# Experiments to resolve the processes coupled to carbon monoxide oxidation in subsurface sediments from R/V JOIDES Resolution IODP-385 drilling expedition in the Guaymas Basin between September and November, 2019

**Website**: https://www.bco-dmo.org/dataset/994698
**Data Type**: Cruise Results
**Version**: 1
**Version Date**: 2026-03-13

**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 |
|---|---|---|
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**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. Samples were collected during IODP Expedition 385 from September to November 2019. The dataset includes time-series measurements of headspace carbon monoxide (CO) concentrations in sediment slurry incubations under multiple experimental treatments, including unamended controls and amendments with sulfate, nitrate, ferrihydrite, molybdate, and bromoethanesulfonate (BES).

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## Coverage

**Location**: Guaymas Basin, Gulf of California
**Spatial Extent**: **N**:27.633 **E**:-111.25 **S**:27.167 **W**:-111.916
**Temporal Extent**: 2019-09-15 - 2019-11-15

## Dataset Description

### Study Results

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.

**Methods & Sampling**

Subsurface sediment samples were collected from 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 (Teske et al. 2021). Profiles and dynamics of carbon monoxide and hydrogen cycling were examined at five sites. Site U1545 (27º38.230'N, 111º53.329'W; water depth 1594.2m) and U1546 (27º37.884'N, 111º52.781'W; water depth 1585.6m) 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 U1545B is considered a reference site since it was free of sill intrusions and unaffected by active hydrothermal circulation. The geothermal gradient at hole U1545B was 227ºC/km. The geothermal gradient at site U1546 was similar to that measured in hole U1545B, 221ºC/km. The sill present at 1546B was clearly thermally equilibrated. Sites U1547 (27º30.413'N, 111º40.734'W; water depth 1739.9 m) and U1548 (27°30.2540′N, 111°40.8601′W; water depth 1738.9m) were 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 (27°28.3383′N, 111°28.7927′W; water depth 1841.2) 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. Of the five sites where we examined CO and $H_2$ dynamics, only site U1547/hole U1547B was impacted by active sill-associated hydrothermal circulation.

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 the 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 and carbon monoxide analysis, duplicate 3 mL sediment samples were collected immediately after core recovery using a cut-off syringe. The subsample was transferred into a 21.5 mL glass vials and incubated and processed according to the procedures of Lin et al. (2012).

To investigate the microbial consumption of CO, we conducted incubation experiments with subsurface sediments collected at 64 mbsf (~18°C) from site U1545 and at 132 mbsf (~75°C) from site U1547. sites U1547 and U1545 are contrasting sites because of their different temperature and geochemical regimes. Sediment slurries were homogenized and prepared in an anaerobic chamber using sterilized artificial seawater medium at a sediment:water ratio of 1:4. Approximately 60 mL of homogenized slurry was pipetted into 120 mL serum bottles, which were sealed with butyl rubber stoppers. To assess coupling of CO consumption with putative electron donors, we amended slurries with 10 mmol $L^{-1}$ sulfate, 1 mmol $L^{-1}$ nitrate, or 50 mmol $L^{-1}$ ferrihydrite. Each treatment was performed in triplicate, and received an additional ~100 ppm CO in the headspace. On the experimental rate table, the average of the three replicates is presented. Biotic reduction of the added substrates may be coupled with biotic CO oxidation generating energy yields suitable for anaerobic respiration and energy production. All treatments, and one control, which received ~100 ppm CO without any other amendments, were incubated in the dark at in situ temperature. During the incubation period the headspace was sampled and CO concentration was measured at different points over time (up to 50 days).

Inhibitors for sulfate reduction (30 mmol $L^{-1}$ molybdate) or methanogenesis (30 mmol $L^{-1}$ 2-bromoethanesulfonic acid; BES), were added to selected sediment slurries to assess the importance of these two processes in CO consumption. Finally, abiotic controls were also conducted with autoclaved slurries (120 degrees C for 40 min) that were supplied with 100 ppm CO in the headspace. During 50 days of incubation, the headspace CO concentration was measured every 3 to 5 days.

**BCO-DMO Processing Description**

- Loaded "CO-H2-experimental-data.csv" as CSV, skipping row 2, treating "nd" and "n.d." as missing values, using filename as resource name
- Added computed fields by row number ranges: Site (U1545 for rows 1-18, U1547 for rows 19-27), Hole (C for rows 1-18, B for rows 19-27), Latitude (27.637 for rows 1-18, 27.507 for rows 19-27), Longitude (-111.889 for rows 1-18, -111.679 for rows 19-27), Water_Depth (1595 for rows 1-18, 1732.2 for rows 19-27), Sediment_Depth (64 for rows 1-18, 132.5 for rows 19-27), and Incubation_temperature (18 for rows 1-18, 75 for rows 19-27) - these metadata fields were not present in the original file and were implied based on row location
- Renamed multiple fields to remove spaces, newlines, and special characters: "Water Depth" to Water_Depth, "Sediment Depth" to Sediment_Depth, "Incubation\ntemperature" to Incubation_temperature, and all multiline CO treatment column headers to clean underscore-delimited names (e.g., "100 ppm CO\n+ 30 mM BES" to CO_100ppm_30mM_BES)
- Set numeric types for Time, Latitude, Longitude, Water_Depth, Sediment_Depth, Incubation_temperature, and all CO concentration fields
- Added BCO-DMO field metadata (descriptions, standard_name_ids, supplied_units) for all fields including CO treatment columns (headspace CO concentrations in ppm), Site, Hole, Latitude, Longitude, Water_Depth, Sediment_Depth, Incubation_temperature, and Time; Latitude, Longitude, and Water_Depth flagged as primary parameters
- Exported file as 994698_v1_exp_co_oxidation.csv

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## Related Publications

Grasshoff, K., Kremling, K., & Ehrhardt, M. (Eds.). (1999). Methods of Seawater Analysis. doi:10.1002/9783527613984
*Methods*

Lin, Y.-S., Heuer, V. B., Goldhammer, T., Kellermann, M. Y., Zabel, M., & Hinrichs, K.-U. (2012). Towards constraining H2 concentration in subseafloor sediment: A proposal for combined analysis by two distinct approaches. Geochimica et Cosmochimica Acta, 77, 186–201. https://doi.org/10.1016/j.gca.2011.11.008
*Methods*

Pilson, M. E. Q. (2012). An Introduction to the Chemistry of the Sea. https://doi.org/10.1017/cbo9781139047203 https://doi.org/10.1017/CBO9781139047203
*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 methods. Guaymas Basin Tectonics and Biosphere. Internet Archive. https://doi.org/10.14379/iodp.proc.385.102.2021
*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*

Weston, N. B., Porubsky, W. P., Samarkin, V. A., Erickson, M., Macavoy, S. E., & Joye, S. B. (2006). Porewater Stoichiometry of Terminal Metabolic Products, Sulfate, and Dissolved Organic Carbon and Nitrogen in Estuarine Intertidal Creek-bank Sediments. Biogeochemistry, 77(3), 375–408. doi:10.1007/s10533-005-1640-1
*Methods*

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## 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 |
| Incubation_temperature | Temperature of the shipboard incubations | degrees Celsius |
| Time | Time of sampling (day of incubation) | days |
| Control_no_CO_added | Average headspace CO concentration in the unamended control incubation (no CO added) | ppm |
| CO_100ppm | Average headspace CO concentration in incubations amended with 100 ppm CO only | ppm |
| CO_100ppm_autoclaved | Average headspace CO concentration in autoclaved (killed) control incubations amended with 100 ppm CO | ppm |
| CO_100ppm_30mM_BES | Average headspace CO concentration in incubations amended with 100 ppm CO and 30 mM BES (methanogenesis inhibitor) | ppm |
| CO_100ppm_30mM_MoO42 | Average headspace CO concentration in incubations amended with 100 ppm CO and 30 mM molybdate (sulfate reduction inhibitor) | ppm |
| CO_100ppm_10mM_SO42 | Average headspace CO concentration in incubations amended with 100 ppm CO and 10 mM sulfate | ppm |

| | | |
|---|---|---|
| CO_100ppm_10mM_SO42_30mM_MoO42 | Average headspace CO concentration in incubations amended with 100 ppm CO, 10 mM sulfate, and 30 mM molybdate (sulfate reduction inhibitor) | ppm |
| CO_100ppm_1mM_NO3 | Average headspace CO concentration in incubations amended with 100 ppm CO and 1 mM nitrate | ppm |
| CO_100ppm_50mM_ferrihydrite | Average headspace CO concentration in incubations amended with 100 ppm CO and 50 mM ferrihydrite | ppm |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Advanced piston coring system (APC) |
| **Generic Instrument Name** | Advanced Piston Corer |
| **Dataset-specific Description** | Sediment samples were collected using an advanced piston coring system (APC) and a half-length APC (Teske et al. 2021). |
| **Generic Instrument Description** | The JOIDES Resolution's Advanced Piston Corer (APC) is used in soft ooze and sediments. The APC is a hydraulically actuated piston corer designed to recover relatively undisturbed samples from very soft to firm sediments. More information is available from IODP (PDF). |

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|---|---|
| **Dataset-specific Instrument Name** | Peak Laboratories LLC (USA) Peak Performer 1 Reduction Gas Analyzer |
| **Generic Instrument Name** | Gas Analyzer |
| **Dataset-specific Description** | Hydrogen and Carbon Monoxide: Peak Laboratories LLC (USA) Peak Performer 1 Reduction Gas Analyzer. |
| **Generic Instrument Description** | Gas Analyzers - Instruments for determining the qualitative and quantitative composition of gas mixtures. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | homogenizer |
| **Generic Instrument Name** | Homogenizer |
| **Dataset-specific Description** | Sediment slurries were homogenized and prepared in an anaerobic chamber using sterilized artificial seawater medium at a sediment:water ratio of 1:4. Approximately 60 mL of homogenized slurry was pipetted into 120 mL serum bottles, which were sealed with butyl rubber stoppers. |
| **Generic Instrument Description** | A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others. |

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## 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... |

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## 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.

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## Funding


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


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