Rates of methanogenesis in subsurface sediments from R/V JOIDES Resolution IODP-385 drilling expedition in the Guaymas Basin between September and November, 2019

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Project
» Pathways and regulation of transformation of low molecular weight carbon compounds in subseafloor sediments from the Guaymas Basin (Gulf of California) (Guaymas Basin Sediments)

Contributors	Affiliation	Role
Joye, Samantha B.	University of Georgia (UGA)	Principal Investigator
Mickle, Audrey	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Deep marine sediments are the largest reservoir of methane on Earth. Yet, the metabolic pathways and activity of methanogenesis in deep, hot sediments remain poorly understood. In this study, we quantified methanogenic activity using five different 14C-labeled substrates, and combined these potential rates with geochemical data to identify the dominant methanogenic pathways and their environmental controls in the subsurface sediments of the Guaymas Basin. Thermodynamic calculations and C1/C2+ ratios indicated that methane in the relatively cooler, shallower layers, was predominantly of biogenic origin. Radiotracer experiments provided direct evidence for the coexistence of multiple methanogenic pathways, hydrogenotrophic, acetoclastic, and methylotrophic, across the sediment column. Methanogenic activity from multiple methanogenic pathways occurred over a wide temperature range (3°C to 80°C), highlighting the unexpectedly high metabolic versatility of methanogens in deep, thermally heated sediments. High methanogenesis rates were detected in near-surface sediments driven predominantly by methylotrophic methanogenesis, followed by hydrogenotrophic pathways. However, these rates declined sharply with depth, particularly within the 40–60°C interval, indicating a transition from mesophilic to thermophilic microbial communities, due to rising temperatures, reductions in gene expression, and decreasing microbial cell densities. Methylotrophic methanogenesis remained detectable down to 320 meters below the seafloor and was the dominant methane-producing pathway at temperatures up to 60°C. In sediments increasingly influenced by sill intrusions, hydrogenotrophic and acetoclastic methanogenesis became the predominant modes of methane production. Methanogenic activity rates from multiple substrates at 80°C were comparable to rates in near-surface sediments. This deep, hot activity is attributed to the presence of active microbial biomass and the enhanced reactivity and bioavailability of organic matter in deep, hydrothermally-heated sediments, which provided abundant substrates for methanogenesis. These findings expand the current understanding of methanogenesis in the deep biosphere and reveal the discovery of the contemporaneous activity of multiple methanogenic pathways in deep, hydrothermally-influenced sediments.

Table of Contents

Coverage

Location: Guaymas Basin, Gulf of California
Spatial Extent: **N**:27.637 **E**:-111.48 **S**:27.472 **W**:-111.889
Temporal Extent: 2019-09-15 - 2019-11-15

Methods & Sampling

Subsurface sediment samples were collected from four drilling sites in the Guaymas Basin, Gulf of California, during IODP Expedition 385 “Guaymas Basin Tectonics and Biosphere” using the research vessel R/V JOIDES Resolution. Rates of methanogenesis were determined at four sites. Sites 1545 (27º38.230’N, 111º53.329’W) and 1546 (27º37.884’N, 111º52.781’W) were located roughly 52 km and 51 km, respectively, northwest of the axial graben of the northern spreading segment. Both sites are highly sedimented and a 75-meter thick inactive (~thermally equilibrated) basaltic/doleritic/gabbroic sill was present at site 1546 between ~355 to 431 meters below the seafloor (mbsf). Site U1545 is considered a reference site since it was free of sill intrusions and unaffected by active hydrothermal circulation. Samples to determine methanogenesis rates were collected from the "B" hole at every site (during IODP expeditions, several, closely-spaced holes are drilled at each site so that sufficient sediment is available for all of the proposed work.

The geothermal gradient at hole U1545B was 227ºC/km. The geothermal gradient at site U1546B was similar to that measured in hole U1545B, 221ºC/km, since the sill at 1546B was thermally equilibrated. Sites U1547 (27º30.413’N, 111º40.734’W) was located inside the periphery of an active, sill-associated hydrothermal mound located about 27 km northwest of the axial graben of the northern spreading segment. Temperatures in hole U1547B exceeded 50°C in the upper 50 mbsf. The geothermal gradient at this site was between 511ºC to 960ºC/km. Site U1549 was located near a cold seep sustained by a deeply buried, thermally equilibrated sill intrusion at several hundred meters depth. The geothermal gradient at hole U1549B was 194ºC/km.

Sediment samples were collected using an advanced piston coring system (APC) and a half-length APC (Teske et al. 2021). After retrieval on deck, sediment cores were sectioned in a designated core cutting area. Approximately 0.5 cm of the outer sediment layer was carefully removed to minimize potential contamination. For analysis of hydrocarbon gases, ~ 5 mL sediment was transferred into 21.5 mL clean, pre-combusted glass vials using a cut-off syringe. The vials were sealed immediately with polytetrafluoroethylene septa and aluminum caps and were incubated for 30 min at 70°C to allow dissolved hydrocarbon gases to equilibrate with the headspace before analysis, as described in Teske et al. (2021). For molecular hydrogen analysis, duplicate 3 mL sediment samples were collected immediately after core recovery using a cut-off syringe. The subsample was transferred into 21.5 mL glass vials and incubated and processed according to the procedures of Lin et al. (2012).

Whole round core (WRC) segments for methanogenic activity measurements were cut from cores retrieved from hole B at each site and stored temporarily in a nitrogen-filled glove bags until processing in the radioisotope tracer isolation space on the R/V JOIDES Resolution. Manipulation of samples for rate measurements were carried out in an anaerobic chamber (95:5 [V/V] N2:H2) to prevent oxygen contamination. Methanogenesis rate assays were carried using modifications of our previous methods (Bowles et al. 2011, Zhuang et al. 2018, Zhuang et al. 2019); the modifications increased the sensitivity of the rate measurements. Replicates for each sample were prepared in quadruplicate (three live and one killed control) for each substrate by transferring 5 cm^3 of sediment from the inner-most part of a whole round core into a modified cut-end Hungate tube (Bellco Glass, 2407-00125) using a sterile cut-end syringe inside an anaerobic chamber (95%:5%, N_2:H_2). The headspace was removed by inserting a modified butyl rubber stopper (Bellco Glass, 2048-11800A) into the cut-end of the tube, and then a septum (Bellco Glass, 2047-11600) and screw cap (Bellco Glass, 2047-16000) were placed on top of the tube to seal the sample. All vials, stoppers, and septa were autoclaved and stored anoxically prior to use as described by Zhuang et al. (2019).

Replicate and control samples were injected with 100 µL of ^{14}C -labeled substrate (American Radiolabeled Chemicals, Inc.). Radiotracer stocks were diluted into pH-adjusted (pH-7) milliQ water to achieve the target activity in a 100 µL injection, which was 390 to 427 kBq for $^{2\text{-}14}C$-acetate, 637 to 1286 for ^{14}C-bicarbonate, 427 to 451 kBq for ^{14}C-formate, 417 to 3294 kBq for ^{14}C-methanol, or 155 to 375 kBq for ^{14}C-methylamine. Prior to the addition of radiotracer, the killed control samples were amended with 4 mL of helium-purged 2M NaOH and homogenized using a vortex. Live and killed samples were incubated for 2-4 weeks at 1 atmosphere and temperatures near *in situ* values (within 10°C of *in situ* temperature). The live incubations were terminated by adding 4 mL of helium-purged 2M NaOH and homogenizing samples using a vortex. All samples were stored at room temperature for shore-based processing and analysis in the University of Georgia laboratory.

Methanogenic activity was determined by converting the ^{14}C-CH_4 produced within each sample to ^{14}C-CO_2 via

combustion at 850°C using a copper oxide catalyst and trapping the evolved ^{14}C-CO_2. For each sample, both atmospheric air and 100 µL of ultra-high purity ^{12}C-CH_4 were added to generate a headspace and serve as a carrier, respectively. The produced ^{14}C-CH_4 was driven into the headspace by vortexing the samples and ^{14}C-$CH_4$was removed from the sample by flushing the headspace with 5% O_2 in a balance of N_2 for 1 hour. Carry-over of ^{14}C-labeled organic substrates or carbon dioxide during ^{14}C-CH_4 recovery would inflate the calculated rates, the carrier gas stream from the sample was purified through a series of substrate-dependent traps between exiting the sample vial and entering the combustion oven. The two parent tracer traps for bicarbonate, acetate, and formate consisted of 2M NaOH in saturated NaCl (trap 1) and NaOH pellets (trap 2). The three parent tracer traps for methylamine consisted of 1M H_2SO_4 (trap 1), 2M NaOH in saturated NaCl (trap 2), and NaOH pellets (trap 3). The four parent tracer traps for methanol were immersed in an ice bath and consisted of 1M NaOH (trap 1), 2M NaOH in saturated NaCl (trap 2), NaOH pellets (trap 3), and silica gel (trap 4). Extra traps were required for methanol due to its inherent volatility.

Downstream of the combustion oven, the evolved ^{14}C-CO_2 was collected in a trap of 1.5 mL of 3-methoxypropylamine (Thermo Scientific, 99% purity, catalog number #AAB24095AU) and 4 mL of scintillation cocktail (Research Products International, Bio-SafeII, catalog number 111195) after passing through an empty trap that was in place to eliminate sample backflow into the combustion column. After one hour, the content of the final trap was transferred to a 7 mL scintillation vial and the ^{14}C-activity was quantified using a liquid scintillation counter. Recovery tests with ^{14}C-CH_4 showed that we recovered $\geq$ 90% of the known activity of added. Blanks (empty tubes) were included in each sample set served to identify any carryover or contamination between runs.

The rate of ^{14}C-substrate turnover to ^{14}C-methane, the turnover constant, *k*, was calculated using the activity of the produced methane (^{14}C-CH_4; disintegrations per minute (dpm)), the activity of the substrate added to the sample (^{14}C-substrate; dpm), and the incubation period (t; days) (Equation 1). Potential rates of methanogenesis (pmol cm^{-3} day^{-1}) were calculated using the equation below, where the turnover constant *k* (day^{-1}) was multiplied by the porosity-corrected substrate concentration in nmol cm^{-3} wet sediment which was derived from multiplying the substrate concentration, nmol mL^{-1}, by the water content, mL water cm^{-3} wet sediment, assuming that the mass of water in grams is equivalent to its volume in mL or cm^{-3}, and the isotopic fractionation factor, α. The following fractionation factors were used: 1.02 for acetate; 1.04 for bicarbonate; 1.07 for methanol; 1.06 for methylamine; 1.02 for formate) were used (Krzycki et al. 1987, Summons et al. 1998, Whiticar 1999). A factor of 1000 was applied to convert the rate from nmol cm^{-3} day^{-1} to pmol cm^{-3} day^{-1}.

> Methanogenesis rate (pmol cm^{-3} d^{-1}) =
> turnover constant (d^{-1}) x [substrate] (nmol cm^{-3}) x α x 1000 (pmol $nmol^{-1}$)

Sample counts were corrected for the killed control by subtracting the minimum detectable dpm (average dpm of killed controls plus three times the standard deviation of the killed controls, dpm) from the measured ^{14}C-CH_4 activity (dpm) of each live sample. Samples with ^{14}C-CH_4 activity (dpm) less than the minimum detectable dpm were considered to be below the detection limit.

The **detection limits for the different methanogenesis rates** were: 1.26 pmol cc^{-1} day^{-1} for H_2/CO_2, 0.05 pmol cc^{-1} day^{-1} for acetate, 0.01pmol cc^{-1} day^{-1} for formate, 0.03 pmol cc^{-1} $day^{-1}$for methanol, and 0.01 pmol cc^{-1} day^{-1} for methylamine.

Data Processing Description

Rate calculations are described in the methods.

BCO-DMO Processing Description

- Imported "OCE-2023575_Methanogenesis.csv" into the BCO-DMO system

[table of contents | back to top]

Related Publications

Bowles, M. W., Samarkin, V. A., & Joye, S. B. (2011). Improved measurement of microbial activity in deep-sea sediments at in situ pressure and methane concentration. Limnology and Oceanography: Methods, 9(10), 499–506. doi:10.4319/lom.2011.9.499
Methods

Krzycki, J. A., Kenealy, W. R., DeNiro, M. J., & Zeikus, J. G. (1987). Stable Carbon Isotope Fractionation by Methanosarcina barkeri during Methanogenesis from Acetate, Methanol, or Carbon Dioxide-Hydrogen. Applied and Environmental Microbiology, 53(10), 2597–2599. https://doi.org/10.1128/aem.53.10.2597-2599.1987
Methods

Solórzano, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method 1 1.This research was fully supported by U.S. Atomic Energy Commission Contract No. ATS (11-1) GEN 10, P.A. 20. Limnology and Oceanography, 14(5), 799–801. doi:10.4319/lo.1969.14.5.0799
Methods

Summons, R. E., Franzmann, P. D., & Nichols, P. D. (1998). Carbon isotopic fractionation associated with methylotrophic methanogenesis. Organic Geochemistry, 28(7–8), 465–475. https://doi.org/10.1016/s0146-6380(98)00011-4 https://doi.org/10.1016/S0146-6380(98)00011-4
Methods

Teske, A., Lizarralde, D., Höfig, T. W., Aiello, I. W., Ash, J. L., Bojanova, D. P., Buatier, M. D., Edgcomb, V. P., Galerne, C. Y., Gontharet, S., Heuer, V. B., Jiang, S., Kars, M. A. C., Khogenkumar Singh, S., Kim, J., Koornneef, L. M. T., Marsaglia, K. M., Meyer, N. R., Morono, Y., … Zhuang, G. (2021). Expedition 385 summary. Guaymas Basin Tectonics and Biosphere. Internet Archive. https://doi.org/10.14379/iodp.proc.385.101.2021
Methods

Torres, M. E., & Kim, J. (2022). Data report: concentration and carbon isotopic composition in pore fluids from IODP Expedition 385. Guaymas Basin Tectonics and Biosphere. Internet Archive. https://doi.org/10.14379/iodp.proc.385.201.2022
Methods

Whiticar, M. J. (1999). Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. Chemical Geology, 161(1–3), 291–314. https://doi.org/10.1016/s0009-2541(99)00092-3 https://doi.org/10.1016/S0009-2541(99)00092-3
Methods

Xu, L., Zhuang, G., Montgomery, A., Liang, Q., Joye, S. B., & Wang, F. (2020). Methyl-compounds driven benthic carbon cycling in the sulfate-reducing sediments of South China Sea. Environmental Microbiology, 23(2), 641–651. Portico. https://doi.org/10.1111/1462-2920.15110
Methods

Zhuang, G., Montgomery, A., Samarkin, V. A., Song, M., Liu, J., Schubotz, F., … Joye, S. B. (2019). Generation and Utilization of Volatile Fatty Acids and Alcohols in Hydrothermally Altered Sediments in the Guaymas Basin, Gulf of California. Geophysical Research Letters, 46(5), 2637–2646. doi:10.1029/2018gl081284 https://doi.org/10.1029/2018GL081284
Results

Zhuang, G., Montgomery, A., Sibert, R. J., Rogener, M., Samarkin, V. A., & Joye, S. B. (2018). Effects of pressure, methane concentration, sulfate reduction activity, and temperature on methane production in surface sediments of the Gulf of Mexico. Limnology and Oceanography, 63(5), 2080–2092. Portico. https://doi.org/10.1002/lno.10925
Methods

Zhuang, G.-C., Elling, F. J., Nigro, L. M., Samarkin, V., Joye, S. B., Teske, A., & Hinrichs, K.-U. (2016). Multiple evidence for methylotrophic methanogenesis as the dominant methanogenic pathway in hypersaline sediments from the Orca Basin, Gulf of Mexico. Geochimica et Cosmochimica Acta, 187, 1–20. https://doi.org/10.1016/j.gca.2016.05.005
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Site	Site name	unitless
Hole	Hole Designation (A, B, C)	unitless
Latitude	Sampling latitude	decimal degrees
Longitude	Sampling longitude	decimal degrees
Water_Depth	Water column depth	m
Sediment_Depth	Depth below the seafloor in meters	mbsf
H2_CO2_1	Methanogenesis rate from H2/CO2 - replicate 1	pmol/cc*d
H2_CO2_2	Methanogenesis rate from H2/CO2 - replicate 2	pmol/cc*d
H2_CO2_3	Methanogenesis rate from H2/CO2 - replicate 3	pmol/cc*d
Acetate_1	Methanogenesis rate from acetate - replicate 1	pmol/cc*d
Acetate_2	Methanogenesis rate from acetate - replicate 3	pmol/cc*d
Acetate_3	Methanogenesis rate from acetate - replicate 3	pmol/cc*d
Formate_1	methanogenesis rate from formate - replicate 1	pmol/cc*d
Formate_1_flag	Flag to indicate sample status; n.s. = no sample available for this substrate; L = sample lost during processing	unitless
Formate_2	Methanogenesis rate from formate - replicate 3	pmol/cc*d
Formate_2_flag	Flag to indicate sample status; n.s. = no sample available for this substrate; L = sample lost during processing	unitless
Formate_3	Methanogenesis rate from formate - replicate 3	pmol/cc*d

Formate_3_flag	Flag to indicate sample status; n.s. = no sample available for this substrate; L = sample lost during processing	unitless
Methanol_1	Methanogenesis rate from methanol - replicate 1	pmol/cc*d
Methanol_2	Methanogenesis rate from methanol - replicate 3	pmol/cc*d
Methanol_3	Methanogenesis rate from methanol - replicate 3	pmol/cc*d
Methylamine_1	Methanogenesis rate from methylamine - replicate 1	pmol/cc*d
Methylamine_1_flag	Flag to indicate sample status; n.s. = no sample available for this substrate; L = sample lost during processing	unitless
Methylamine_2	Methanogenesis rate from methylamine - replicate 3	pmol/cc*d
Methylamine_2_flag	Flag to indicate sample status; n.s. = no sample available for this substrate; L = sample lost during processing	unitless
Methylamine_3	Methanogenesis rate from methylamine - replicate 3	pmol/cc*d
Methylamine_3_flag	Flag to indicate sample status; n.s. = no sample available for this substrate; L = sample lost during processing	unitless

[table of contents | back to top]

Deployments

IODP-385

Website	https://www.bco-dmo.org/deployment/869491
Platform	R/V JOIDES Resolution
Start Date	2019-09-16
End Date	2019-11-16
Description	Guaymas Basin Tectonics and Biosphere - International Ocean Discovery Program Expedition 385, General information: https://iodp.tamu.edu/scienceops/expeditions/guaymas_basin_tectonics_bio...

[table of contents | back to top]

Project Information

Pathways and regulation of transformation of low molecular weight carbon compounds in subseafloor sediments from the Guaymas Basin (Gulf of California) (Guaymas Basin Sediments)

Coverage: Guaymas Basin (Gulf of California)

NSF Award Abstract:
This research will explore carbon cycling in one of the largest carbon reservoirs on Earth, marine sediments, located at bottom of the ocean. This carbon is recycled gradually over time through interacting geological, chemical, and biological processes. This project will document how each of these processes transforms carbon in marine sediments from the Guaymas Basin (Gulf of California). This setting offers the chance to study carbon cycling across a broad range of chemical and temperature gradients, providing an opportunity to tease apart the factors regulating carbon cycling in marine sediments. This project will investigate the role of ocean sediments in the global carbon cycle. These research objectives represent key science priorities in a time of global environmental change. For outreach activities, the scientist, in collaboration with Jim Toomey Education, would continue the "Adventures of Zack and Molly" educational video series. In this instance, the video would document results from this study and its broader significance. The scientist also would create a learning guide for teachers. Both the video and the learning guide would be disseminated to educators. One graduate and one undergraduate student would be supported and trained as part of this project.

Subsurface sediments in the Guaymas Basin (Gulf of California) offer an accessible window for investigating carbon cycling in a dynamic, yet tractable, marine environment. This work will study how heating of subsurface sediments affects the production, consumption, and fate of low molecular weight dissolved organic carbon. The research will track the fate of key carbon species – including formate, acetate, and methanol – as they are processed through a gauntlet of microbial-mediated processes. Samples were collected during Expedition 385 of the International Ocean Discovery Program in September-October 2019. Some experiments were conducted on the research vessel and additional experiments will be conducted in the laboratory. The study will constrain the magnitudes of transformation and the fate of low molecular weight carbon substrates using a combination of direct rate, pool size, and stable isotopic measurements coupled to thermodynamic modeling and probative laboratory experiments. Key topics for investigation include: (1) What is the dominant production mode for organic compounds in subsurface sediments? (2) What are the dominant pathways of methanogenesis along geochemical and temperature gradients? (3) What are the temperature limits of microbially-driven carbon cycling processes? (4) How does the fate of organic compounds change along geochemical and/or temperature gradients?

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2023575

[table of contents | back to top]