rate for *Methanocaldococcus jannaschii, Methanothermococcus thermolithotrophicus, Methanocaldococcus bathoardescens* (from Ver Eecke et al., 2013) grown with varying concentrations of NH4Cl in otherwise nitrogen-free medium

Relevant Reference: Topcuoglu, BD, Stewart LC, Morrison HG, Butterfield DA, Huber JA and Holden JF (2016) Hydrogen Limitation and Syntrophic Growth among Natural Assemblages of Thermophilic Methanogens at Deep-sea Hydrothermal Vents. Front. Microbiol. 7:1240. doi: 10.3389/fmicb.2016.01240

Related Datasets:
Axial Seamount hydrothermal sampling sites
Thermophile abundance - MPN

Methods & Sampling

In August 2012, September and October 2013, August 2014, and August 2015, 7-40°C diffuse hydrothermal fluids were collected from 10 vent sites at 1515-1716 m depths from Axial Seamount on the Juan de Fuca Ridge. The fluid samples were drawn into 650 ml Tedlar plastic bags with polyethylene valves within rigid

housings using the NOAA Hydrothermal Fluid and Particle Sampler. The sampler pumped vent fluid through a titanium nozzle and recorded the temperature of the fluid within the intake nozzle once every second during pumping. Samples were collected using the research submarines Jason II (dives J820-J826) and ROPOS. Background seawater was collected by shipboard hydrocasts at 1500 m depth directly over the caldera (25 m above the bottom) and 3 km west of the summit with 10 L Niskin bottles. The hydrothermal fluid and background seawater samples were divided for cultivation-dependent Most Probable Number (MPN) concentration estimates of thermophiles and hyperthermophiles (100 ml), microcosm incubations (400 ml), and total cell counts (40 ml). All operations at sea occurred on the research vessels Marcus G. Langseth, Thomas G. Thompson, Falkor, and Ronald H. Brown.

For each sample site, 25 ml of hydrothermal fluid or background seawater was added without exposure to air to each of 16 sealed 60 ml serum bottles that had been pre-flushed with either H_2:CO_2 (80%:20%) or N_2:CO_2 (80%:20%), depending on the headspace composition used for incubation (see table in full methods pdf). The bottles were divided into four sets of four bottles with a pair of bottles from each set incubated at 55°C and 80°C for up to a week or until visibly turbid. Three of the four sets of microcosms (sets A-C) were incubated each of the four study years. Set A was flushed and filled with 200 kPa of H_2:CO_2 yielding an estimated aqueous H_2 concentration of 1.2 mM at their incubation temperatures based on calculations using the geochemical prediction software Geochemist's Workbench. Sets B and C were flushed and filled with 200 kPa of N_2:CO_2, and half of these bottles (set B) were given 1 ml of H_2:CO_2 in exchange for 1 ml of N_2:CO_2 to produce an estimated aqueous H_2 concentration of 20 mM at their incubation temperatures. In 2012 and 2013, the remaining four serum bottles (set D) were amended with 4.7 mM NH_4Cl (2012 only) or 47 mM NH_4Cl (2013 only) and flushed and filled with 200 kPa of H_2:CO_2 to test for growth stimulation by ammonium. The NH_4Cl concentration was based on that added to our defined methanogen growth medium (see below). In 2014 and 2015, the remaining four serum bottles (set E) were amended with 0.5% (wt vol^{-1}) tryptone plus 0.01% (wt vol^{-1}) yeast extract and flushed and filled with 200 kPa of N_2:CO_2 to test for H_2 syntrophy. All samples were reduced with 0.025% (wt vol^{-1}) each of cysteine-HCl and $Na_2S•9H_2O$. Growth of methanogens was determined by analyzing for CH_4 in the headspace using gas chromatography once the cells in the bottle had reached stationary growth phase. In 2015, an aliquot of the 80°C and 55°C tryptone/no H_2 samples (set E) that showed CH_4 production were filtered onto 0.2-µm pore size nucleopore filters prestained with Irgalan black (Sterlitech, Kent, WA, USA), stained with acridine orange, and examined using epifluorescence microscopy. In 2015, the 80°C and 55°C tryptone/no H_2 samples from the Marker 113 vent site were also separately filtered through Sterivex GP 0.22 µm sterile filter units (Millipore, Billerica, MA, USA) and frozen at -80°C until analyzed. In2015, 10 ml of hydrothermal fluid was added to sealed Balch tubes without exposure to air, amended separately with 0.1% (wt vol^{-1}) sodium formate and 0.5% (wt vol^{-1}) sodium acetate, flushed and filled with 200 kPa N_2:CO_2, and incubated in duplicate at 80°C and55°C for up to seven days to determine if these substrates can support methanogenesis at high temperatures.

Total cell counts in the original hydrothermal fluids were done by preserving in duplicate 18 ml of hydrothermal fluid with 1.8 ml of 37% formaldehyde. Samples were stored at 4°C for less than a month prior to counting by epifluorescence microscopy as described above.

Full methodology

Data Processing Description

BCO-DMO Processing notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- replaced blank cells with NA (not applicable)
- split +/- error into two columns

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Data Files

File
thermophile_growth.csv (Comma Separated Values (.csv), 1.09 KB) MD5:b84f5666ea7cae5331ca79a2d2a8b1f9 Primary data file for dataset ID 664306

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Parameters

Parameter	Description	Units
species	experimental species	unitless
NH4Cl	concentration of ammonium chloride used in experiment	micromoles or millimoles
growth_rate	growth rate	(k) (h-1)
growth_rate_err	growth rate error: 95% confidence interval (alpha = 0.05)	(k) (h-1)
yield	yield	10^12 cells mol-1 CH4
yield_err	yield error: 95% confidence interval (alpha = 0.05)	10^12 cells mol-1 CH4
CH4_prod_rate	methane production rate	picomoles methane/cell/hr (pmol CH4 cell-1 h-1)
CH4_prod_rate_err	methane production rate error: 95% confidence interval (alpha = 0.05)	picomoles methane/cell/hr (pmol CH4 cell-1 h-1)

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Instruments

Dataset-specific Instrument Name	phase-contrast light microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Used to confirm growth of cultures
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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Deployments

TN327

Website	https://www.bco-dmo.org/deployment/664100
Platform	R/V Thomas G. Thompson
Start Date	2015-08-14
End Date	2015-08-29
Description	NOAA New Millennium Observatory (NeMO) 2015/Rapid Response to an Eruption

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Project Information

Event response to an eruption at Axial Seamount (NeMO2015)

Website: http://axial2015.blogspot.com

Coverage: Axial Seamount, Juan de Fuca Ridge, northeastern Pacific Ocean (46.06°N 130.00°W)

On 24 April 2015, the NSF-funded Ocean Observatories Initiative's (OOI) Cabled Array detected the onset of a probable eruption at Axial Seamount, heralded by a swarm of >8000 small earthquakes and a rapid subsidence of the seafloor by >2.4 meters at the center of the caldera. Evidence that lava was erupted in or near the summit caldera includes a dramatic temperature rise recorded by instruments on the OOI Cabled Array-- up to 0.6-0.7°C above ambient sustained for weeks after the event. This eruption is likely to have significantly perturbed the hydrothermal and biological systems in and around the summit caldera, and provides the rare opportunity to address time-critical scientific questions that can only be investigated with the near-term seafloor investigations. A currently scheduled NSF and NOAA funded cruise to Axial Seamount on R/V Thompson with ROV Jason and AUV Sentry in August 2015 provides an excellent opportunity for such a response. This study adds 3 days onto this cruise to facilitate time-critical event response science.

Detailed seafloor mapping with shipboard multi-beam sonar and near-bottom Sentry surveys will cover areas of the caldera and adjacent rift zones that are expected eruption site(s). Fresh rock, if located, will be sampled and dated using the 210Po-210Pb technique. Hydrothermal plumes will be discerned with CTD casts and sensor tows. A mooring will be deployed with Miniature Autonomous Plume Recorders to measure temperature, light attenuation, and redox potential. The at-sea team plans to make samples and data available to the broader science community for targeted research on seafloor processes.

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Program Information

Ocean Observatories Initiative (OOI)

Website: http://oceanobservatories.org/

The Ocean Observatories Initiative (OOI) is a science-driven ocean observing network that delivers real-time data to address critical science questions regarding the world's oceans. Funded by the National Science Foundation to encourage scientific investigation, OOI data are freely available online to anyone with an Internet connection. OOI was designed as a long-term project to collect ocean data for up to 30 years. This longevity makes it possible to measure and directly observe both short-lived episodic events and longer-term changes occurring in the ocean. Such data make it possible to better understand ocean processes and how the ocean is changing.

The OOI has five active research arrays that comprise the three major observatory elements linked together by instrument, infrastructure, and information management systems. Global Ocean Arrays consist of moored

arrays and autonomous vehicles that provide time-series observations and mesoscale spatial sampling at sparsely sampled, high-latitude regions critical to our understanding of climate, the carbon cycle, and ocean circulation. The Regional Cabled Array consists of fiber-optic cables off the Oregon coast that provide unprecedented power, bandwidth, and communication to seafloor instrumentation and profiler moorings, enabling monitoring of volcanic and hydrothermal activity, methane seeps, earthquakes, and myriad ocean processes in coastal and blue water environments. Coastal Arrays consist of cross-shelf moored arrays and autonomous vehicles that observe the dynamic coastal environment, enabling examination of upwelling, shelf break fronts, and cross-shelf exchanges.

These marine arrays are outfitted with more than 900 instruments — of 45 different types — measuring more than 200 different parameters. These instruments gather physical, chemical, geological, and biological data – from the air-sea interface to the seafloor. The data collected are transmitted through a cyberinfrastructure, an information management system that allows users to access real- to near real-time data from suites of sensors. The OOI provides annotations and automated quality control for data streams and is working to meet the IOOS Quality Assurance of Real Time Ocean Data (QARTOD) standards.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1547004

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