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Effects of Temperature and Pressure on Sulfate Reduction and Anaerobic Oxidation of Methane in Hydrothermal Sediments of Guaymas Basin†

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Rates of sulfate reduction (SR) and anaerobic oxidation of methane (AOM) in hydrothermal deep-sea sediments from Guaymas Basin were measured at temperatures of 5 to 200°C and pressures of 1×10^5, 2.2×10^7, and 4.5×10^7 Pa. A maximum SR of several micromoles per cubic centimeter per day was found at between 60 and 95°C and 2.2×10^7 and 4.5×10^7 Pa. Maximal AOM was observed at 35 to 90°C but generally accounted for less than 5% of SR.

Hydrothermal sediments of the Guaymas Basin contain highly diverse anaerobic thermophilic microorganisms, including methanogens, sulfate-reducing bacteria, and presumably also methanotrophs (2, 6, 15, 18, 19, 23). Thermogenic reactions in the subsurface sediments provide a complex mixture of methane, other hydrocarbons, and volatile fatty acids as substrates for these microorganisms (8). Acetate concentrations in Guaymas sediments are extremely variable, ranging from 10 to >1,000 μM, with maximum concentrations occurring in hot sediments (12). Methane concentrations are 12 to 16 mM (22), but various other hydrocarbons derived during thermal alteration of organic material have also been found in high concentrations (11, 16, 17). In previous investigations, high rates of sulfate reduction (SR) were found at sediment surfaces, where freshly deposited organic material as well as bottom-water sulfate is available. Subsurface maxima in SR are found where upward moving fluids advect high concentrations of volatile fatty acids and a variety of hydrocarbons (4, 7, 8, 12, 16, 21). It is not known whether methane is a relevant electron donor for SR in hydrothermal sediments because the activity of thermophilic methanotrophs in Guaymas sediments has not yet been investigated. So far, high rates of methane-dependent SR have been found only in cold environments, at in situ temperatures of −1.5 to +12°C (5). Accordingly, physiological experiments with anaerobic-methanotrophic (ANME) groups from cold seeps have shown temperature optima for the anaerobic oxidation of methane (AOM) of between 5 and 15°C (13, 14). However, typical biomarker and 16S rRNA gene signatures of AOM consortia suggest the presence of thermophilic methanotrophs in hydrothermal surface sediments of Guaymas Basin (15, 19). The primary aims of the present study were to reveal the effects of temperature and pressure on SR and AOM in such sediments and to test whether SR is fueled by methane.

Hydrothermal sediment was retrieved from a vented site, covered with a *Beggiatoa* mat, by the submersible ALVIN (dive 3780, cruise AT-07, 5 May 2002; 27°0′32″N, 111°24′26″W; 2,013 m water depth). The maximum temperature recorded at this site was 130°C at a sediment depth of 20 cm (D. Albert, personal communication), a setting similar to that of previous sampling of vent cores (3, 5, 19). The core was very gassy and expanded during retrieval. Immediately after recovery, the complete sediment sample was stored under anoxic conditions at 4°C until further measurements were performed in the home laboratory. For the experiments, 1 part of Guaymas sediment was mixed and diluted with 4 parts of a standard mineral salt solution (14). All preparations were carried out inside a glove box under an atmosphere of N_2-CO_2. Methane-saturated slurries were prepared by equilibrating the sediment slurry with a 100% methane headspace in a glass flask. Because AOM occurs in two steps (oxidation of methane to carbon dioxide and reduction of sulfate to sulfide), the radiotracers $^{14}CH_4$ and $^{35}SO_4$ were added in trace amounts to replicate subsamples for measurement of carbon dioxide and sulfide production. Measurements of SR and AOM were performed according to methods described elsewhere (10, 20). All data were calculated as activity per volume of undiluted sediment. For SR, two replicates were incubated for each temperature or pressure setting. For AOM, two (experiments 2 and 3) or four (experiment 4) replicates were used for each temperature setting. Abiotic controls, obtained by fixing the sample prior to addition of tracer, were also employed. The average control value plus the standard deviation was substracted from each data point value before calculation of activity. The effects of temperature and pressure on SR and AOM were studied by using a high-pressure thermal gradient block (10). Experiment 1 was carried out to measure the combined effects of pressure and temperature on SR (Table 1). In addition, temperature gradient experiments were carried out with different preincubations times (experiment 2, 1 day; experiments 3 and 4, 7 days) and methane concentrations (experiments 2 and 3, 0.1 mM; experiment 4, 1 mM).

The temperature maximum of SR was between 60 and 90°C

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TABLE 1. Effect of pressure on SR rates at high temperatures (experiment 1)

Pressure (Pa)	SR rate at temp (°C)[a]:										
	73	80	85	95	100	105	115	120	130	...	195
1×10^5	154	90	76	ND	ND	ND	ND	ND	ND		ND
1×10^6	ND	128	75	100	13	0.2	0.3	ND	ND		ND
2.2×10^7	ND	645	ND	2,786	5,564	0.0	1.8	0.1	0.4	...	0.1
4.5×10^7	ND	2,805	2,465	6,660	3,619	1.8	0.4	0.0	0.3	...	0.3

[a] Values are in units of nanomoles per cubic centimeter per day. ND, not determined.

with a peak at 80°C, similar to results for intact sediment cores in previous studies (3, 21). At 80°C and 2.2×10^7 Pa, which represent the in situ conditions at the sampled site, SR was around 650 nmol cm^{-3} day^{-1} (Table 1). A maximum SR of almost 6,700 nmol cm^{-3} day^{-1} was reached at 4.5×10^7 Pa and 95°C. Under these conditions, the SR rate was 40 times higher than that in the 10^5- and 10^6-Pa incubations at the same temperatures. These rates are among the highest SR ever observed in a marine setting, comparable to the methane-driven SR values measured in *Beggiatoa* mats at cold seeps (1). SR ceased above 102°C even at high pressures (Table 1). Experiment 2 showed that a 1-day preincubation was insufficient for the microorganisms to adjust to the original temperature conditions, as SR and AOM rates were around 1 nmol cm^{-3} day^{-1} over the whole temperature range. In experiment 3, SR reached values similar to in situ (21) or ex situ (3) SR in samples from similar settings. The SR rate was significantly lower ($P < 0.001$) at low to intermediate temperatures (5 to 53°C; SR range, 0 to 15 nmol cm^{-3} day^{-1}; average, 3.2 ± 2.5 nmol cm^{-3} day^{-1}; $n = 12$) than at higher temperatures (62 to 85°C; SR range, 75 to 200 nmol cm^{-3} day^{-1}; average, 95 ± 37 nmol cm^{-3} day^{-1}; $n = 8$). Most interestingly, AOM rates were around 1% of SR rates at all temperatures. However, both processes exhibited a similar trend, as AOM rates in 62 to 85°C incubations (average, 1.6 ± 0.5 nmol cm^{-3} day^{-1}; $n = 8$) were significantly higher ($P < 0.001$) than those for samples incubated at 5 to 53°C (average, 0.6 ± 0.2 nmol cm^{-3} day^{-1}; $n = 12$). However, the identification of a clear temperature maximum was difficult because of the very low AOM rates. In experiment 4, samples were preincubated for a week at a methane concentration of ca. 1 mM. AOM rates increased to an average of 12 ± 5 nmol cm^{-3} day^{-1} at temperatures of 31 to 62°C ($n = 8$). At higher temperatures (>87°C), AOM rates declined again, with only one of four replicates showing measurable activity. At lower temperatures (<31°C), AOM rates were significantly lower ($P < 0.005$; average, 1.4 ± 1.7 nmol cm^{-3} day^{-1}; $n = 8$), comparable to those for experiment 3 at similar temperatures ($P = 0.17$). Hence, our results suggest that a maximum for anaerobic oxidation of methane occurs at 30 to 60°C in the hydrothermal sediments of Guaymas Basin (Fig. 1). However, AOM contributed only 1 to 5% to SR at all temperatures, which is far from the 1:1 stoichiometry usually attributed to active AOM zones, where methane is the main substrate fueling SR (1, 5, 13, 14). The AOM rate was much higher in sediments from active cold seeps, reaching several hundred to 1,000 nmol cm^{-3} day^{-1} (13, 14, 20), compared to the turnover of a few nanomoles at Guaymas vents. However, at a cold-seep site, where other hydrocarbons were available in addition to methane, AOM was also reduced to 1 to 10% of SR (9). This may indicate that C_2:C_5 or higher hydrocarbons are a favorable substrate for sulfate reducers, outcompeting anaerobic methanotrophs in such environments.

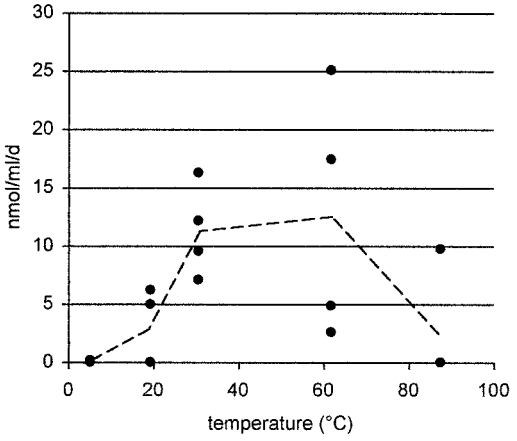

FIG. 1. Effects of temperature on AOM in hydrothermal sediments of Guaymas Basin (experiment 4). Each point represents one replicate subsample ($n = 4$ per setting). ●, AOM; – – –, average AOM.

There remains the question of whether the ANME-1 and ANME-2 groups detected in the surface sediments of *Beggiatoa* cores from Guaymas Basin (15, 19) are also present in hydrothermal subsurface sediments and are responsible for AOM at temperatures above 30°C. The previously observed substantial decrease in archaeal methanotroph biomarker lipids with increasing sediment depth (i.e., increasing temperature) (15, 19) indicates that the methane-rich, hot subsurface sediments are not a preferred environment for ANME populations. In conclusion, although AOM proceeds at higher temperatures in subsurface sediments of the Guaymas basin, it is clearly not the dominant carbon cycling process.

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