

Methane enrichment in low-temperature hydrothermal fluids from the Suiyo Seamount in the Izu-Bonin Arc of the western Pacific Ocean

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[1] The gas geochemistries of high-temperature and low-temperature hydrothermal fluids (HTHFs and LTHFs, respectively) were studied for the Suiyo Seamount hydrothermal system (140°39'E, 28°33'N) in the southern Izu-Bonin (Ogasawara) Arc of the western Pacific Ocean. HTHFs (290 ± 20°C) were collected from four active vents using a gas-tight fluid sampler, which prevented the loss of volatile components through degassing during sample processing. The end-member CH₄ concentrations were homogeneous across the vent fields. LTHFs were collected from four diffuse flow zones using a funnel, which was deployed on the seafloor with replacement of the seawater inside the funnel by the LTHF. Strong linear relationships were found among the concentrations of CH₄, ΣCO₂, and Si of the obtained samples, which ensured that the end-member chemical composition of the LTHFs could be accurately estimated. While the ΣCO₂ concentrations of the HTHFs and LTHFs were similar, all four LTHFs showed enrichment of CH₄ as compared to the HTHFs. The carbon isotopic composition of CH₄ also revealed a significant difference: the LTHFs (δ¹³C(CH₄) = −4.8 ± 0.3‰PDB) showed ¹³C enrichment, as compared to the HTHFs (δ¹³C(CH₄) = −5.8 ± 0.4‰PDB). The ¹³C enrichment can be explained by a combination of microbial CH₄ production and oxidation after formation of the LTHFs. On the basis of the observed differences in CH₄ concentrations and carbon isotopic compositions of the HTHFs and LTHFs, microbial methane oxidation and production are estimated to have comparable impacts. These results support the notion of a subseafloor microbial consortium of methanogens and methanotrophs.

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1. Introduction

[2] Hydrothermal fluid venting from the seafloor was discovered at the spreading axes of the Galapagos Ridge in 1977 [Corliss *et al.*, 1979; Edmond *et al.*, 1979b]. Thereafter, deep-sea hydrothermal systems have been considered as one of the most interesting and important subjects for earth scientists. Numerous studies have revealed that hydrothermal activity is a ubiquitous concomitant phenomenon for shallow magmatic heat sources. The chemical

composition of the pure hydrothermal fluid in a deep reservoir depends primarily on the nature of the rocks in the reservoir. The seawater-rock interaction abiotically produces high levels of hydrogen, methane, and light hydrocarbons through the Fischer-Tropsch reaction [Welhan and Craig, 1983; Welhan, 1988; Charlou *et al.*, 2002; Simoneit *et al.*, 2004; Proskurowski *et al.*, 2008b]. The chemical composition of low-temperature hydrothermal fluids (LTHFs) is controlled mainly by mixing of the pure hydrothermal fluid in the deep reservoir with ambient seawater, although it can also be modified by subsurface processes during circulation, which include mineral precipitation and recrystallization [Edmond *et al.*, 1979b; Edmond *et al.*, 1979a], phase separation [Von Damm and Bischoff, 1987; Massoth *et al.*, 1989; Butterfield *et al.*, 1990], and microbial metabolism [Deming and Baross, 1993; Takai and Horikoshi, 1999].

[3] Microbial metabolism may play an important role in fluid chemistry during hydrothermal circulation, as recent studies have pointed to the existence of an enormous biosphere lying beneath the seafloor of submarine hydrothermal fields, which is supported by the continuously mixing environment of the reduced state of high-temperature hydrothermal

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fluids (HTHF) in the subsurface layer and the cooler, oxidized seawater of the ocean [Deming and Baross, 1993; Shock and Schlute, 1998; Takai and Horikoshi, 1999; Proskurowski et al., 2008a]. A common ancestor of life is likely to have been a thermophilic or hyperthermophilic chemolithoautotroph that lived in a hydrothermal system [Pace, 1991]. Most microorganisms obtain carbon and energy from the oxidation of organic matter to CO₂. Submarine hydrothermal vent fluids contain significant amounts of dissolved CH₄ [Welhan and Craig, 1979], which is suitable organic matter for microbial metabolism [Jannasch and Mottl, 1985]. When microbes utilize CH₄ as an organic material for metabolism (equation (1)), biogenic fractionation generates ¹²C-enriched CO₂. Therefore, the remaining CH₄ is enriched for ¹³C. On the other hand, the utilization of CO₂ together with H₂ by methanogenic archaea produces CH₄, resulting in ¹²C-enriched CH₄ (equation (2)).



Thus, characterization of the levels of dissolved CH₄ and ΣCO₂ (CO₂ gas plus dissolved CO₂, HCO₃⁻, and CO₃²⁻) provides useful information regarding the microbial processes in deep-sea hydrothermal systems.

[4] We conducted the present study using samples from active hydrothermal fields at the top of the Suiyo Seamount, which is a submarine volcano that lies about 1000 km from the nearest landmass. A previous study on the fatty acid compositions of sediment samples [Yamanaka et al., 2001] revealed that from the contribution of terrigenous organic compounds was almost negligible. Therefore, we consider the Suiyo hydrothermal field to be an extreme seafloor environment, in which organic materials are supplied exclusively by chemolithoautotrophic microbes, and which resembles the early Earth [Holm and Andersson, 1998]. Many novel thermophilic and hyperthermophilic microbes (archaea and bacteria) have been isolated from hydrothermal vent chimneys and fluids, and in situ incubators have been deployed at the Suiyo Seamount site [Kasai et al., 2003; Nakagawa et al., 2003; Sako et al., 2003; Takai et al., 2003a; Takai et al., 2003b; Higashi et al., 2004; Mori et al., 2004; Nakagawa et al., 2004a; Nakagawa et al., 2004b; Kuwabara et al., 2005; Hara et al., 2005; Elsaied et al., 2007; Kuwabara et al., 2007]. These studies imply that the subseafloor of the Suiyo Seamount contains a microbial community that is similar to that postulated to have been present during the early periods of life on Earth [Hara et al., 2005; Yamagishi, 2005]. The microbial activities reported for the hydrothermal plume above the Suiyo Seamount indicate the presence of sulfur-oxidizing and methane-oxidizing microbes, as well as carbon monoxide-producing organisms [Sunamura et al., 2004; Utsumi et al., 2004; Tsunogai et al., 2005].

[5] Fluid chemistry showed that all of the fluid samples obtained during the present study have a single source in the hydrothermal system [Ishibashi et al., 2003; Kishida et al., 2004]. The calculated Si end-member for all the collected samples indicated a quartz equilibrium at 300°C. The level

of Cl end-member at this seamount was found to be significantly greater than that in seawater. Such chemical features of hydrothermal fluids have been observed for several years [Ishibashi and Urabe, 1995; Kishida et al., 2004].

[6] We studied the concentrations and stable carbon isotopic compositions of the CH₄ and ΣCO₂ species in HTHFs and LTHFs. We found that the estimated end-member compositions of the HTHFs and LTHFs were different. We discuss (1) the processes that potentially cause this difference; (2) the estimated levels of microbiological consumption and production of methane; and (3) the composition of the microbial ecosystem that utilizes methane.

2. Study Area

[7] The Izu-Bonin (Ogasawara) Arc, which lies south of Japan, is a well-developed intraoceanic island arc that is associated with the Izu-Bonin subduction system (Figure 1a). The chain of active volcanic islands and submarine volcanoes, which is about 1200 km in length, extends to the northern end of the Mariana arc [Yuasa, 1985]. This subduction system lies on the eastern rim of the Philippine Sea plate, where the Pacific plate subducts toward the northwest (Figure 1a).

[8] The Suiyo Seamount (Suiyo SMT, 140°39'E, 28°33'N) lies on the volcanic front in the middle part of the Izu-Bonin Arc [Taylor et al., 1990]. The Suiyo Seamount has two major peaks, the eastern and western peaks (Figure 1b). Numerous small sulfide chimneys venting clear hydrothermal fluid at 230°C were discovered on the slope of the caldera of the western peak at a depth of 1320 m in 1991 [Kasuga and Kato, 1992]. In 1992, vigorous hydrothermal activity with HTHFs (260–311°C) was detected at the caldera floor at a depth of 1370 m [Watanabe et al., 1994; Watanabe and Kajimura, 1993; Watanabe and Kajimura, 1994]. An active hydrothermal field is distributed within an approximately 300 × 150 m area in the NNW–SSE direction south of the huge rolling stone of dacitic lava, the so-called “Big Rock” [Watanabe et al., 1994; Kakegawa et al., 2005; Marumo et al., 2005; Kinoshita et al., 2006], as shown in Figure 1c. This area comprises structures typical of hydrothermal fields, such as sulfide chimneys of up to ~30 cm that emit black HTHFs (AP01 and Marker 223), and mounds of shimmering clear LTHFs (Marker 300 and the neighborhoods of the high-temperature hydrothermal vents). Macrofaunal assemblages (mussel colonies), which are seen in most vent environments [Van Dover, 2000], have been observed around the hydrothermal vents, and in particular, dense mussel colonies (“Shell Carpet”) have been noted in the warm shimmering vent fluid around Marker 300 (Figure 1c).

[9] As part of the Archaean Park Project, the seafloor of the Suiyo hydrothermal field was drilled, to allow direct collection of purer hydrothermal fluids from the subsurface of the hydrothermal system [Urabe et al., 2001]. Seven boreholes (site names APSK01–07) were drilled in the hydrothermal field of the Suiyo Seamount in June 2001 using a portable marine drill (Benthic Multicoring System) [Matsumoto and Sarata, 1996]. All the artificially drilled vents, with the exception of APSK02 discharged hydrothermal fluids; their temperatures are listed in Table 1. The surface sediments at these boring sites consist primarily of

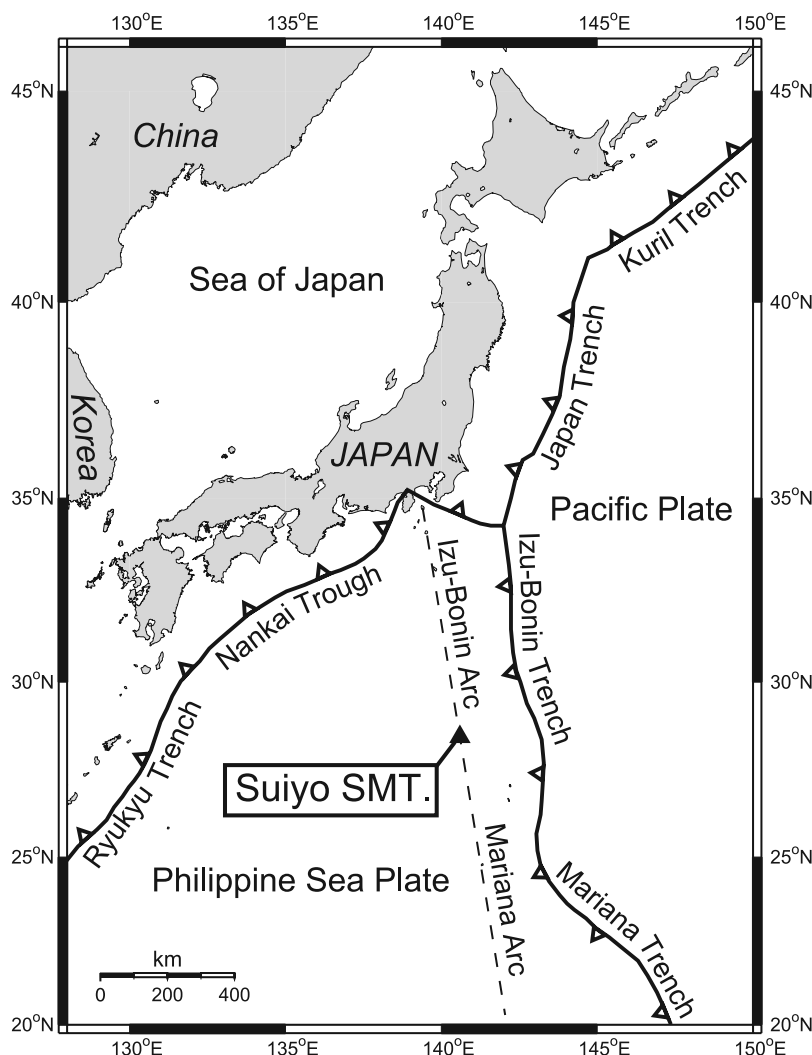


Figure 1a. The Izu-Bonin (Ogasawara) Arc on the eastern edge of the Philippine Sea plate (after Taylor *et al.* [1990]). The Suiyo Seamount (triangle) is located at 28°33'N, 140°39'E.

sandy volcanoclastics, below which unaltered volcanic rocks dominate the mainly pumice deposits. Beneath this zone, mixed-layer clay minerals, chlorite and montmorillonite, which are presumed to be altered dacite, are the dominant hydrothermal minerals, and anhydrite cements the rocks and fills the pore spaces. Sulfide grains, mostly pyrite, have been observed in several zones, representing possible vestiges of hydrothermal veining, followed by zones that are less altered, more porous, and containing a mixture of mica and chlorite. These are the predominant features of the Suiyo hydrothermal field [Takano *et al.*, 2004].

3. Sampling

3.1. Equipment

3.1.1. Fluid Accumulation Sampler (FAS)

[10] The Fluid Accumulation Sampler (FAS) was deployed over a vent site with the purpose of channeling the effluent from the seafloor into a semiencllosed environment (Figure 2). The sampler holds 12 acrylic cylindrical bottles (100 cm³), a revolving switch valve, a pump, a controller unit that contains the electronics and batteries, and a bell jar. The internal volume of each cylinder is

initially filled with distilled water. An inlet tube is inserted through a hole in the top of the bell jar, and the distilled water in the cylindrical samplers is replaced through the introduction of the accumulated fluid samples within the jar. Sampling intervals are preprogrammed before dive commencement. It takes about 2 min to fill one cylinder. The sampler has a dead space of about 3 cm³ in the inlet tube, which is filled with bottom seawater before deployment. Temperature changes are monitored during the deployment period by a temperature sensor inside the bell jar.

3.1.2. WHATS

[11] The Water and Hydrothermal *Atsuryoku* Tight Sampler (WHATS) was designed to hold collected gas and gas-rich fluids during the ascent of a submersible. *Atsuryoku* means “pressure” in Japanese. A detailed description of this sampler is presented elsewhere [Tsunogai *et al.*, 2003; Saegusa *et al.*, 2006]. It takes about 7 min to fill one sample bottle with capacity of 150 cm³.

3.2. Samples

3.2.1. LTHFs

[12] LTHFs were sampled using the FAS and the remotely operated submersible *Hakuyo 2000* (SNK OCEAN Co.,

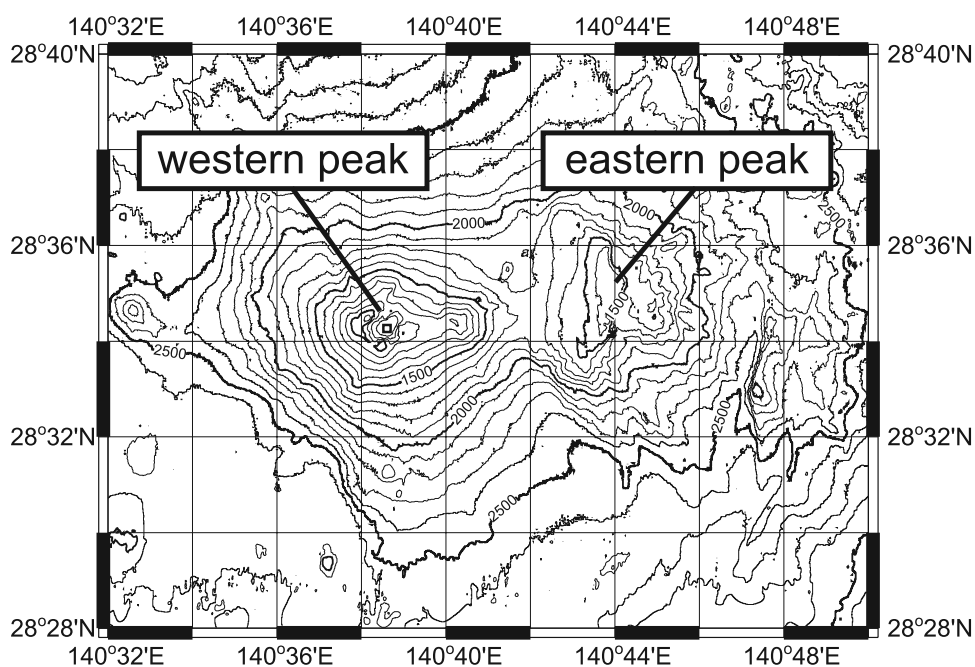


Figure 1b. A topographic map of the Suiyo Seamount. The square on the western peak indicates the area of Figure 1c. Contour interval is 100 m.

Ltd.) and the submersible *Shinkai 2000* of the Japan Marine Science Technology Center (JAMSTEC) during the 2001 Archaean Park Project. In Table 2, the sample names are listed together with the sampling site, fluid temperature measured during sampling, and the analytical results for the chemical and isotopic compositions. The sampler was deployed according to the following concepts: (1) a relatively flat seafloor where the bell jar could be positioned as firmly as possible, to avoid influx of ambient seawater; and (2) at a shimmering vent, for which the temperature of the shimmering fluid was above that of the ambient seawater (3°C), thereby confirming the discharge of LTHF.

[13] During dive HY11, the FAS was placed for about 7 h over a vent around the APSK04 site at a depth of 1385 m. Ten fluid samples were taken from the bell jar at intervals of 15 min. During Dive 1293, the sampler was set for 2.3 h over a densely populated colony of clams and bacterial mats, awash in warm shimmering vent fluid, around Marker 300 on the “Shell Carpet” at a depth of 1379 m. During Dive 1297, the sampler was placed for 3.3 h over a densely populated colony of clams and bacterial mats, from which shimmering hot water was discharged, around the APSK07 site at a depth of 1383 m. During Dive 1312, the sampler was deployed for 2.8 h over a white patch, presumably composed of sulfate minerals, around the APSK03 site at a depth of 1368 m.

[14] Each of the recovered fluid samples was immediately transferred to two 30-cm³ glass vials (one for measurements of dissolved CH₄ content and $\delta^{13}\text{C}(\text{CH}_4)$, and the other for assaying ΣCO_2 content and $\delta^{13}\text{C}(\text{CO}_2)$), with sufficient overflow to avoid air contamination in the onboard laboratory of the tender ship. After the samples were poisoned with HgCl₂ (the final concentration of mercury was $\sim 100 \text{ mg L}^{-1}$), the glass vials were capped with a gray butyl rubber septum without headspace. The residual

fluid was transferred as soon as possible to a 30-cm³ polypropylene bottle for measurements of pH, titration alkalinity, salinity, and Si content, as well as for onshore measurements of the major cations and anions. All samples were stored in the dark and kept at 4°C until analysis.

3.2.2. HTHFs

[15] HTHFs were collected using the WHATS in 2001 from several active vents within the caldera (Table 1), during dives by the manned submersible *Shinkai 2000* (JAMSTEC). The gas and liquid phases were immediately isolated in the laboratory aboard the tender ship *Natsushima* using the following procedures. First, the sampler was connected to a vacuum line made of stainless steel, and the fluid sample was transferred to a 2-L evacuated stainless steel container. The fluid sample was then acidified by adding amidosulfuric acid, to convert all dissolved carbonate species to CO₂ in the gas phase, and mixed with HgCl₂, to deposit the H₂S as HgS. The gas phase was transferred to a 150-cm³ evacuated stainless steel container for CH₄ and ΣCO_2 content and isotope measurements. After the gas phase was obtained, the liquid phase was filtered (mesh size, 0.45 μm) and placed in a 50-cm³ polypropylene bottle for assays of major cations and anions.

4. Analytical Methods

4.1. Concentration and Isotopic Composition of Gases

[16] The concentrations and stable carbon isotopic compositions of CH₄ in both the liquid and gas samples were measured using the Finnigan MAT 252 isotope-ratio-monitoring continuous-flow mass spectrometer [Tsunogai *et al.*, 2000]. The hydrocarbon content of the sample was calculated by comparing the ⁴⁴CO₂ output with that of a working standard gas, which contained $\sim 875 \text{ ppm CH}_4$, $\sim 44 \text{ ppm C}_2\text{H}_6$, and 21 ppm C₃H₈ in nitrogen, derived from

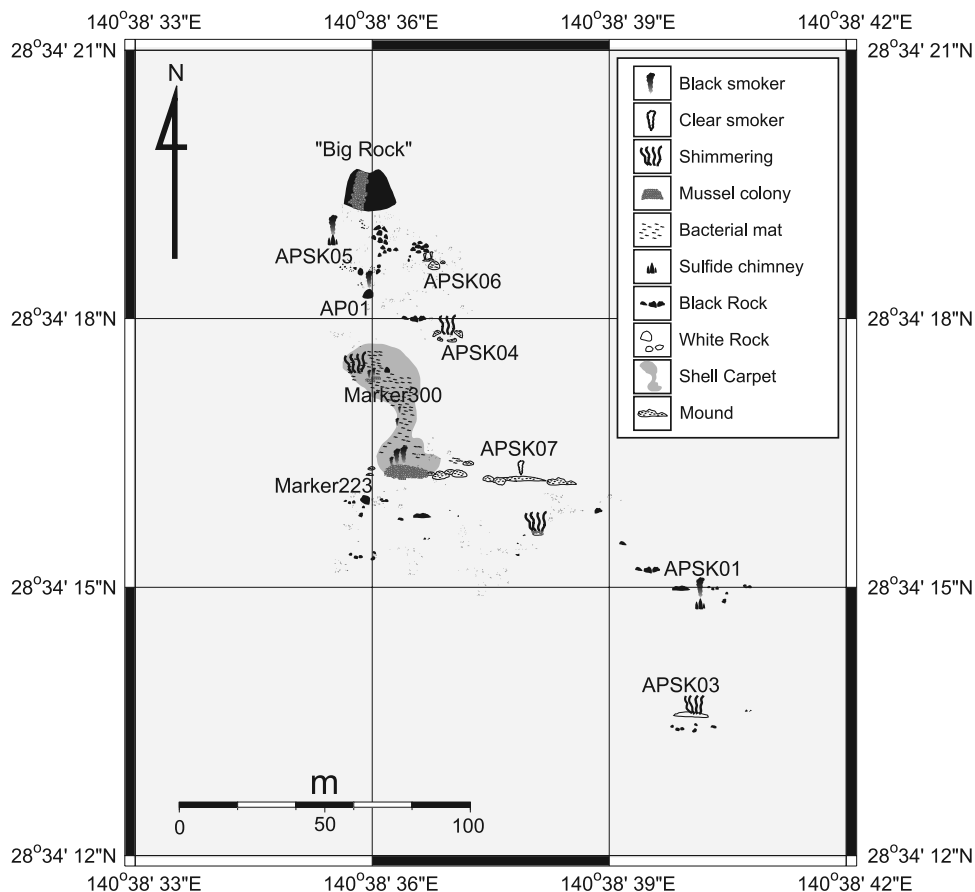


Figure 1c. A map showing the distribution of hydrothermal activities at the bottom of the caldera of the Suiyo Seamount.

Table 1. Chemical and Isotopic Compositions of High-Temperature Hydrothermal Fluids Collected by WHATS From Suiyo Seamount Hydrothermal Vents^a

| Date | Dive | Sample | Site | Maximum Temperature (°C) | Si (mmol/kg) Color. | pH Elec. | Sal. (‰) Ref. | Alk. (mmol/kg) Titr. | Mg (mmol/kg) ICP-AES | CH ₄ (μmol/kg) CF-IRMS | δ ¹³ C(CH ₄) (‰PDB) CF-IRMS | ΣCO ₂ (mmol/kg) CF-IRMS | δ ¹³ C(CO ₂) (‰PDB) CF-IRMS |
|------------|-------|--------|------------|--------------------------|---------------------|----------|---------------|----------------------|----------------------|-----------------------------------|----------------------------------------------------|------------------------------------|----------------------------------------------------|
| 2001.9.2 | D1298 | W-1 | APSK01 | 298.5 | 11.9 | 3.80 | 40.5 | -0.15 | - | 113 | -5.7 | 39.5 | -0.5 |
| 2001.9.2 | D1298 | W-2 | APSK01 | 172.0 | 9.85 | 4.35 | 40.0 | 0.05 | - | 94.2 | -7.1 | 25.7 | -0.7 |
| 2001.10.11 | D1307 | W-4 | APSK03 | 223.0 | 9.50 | 5.13 | 40.0 | 1.52 | 6.6 | 91.3 | -6.0 | 17.1 | -0.9 |
| 2001.9.15 | D1303 | W-1 | APSK04 | 285.5 | 12.2 | 4.29 | 37.5 | -0.02 | - | 16.6 | -9.5 | 6.17 | -0.2 |
| 2001.9.15 | D1303 | W-2 | APSK04 | 276.0 | 11.8 | 4.09 | 40.0 | -0.04 | - | 64.8 | -7.4 | 42.1 | -0.4 |
| 2001.8.26 | D1293 | W-1 | APSK05 | 156.0 | - | - | 38.0 | - | - | 40.1 | -9.1 | 22.1 | -0.9 |
| 2001.9.14 | D1302 | W-3 | APSK05 | 111.0 | 1.19 | 6.60 | 36.5 | 2.08 | - | 26.0 | -6.1 | 5.16 | -1.8 |
| 2001.10.6 | D1305 | W-1 | APSK05 | 305.0 | 13.1 | 4.17 | 41.5 | -0.08 | 0.6 | 127 | -5.7 | 39.5 | -0.4 |
| 2001.10.6 | D1305 | W-2 | APSK05 | 305.0 | 13.4 | 3.81 | 41.5 | -0.15 | 0.6 | 170 | -6.2 | 46.7 | -0.4 |
| 2001.9.6 | D1301 | W-1 | APSK06 | 50.0 | 1.71 | 6.48 | 35.0 | 2.12 | - | 18.4 | -4.2 | 8.06 | -1.3 |
| 2001.9.14 | D1302 | W-1 | APSK06 | 265.5 | 10.8 | 4.72 | 40.5 | 0.09 | - | 140 | -5.8 | 40.3 | -0.6 |
| 2001.9.14 | D1302 | W-2 | APSK06 | 300.0 | 10.6 | 4.78 | 40.5 | 0.10 | - | 168 | -5.4 | 40.3 | -0.5 |
| 2001.10.12 | D1308 | W-4 | APSK07 | 280.0 | 12.6 | 3.99 | 35.0 | -0.02 | 2.3 | 150 | -6.1 | 40.1 | -0.4 |
| 2001.8.30 | D1297 | W-2 | Marker 223 | 292.0 | - | - | 38.0 | 0.08 | - | 79.6 | -6.1 | 35.7 | -0.5 |
| 2001.9.2 | D1298 | W-3 | Marker 223 | 291.5 | 11.2 | 3.83 | 40.5 | -0.11 | - | 158 | -6.3 | 38.1 | -0.5 |
| 2001.9.2 | D1298 | W-4 | Marker 223 | 293.3 | 10.3 | 3.83 | 40.5 | -0.11 | - | 135 | -5.5 | 39.3 | -0.5 |
| 2001.10.6 | D1305 | W-4 | Marker 223 | 225.0 | 13.0 | 3.82 | 40.5 | -0.14 | 0.5 | 230 | -5.3 | 49.3 | -0.5 |
| 2001.9.5 | D1300 | W-2 | Marker 300 | 4.0 | 0.16 | 7.53 | 34.0 | 2.44 | - | 0.66 | -5.3 | 3.67 | -1.1 |
| 2001.8.30 | D1297 | W-3 | AP01 | 312.6 | 12.6 | - | 40.0 | -0.10 | 0.8 | 79.2 | -5.3 | 37.9 | -0.5 |
| 2001.8.30 | D1297 | W-4 | AP01 | 312.6 | 12.6 | - | 41.5 | -0.03 | 1.3 | 169 | -6.0 | 38.0 | -0.5 |
| 2001.9.15 | D1303 | W-3 | AP01 | 308.0 | 12.6 | 3.95 | 40.5 | -0.07 | - | 164 | -6.0 | 44.5 | -0.4 |
| 2001.9.15 | D1303 | W-4 | AP01 | 307.5 | 12.7 | 3.86 | 40.5 | -0.08 | - | 166 | -5.8 | 41.2 | -0.5 |

^aAnalytical method: Si (colorimetry), pH (pH meter), salinity (refractometer), alkalinity (potentiometric titration) onboard analysis, Mg (ICP-AES), CH₄, δ¹³C(CH₄), ΣCO₂, and δ¹³C(CO₂) (Continuous flow isotope-ratio-monitoring gas chromatography/mass spectrometry) onland analysis.

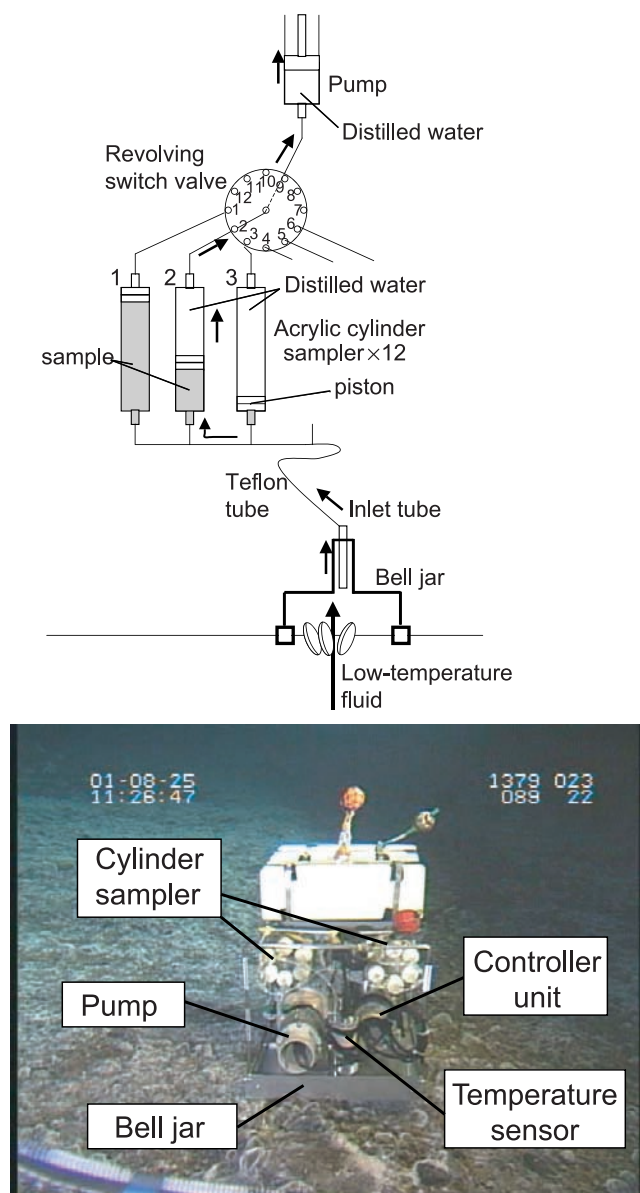


Figure 2. (top) Schematic diagram showing the “Fluid Accumulation Sampler.” (bottom) A view of the Fluid Accumulation Sampler over a dense clam colony with bacteria mats, the so-called “Shell Carpet,” in the Suiyo Seamount during Dive 1293. (Photographs provided by JAMSTEC.)

the NIST RM 8560 (IAEA NGS2) standard. The precision of the concentration determination (1 sigma value for 10 determinations) was estimated to be 6.5% at a concentration of 2 nmol/kg of CH₄. The $\delta^{13}\text{C}$ values were calibrated using the NGS2 CH₄ standard. The detection limits of this isotope analysis are 200 pmol for isotope ratio standard deviations of 0.3‰, which corresponds to 6 nmol/kg CH₄ in seawater samples, in a 30-cm³ vial. The analytical blank associated with the method, being less than 1 pmol, is negligible.

[17] The content and carbon isotopic composition of ΣCO_2 in the gas samples were measured using the Finnigan MAT 252 isotope-ratio-monitoring continuous-flow mass

spectrometer [Ijiri *et al.*, 2003]. The ΣCO_2 content of the sample was calculated by comparing the ⁴⁴CO₂ output with that of a working standard gas, which contained ~1095 ppm CO₂ in nitrogen, derived from the Oztech standard. The precision of the isotope analysis (1 sigma value for 10 determinations) was estimated to be 2% at a concentration level of 2 mmol/kg of ΣCO_2 . The $\delta^{13}\text{C}$ values were calibrated by using the Oztech CO₂ standard (−11.0‰ PDB). The detection limits of this content analysis were 5 nmol for isotope ratio standard deviations of 0.3‰. The analytical blank associated with the method, being less than 4 pmol, was negligible.

[18] The concentrations of ΣCO_2 in the liquid samples were measured with a coulometer (UIC Inc.). The precision of the determination (1 sigma value for 10 determinations) was estimated to be less than 3%. The carbon isotopic composition of ΣCO_2 in liquid samples was analyzed using the Finnigan MAT 252 isotope-ratio-monitoring dual-inlet mass spectrometer [Kroopnick, 1971]. The precision of the isotope analysis (1 sigma value for 10 determinations) was estimated to be 0.04‰. The carbon isotope composition is reported as a δ value relative to the PDB standard, where

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000\text{‰},$$

with R being the isotope ratio ¹³C/¹²C.

4.2. Major Components of the Liquid Samples

[19] The Mg concentrations in the fluid samples were measured by inductively coupled plasma (ICP) emission spectrophotometry. The analytical precision was estimated to be within 5% for Mg. Samples were analyzed aboard the ship for alkalinity, pH, and Si. Alkalinity was determined by potentiometric titration with 0.1 M HCl. Si was measured by spectrophotometry using the silicomolybdate complex method. The analytical precisions were estimated to be within 0.5% for pH, 5% for alkalinity, and 7% for Si.

5. Results

[20] The chemical and isotopic compositions of the HTHFs and LTHFs are shown in Tables 1 and 2, respectively. We also determined the end-members of the HTHFs and LTHFs.

5.1. End-Members of the HTHFs

[21] To determine the end-members of the HTHFs, we assessed the purities of the samples collected by the WHATS, by measuring the Si and Mg concentrations in the hydrothermal samples. The Si concentration of pure hydrothermal fluids from the Suiyo hydrothermal system were determined on the basis of the Si-Mg relationship observed for the fluid samples from numerous vents in the Suiyo Seamount during these expeditions, since there was a linear relationship between the Si and Mg concentrations [Kishida *et al.*, 2004]. Correction for the addition of seawater to the pure hydrothermal fluid was carried out by extrapolating Mg to 0 based on the assumption that the Mg concentration in the pure hydrothermal fluid was zero [Von Damm *et al.*, 1985]. The end-member Si concentration was estimated as 12.8 ± 0.3 mmol/kg, and the hydrothermal system was assumed to have only a single source of

Table 2. Chemical and Isotopic Compositions of Low-Temperature Hydrothermal Fluids Collected by Fluid Accumulation Sampler From Suiyo Seamount Hydrothermal Vents^a

| Date | Dive | Sample | Time | Depth | Temp. | Note | Si mmol/kg | pH | Cl mmol/kg | Sal. ‰ | Alk. mmol/kg | Mg mmol/kg | CH ₄ μmol/kg | δ ¹³ C(CH ₄) ‰PDB | ΣCO ₂ mmol/kg | δ ¹³ C(CO ₂) ‰PDB |
|------------|-------|--------|------|-------|-------|------------------------------|---------------|--------|---------------|-----------|-----------------|---------------|----------------------------|---------------------------------------------|-----------------------------|---------------------------------------------|
| | | | | m | °C | | Color. | Elec. | Tit. | Ref. | Tit. | ICP-AES | CF-IRMS | CF-IRMS | Coulometer | Dual Inlet |
| 2001.8. 8 | HY11 | T1 | 0900 | 960 | 4.1 | descending | 0.13 | 7.53 | 544 | - | 2.36 | 52.2 | 0.02 | -8.1 | 2.23 | - |
| | | T2 | 0930 | 1363 | 3.5 | before release in cage | 0.14 | 7.55 | 549 | - | 2.39 | 50.9 | 0.03 | -7.1 | - | - |
| | | T3 | 1000 | 1383 | 3.4 | on working in cage | 0.15 | 7.59 | 554 | - | 2.41 | 51.3 | 0.06 | -5.4 | 2.37 | - |
| | | T4 | 1030 | 1385 | 9.1 | set at 1022 | 0.50 | 6.43 | 549 | - | 2.25 | 49.1 | 7.83 | -5.0 | 3.22 | - |
| | | T5 | 1100 | 1385 | 9.9 | APSK04 | 0.63 | 6.34 | 552 | - | 2.15 | 49.6 | 9.69 | -5.0 | 3.53 | - |
| | | T6 | 1130 | 1385 | 8.0 | | 0.36 | 6.75 | 547 | - | 2.31 | 50.4 | 4.03 | -5.5 | 2.78 | - |
| | | T7 | 1200 | 1385 | 11.4 | | 0.52 | 6.37 | 557 | - | 2.16 | 50.4 | 5.87 | -5.1 | 3.31 | -0.68 |
| | | T8 | 1230 | 1385 | 8.4 | | 0.39 | 6.57 | 547 | - | 2.26 | 50.2 | 4.45 | -5.1 | 2.97 | - |
| | | T9 | 1300 | 1385 | 9.0 | | 0.43 | 6.47 | 571 | - | 2.24 | 50.3 | 4.25 | -5.2 | 3.07 | - |
| | | T10 | 1400 | 1385 | - | failed | - | - | - | - | - | - | - | - | - | - |
| | | T11 | 1500 | 1385 | 7.4 | | 0.35 | 6.70 | 549 | - | 2.26 | 50.6 | 3.30 | -5.2 | 2.81 | -0.67 |
| | | T12 | 1600 | 1385 | 8.1 | rec. at 1934 | 0.30 | 6.74 | 552 | - | 2.23 | 51.5 | 2.83 | -5.4 | 2.69 | -0.53 |
| 2001.8.26 | D1293 | T1 | 1045 | 1380 | 3.1 | | 0.13 | 7.65 | 540 | 34.5 | 2.40 | - | 0.02 | -16.7 | 2.19 | - |
| | | T2 | 1100 | 1371 | 3.1 | | 0.13 | 7.56 | 541 | 34.5 | 2.50 | - | 0.05 | -6.4 | 2.43 | - |
| | | T3 | 1115 | 1372 | 3.1 | | 0.13 | 7.59 | 540 | 34.5 | 2.54 | - | 0.06 | -5.6 | 2.49 | - |
| | | T4 | 1130 | 1379 | 3.3 | set at 1127 | 0.14 | 7.58 | 542 | 34.5 | 2.54 | - | 0.09 | -5.1 | 2.49 | - |
| | | T5 | 1145 | 1379 | 3.2 | Marker 300 | 0.14 | 7.52 | 546 | 34.5 | 2.67 | - | 0.10 | -5.0 | 2.46 | - |
| | | T6 | 1200 | 1379 | 3.2 | failed | (0.14) | (7.57) | (540) | (34.5) | (2.63) | - | (0.07) | (-10.2) | (2.44) | - |
| | | T7 | 1220 | 1379 | 3.3 | | 0.13 | 7.57 | 540 | 34.5 | 2.61 | - | 0.07 | -5.2 | 2.36 | - |
| | | T8 | 1240 | 1379 | 3.6 | | 0.14 | 7.51 | 539 | 35.0 | 2.59 | - | 0.19 | -4.9 | 2.37 | - |
| | | T9 | 1300 | 1379 | 3.7 | | 0.15 | 7.48 | 539 | 35.5 | 2.54 | - | 0.29 | -6.1 | 2.38 | - |
| | | T10 | 1330 | 1379 | 4.1 | rec. at 1347 | 0.16 | 7.29 | 540 | 34.5 | 2.45 | - | 0.44 | -5.8 | 2.43 | -0.76 |
| | | T11 | 1400 | 1368 | 3.7 | | 0.16 | 7.40 | 538 | 34.5 | 2.50 | - | 0.42 | -5.8 | 2.44 | -0.79 |
| | | T12 | 1430 | 945 | 5.0 | ascending | 0.08 | 7.92 | 549 | 35.0 | 2.64 | - | 0.19 | -13.5 | 2.24 | - |
| 2001.8.30 | D1297 | T1 | 1100 | 1383 | - | Set at 10:55 | 0.75 | 6.18 | 549 | 35.0 | 2.38 | 50.6 | 11.8 | -5.3 | 4.36 | -0.55 |
| | | T2 | 1110 | 1383 | - | APSK07 | 0.98 | 6.02 | 546 | 35.0 | 2.32 | 49.6 | 19.2 | -5.0 | 5.17 | -0.55 |
| | | T3 | 1120 | 1383 | - | | 0.57 | 6.36 | 547 | 35.0 | 2.49 | 52.0 | 8.94 | -5.4 | 3.73 | -0.62 |
| | | T4 | 1130 | 1383 | - | | 0.56 | 6.33 | 545 | 35.0 | 2.44 | 51.4 | 8.36 | -5.3 | 3.73 | -0.61 |
| | | T5 | 1145 | 1383 | - | | 0.71 | 6.14 | 540 | 35.5 | 2.43 | 50.2 | 12.4 | -5.2 | 4.20 | -0.68 |
| | | T6 | 1200 | 1383 | - | | 0.97 | 6.15 | 555 | 35.5 | 2.29 | 48.8 | 17.6 | -5.1 | 5.21 | - |
| | | T7 | 1215 | 1383 | - | | 1.15 | 5.89 | 547 | 35.5 | 2.18 | 48.2 | 20.7 | -5.2 | 5.39 | - |
| | | T8 | 1230 | 1383 | - | | 0.62 | 6.30 | 542 | 35.0 | 2.36 | 51.6 | 8.55 | -5.3 | 3.82 | -0.62 |
| | | T9 | 1250 | 1383 | - | | 0.85 | 6.05 | 556 | 35.5 | 2.30 | 49.2 | 14.1 | -5.2 | 4.72 | -0.54 |
| | | T10 | 1310 | 1383 | - | | 1.07 | 5.95 | 553 | 35.0 | 2.19 | 48.9 | 18.6 | -4.9 | 5.44 | -0.64 |
| | | T11 | 1330 | 1383 | - | | 1.07 | 5.95 | 545 | 35.0 | 2.26 | 48.8 | 19.4 | -5.3 | 5.60 | - |
| | | T12 | 1400 | 1383 | - | rec. at 1408 | 0.84 | 6.26 | 553 | 36.0 | 2.25 | 50.3 | 11.9 | -5.2 | 4.60 | -0.42 |
| 2001.10.24 | D1312 | T1 | 1130 | 1380 | 3.4 | | 0.13 | 7.64 | 551 | - | 3.27 | 51.2 | 0.05 | -6.8 | 2.20 | - |
| | | T2 | 1145 | 1365 | 3.3 | | 0.14 | 7.60 | 540 | - | 2.62 | 51.6 | 0.06 | -6.5 | 2.19 | - |
| | | T3 | 1200 | 1368 | 4.1 | set at 1202 | 0.20 | 7.09 | - | - | 2.59 | 51.4 | 1.19 | -8.1 | 2.31 | -0.08 |
| | | T4 | 1215 | 1368 | 4.5 | APSK03 | 0.19 | 7.11 | 547 | - | 2.60 | 51.2 | 1.37 | -7.2 | 2.32 | -0.16 |
| | | T5 | 1230 | 1368 | 3.7 | | 0.16 | 7.32 | 539 | - | 2.60 | 51.2 | 0.53 | -9.8 | 2.26 | -0.23 |
| | | T6 | 1245 | 1368 | 4.7 | | 0.23 | 6.89 | 540 | - | 2.64 | 51.2 | 2.00 | -6.2 | 2.45 | - |
| | | T7 | 1300 | 1368 | 4.8 | | 0.26 | - | - | - | - | - | 2.48 | -6.2 | 2.64 | +0.24 |
| | | T8 | 1315 | 1368 | 5.7 | | 0.46 | 6.43 | 563 | - | 2.47 | 49.8 | 5.09 | -5.1 | 3.18 | - |
| | | T9 | 1330 | 1368 | 6.7 | | 0.35 | 6.58 | - | - | 2.54 | 50.1 | 3.88 | -5.4 | 2.84 | +0.61 |
| | | T10 | 1345 | 1368 | 6.7 | | 0.41 | 6.46 | 548 | - | 2.51 | 49.9 | 4.39 | -5.4 | 2.98 | +0.72 |
| | | T11 | 1400 | 1368 | 7.0 | | 0.46 | 6.38 | 560 | - | 2.45 | 49.6 | 5.40 | -5.2 | 3.20 | +0.57 |
| | | T12 | 1415 | 1368 | 5.3 | rec. at 1450 | 0.27 | 6.71 | 547 | - | 2.59 | 51.1 | 2.74 | -5.2 | 2.62 | +0.89 |

^aAnalytical method: Si (colorimetry), pH (pH meter), chlorinity (Mohr titration), salinity (refractometer), alkalinity (potentiometric titration) onboard analysis, Mg (ICP-AES), CH₄ and δ¹³C(CH₄) (Continuous flow isotope-ratio-monitoring gas chromatography/mass spectrometry), ΣCO₂ (coulometer), and δ¹³C(CO₂) (dual inlet isotope-ratio-monitoring mass spectrometry) onland analysis.

Table 3. Concentrations and Isotopic Compositions of Dissolved CH₄ and ΣCO₂ of End-Member High-Temperature Hydrothermal Fluid

| Site | Sample | Temp. (°C) | Si (mmol/kg) | CH ₄ (μmol/kg) | δ ¹³ C(CH ₄) (‰PDB) | ΣCO ₂ (mmol/kg) | δ ¹³ C(CO ₂) (‰PDB) |
|------------|------------------------------|------------|--------------|---------------------------|--------------------------------------------|----------------------------|--------------------------------------------|
| APSK05 | D1305 W-1, -2 | 305 | 13.2 ± 0.3 | 150 ± 40 | -5.9 ± 0.4 | 43 ± 6 | -0.43 ± 0.02 |
| APSK07 | D1308 W-4 | 280 | 12 ± 2 | 150 ± 10 | -6.1 ± 0.3 | 40 ± 1 | -0.4 ± 0.3 |
| Marker 223 | D1298 W-3, -4, D1305 W-4 | 270 ± 40 | 11 ± 2 | 170 ± 50 | -5.7 ± 0.6 | 42 ± 7 | -0.50 ± 0.05 |
| AP01 | D1297 W-3, -4, D1303 W-3, -4 | 310 ± 3 | 12.6 ± 0.1 | 140 ± 50 | -5.8 ± 0.3 | 40 ± 4 | -0.47 ± 0.08 |
| Average | | 290 ± 20 | 12 ± 1 | 150 ± 40 | -5.8 ± 0.4 | 40 ± 5 | -0.4 ± 0.2 |

hydrothermal fluids, as mentioned previously [Ishibashi *et al.*, 2003].

[22] We successfully captured some hydrothermal fluid samples without artificial mixing, as evidenced by a Si concentration close to the end-member value (12.8 mmol/kg). For the four active sites (APSK05, APSK07, Marker 223, and AP01), the concentrations and isotopic compositions of dissolved CH₄ and ΣCO₂ were averaged site by site, the values of which together with the average temperatures are shown in Table 3. Both the concentrations and isotopic compositions were similar across the four sites. These results are in agreement with those of the major element fluid chemistry [Ishibashi *et al.*, 2003] and suggest that the microbial activities related to CH₄ and ΣCO₂ are low or homogenous in the HTHFs. Clear microbial diversity has been reported between a borehole and natural vent sites based on 16S rDNA clone libraries [Higashi *et al.*, 2004]. These results imply that the microbial activities in the HTHFs are minimal.

5.2. End-Members of the LTHFs

[23] Correction for the addition of seawater to the pure hydrothermal fluid is an important step in considering other subsurface processes. The concentration of each chemical species in the undiluted pure hydrothermal fluid can be estimated by extrapolating the linear relationship from the data on each LTHF sample back to Mg = 0 [Von Damm *et al.*, 1985]. However, the analytical error for Mg (±1 mmol/kg) is too large to correct for the addition of seawater to the hydrothermal fluid. The linearity noted between the Si and Mg concentrations suggests that Si is one of the conservative components [Kishida *et al.*, 2004]. The analytical error associated with the Si measurement is much smaller than that associated with the Mg measurement, so we adopted the Si concentration of 12.8 ± 0.3 mmol/kg, the end-member Si concentration in the pure Suiyo Seamount hydrothermal fluid, for estimating the end-member fluid compositions of other components of the LTHFs. This approach to estimating the end-member fluid compositions has previously been applied to diffuse fluids, using the end-member Si concentration to estimate the chemical composition of the pure hydrothermal fluid [Von Damm and Lilley, 2004].

[24] Among the samples collected from a diffuse flow field, the concentrations of volatile components (CH₄ and ΣCO₂) showed linear relationships with the Si concentration (Figures 3 and 4). The end-member compositions of CH₄ and ΣCO₂ for each site were calculated by extrapolation of the relationship determined by the least squares fit to Si = 12.8 ± 0.3 mmol/kg. The estimated end-member concentrations of CH₄ and ΣCO₂ are listed in Table 4. As for D1293, all the collected samples were too diluted by seawater to allow accurate estimations of the end-member values.

[25] The end-member ΣCO₂ concentrations of the LTHFs (36–46 mmol/kg) were in the same range as those of the HTHFs (40–43 mmol/kg) within the range of the analytical error (Tables 3 and 4). This agreement suggests that LTHFs are formed by dilution of the original hydrothermal fluid with seawater. Moreover, the strong agreement noted between the end-member ΣCO₂ concentrations calculated from two different types of hydrothermal fluids confirm the reliabilities of the two sampling devices used in the present study. In contrast, the end-member concentrations of dissolved CH₄ in the LTHFs (150–210 μmol/kg) were higher than those in the HTHFs (140–170 μmol/kg) above the analytical error (Tables 3 and 4).

[26] The carbon isotopic compositions of the CH₄ in the LTHFs were compared with those in the HTHFs. To estimate the CH₄ carbon isotopic composition of the LTHF end-members, the δ¹³C of CH₄ was plotted against 1/CH₄ (reciprocal of the CH₄ concentration) for the collected samples at each site (Figure 5). All the plots should be distributed along a straight line if simple mixing between the end-member and the ambient seawater is the sole process that determines the methane dissolved in the collected samples (from the sampling chamber in a diffuse flow field). For the regression factors (Figure 5), strong relationships were identified (with the exceptions of two samples from D1293 (Figure 5b)) for each site, which enabled the determination of the methane carbon isotopic compositions by extrapolation of the least squares fit to (1/CH₄) = 0. The calculated δ¹³C values were -4.8 ± 0.3‰ for APSK04 (HY11), -4.6 ± 0.6‰ for Marker 300 (D1293), -4.9 ± 0.3‰ for APSK07 (D1297), and -4.7 ± 0.2‰ for APSK03 (D1312) (Table 4). In comparison with the methane carbon isotopic composition of the HTHF end-member (-5.8 ± 0.4‰, indicated by an asterisk in Figure 5), the LTHF end-member comprised methane that was significantly enriched in ¹³C.

[27] The chemical and isotopic compositions of the ambient seawater averaged 50 nmol/kg and -6.3 ± 0.7‰, respectively, based on the fluids (HY11 T2 and 3, D1293 T2 and 3, D1312 T1 and 2) obtained using the FAS (Table 2). For D1312, the background seawater of the mixing line was a local source rather than the ambient seawater, as mentioned above, but the mixing line was apparently straight, such that there is no doubt about the value for the hydrothermal end-member (Figure 5d).

6. Discussion

6.1. Processes That Control the Methane Concentrations of Hydrothermal Fluids

[28] The end-member methane concentrations of the LTHFs were higher than those of the HTHFs. The mechanisms that increase the methane concentration in hydrothermal fluids

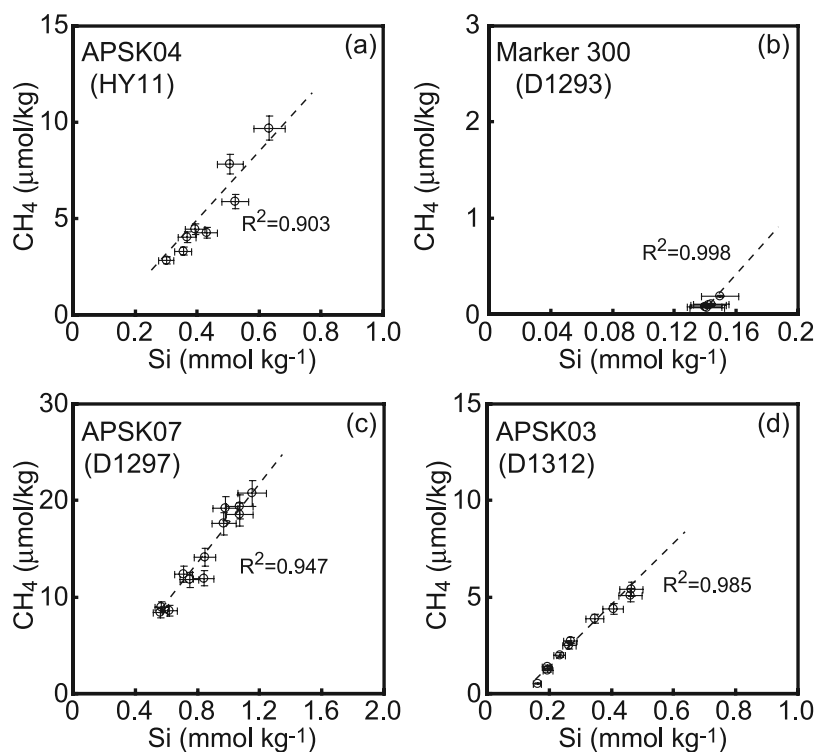
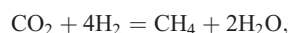


Figure 3. Plots of CH₄ concentration versus Si concentration in the low-temperature fluid samples recovered by the Fluid Accumulation Sampler during deployment for (a) HY11, (b) D1293, (c) D1297, and (d) D1312. The dashed line is the regression line of all data together with the correlation coefficient (R^2).

in sediment-starved hydrothermal systems include: (1) phase separation [Massoth *et al.*, 1989]; (2) abiogenic production [Welhan and Craig, 1983]; and (3) microbial production [Deming and Baross, 1993].

[29] During ascent, as its temperature reaches the boiling point, the hydrothermal fluid is separated into gaseous and aqueous phases. Subsequently, the gaseous phase mixes with cooler seawater to form a vapor-rich fluid, owing to its fractionation during boiling. However, according to the Rayleigh distillation model phase-segregated (gas phase) fluids should contain CH₄ enriched in ¹²C due to fractionation during boiling, which is inconsistent with our observation. In our study, the $\delta^{13}\text{C}$ value of methane as the end-member of the LTHFs showed enrichment for ¹³C relative to that of the HTHFs (Tables 3 and 4). Indeed, the temperature at the time of sampling of the Suiyo Seamount ($290 \pm 20^\circ\text{C}$; Table 4) was below the boiling point (330°C) for seawater at ambient pressure (140 bar) [Bischoff and Rosenbauer, 1988], and no decrease in salinity or chloride concentration was observed for any of the samples (Table 2), which contradicts the idea of gas phase fluid venting induced by phase separation.

[30] Abiogenic methane production is generally observed in sediment-starved seafloor hydrothermal fluids. The $\delta^{13}\text{C}$ of methane is typically -15‰ to -25‰ PDB [Welhan and Craig, 1983; Evans *et al.*, 1988]. The Fischer-Tropsch synthesis reaction,



has been demonstrated experimentally at 127°C , with reported kinetic isotopic fractionation of 50‰ to 100‰

[Lancet and Anders, 1970]. Kinetic isotopic fraction during abiogenic CH₄ formation was determined as: $\alpha = (^{13}\text{CH}_4/^{12}\text{CH}_4)/(^{13}\text{CO}_2/^{12}\text{CO}_2) = 0.940$ to 0.950 at 200°C and $\alpha = 0.960$ to 0.965 at 300°C , based on an experiment performed in the presence of Ni-Fe alloy as catalyst [Horita and Berndt, 1999]. The equilibrium ¹³C/¹²C fractionations expected between ΣCO_2 and CH₄ were evaluated as α (equil) = 0.970 at 200°C and α (equil) = 0.982 at 300°C [Horita, 2001]. Abiogenic methane has also been found in slow-spreading ridges, such as Rainbow, Lost City, and Logatchev, where the $\delta^{13}\text{C}$ s of methane vary from -15.8‰ to -8.8‰ PDB [Charlou *et al.*, 2002; Kelley *et al.*, 2005; Charlou *et al.*, 1998]. Therefore, abiogenic methane production cannot be the source of the higher CH₄ concentration and heavier isotopic values of CH₄ observed for the LTHF samples from Suiyo Seamount.

[31] Methanogenic archaea can produce CH₄ from H₂ and CO₂. The carbon isotopic fractionation from CO₂ to CH₄ by cultured microbes is reported to be -20‰ to -30‰ PDB [House *et al.*, 2003]. The observed isotopic composition of ΣCO_2 in all samples collected from the Suiyo hydrothermal system in the present study was $-0.3 \pm 0.5\text{‰}$. Since the $\delta^{13}\text{C}$ of microbially produced methane from CO₂ should be -20.3‰ to -30.3‰ PDB, microbial methane production in the hydrothermal circulation cannot explain the ¹³C enrichment in the LTHF samples compared to the $\delta^{13}\text{C}$ data for the hydrothermal end-member.

6.2. Combination of Microbial Production and Oxidation of Methane

[32] Thus, the CH₄ enrichment and ¹³C enrichment in $\delta^{13}\text{C}(\text{CH}_4)$ for the LTHFs are not explained by any of the

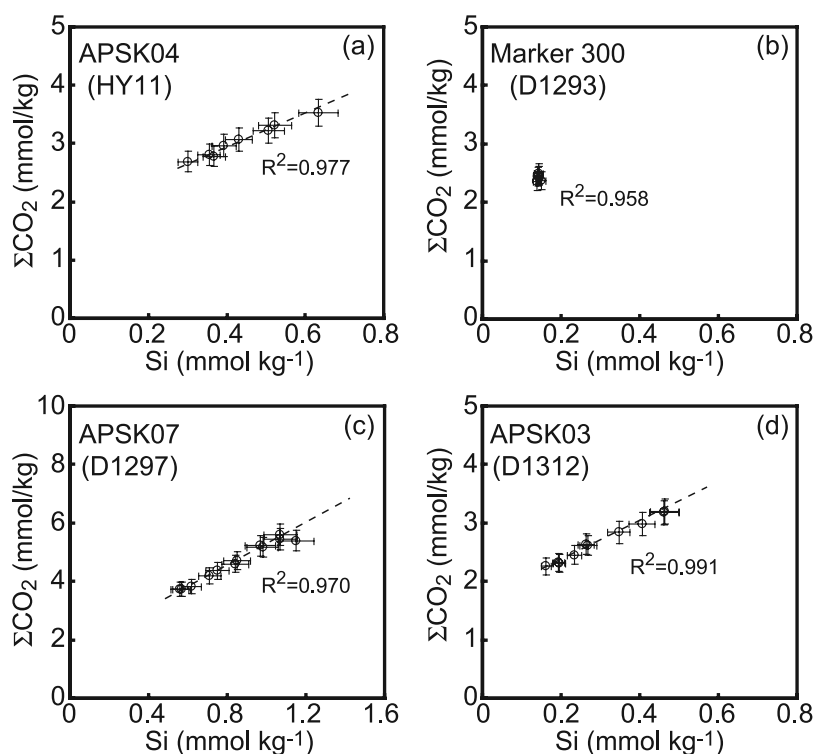


Figure 4. Plots of ΣCO_2 concentration versus Si concentration in the low-temperature fluid samples recovered by Fluid Accumulation Sampler during deployment for (a) HY11, (b) D1293, (c) D1297, and (d) D1312. The dashed line is the regression line of all data together with the correlation coefficient (R^2).

above mentioned processes. However, the hydrothermal vents of the Suiyo Seamount contain a number of methanogenic hyperthermophilic archaeal clones [Higashi *et al.*, 2004], which are closely related to *Methanococcaldococcus infernos*, which grows at temperatures of between 49°C and 89°C, with an optimum growth temperature of 85°C [Jeanthon *et al.*, 1998], and *Methanococcaldococcus jannaschii*, which grows at 50°C to 86°C with an optimum growth temperature of 85°C [Jones *et al.*, 1983]. On the other hand, microbial methane oxidation was measured using direct sampling of the hydrothermal vent fluid from the Suiyo Seamount [Utsumi *et al.*, 2004]. Of the three sites from which LTHFs were collected, the site with the highest temperature (Table 2) showed the highest CH₄ end-member concentration (Table 4), which supports the notion of microbial influence on the CH₄ concentration of the LTHFs. Since methane enrichment and ¹³C enrichment of methane could be due to a combination of microbial methane production and oxidation after formation of the LTHFs, we estimated methane production and consumption by microbial processes.

[33] For calculations of combinatory production and oxidation of methane in the hydrothermal system during

circulation, the carbon isotopic fractionation (ε) of methane production and kinetic isotopic effect (KIE) α of methane oxidation were taken from previous studies [House *et al.*, 2003; Utsumi *et al.*, 2004; Templeton *et al.*, 2006].

[34] For methane production, the carbon isotopic fractionation ε value for the conversion of CO₂ to CH₄ by the methanogenic microbe *Methanococcaldococcus jannaschii*, which is closely related to the clones from the Suiyo Seamount [Higashi *et al.*, 2004], has been reported [House *et al.*, 2003], and 25‰PDB was adopted as a representative value of ε in the present study.

[35] The value of KIE (α) due to both aerobic and anaerobic methane oxidation has been reported in several microbiological and geochemical studies [e.g., Whiticar and Faber, 1986], with α defined as the ratio of relative separation rates of molecules that contain different isotopes,

$$\alpha = k_{12}/k_{13},$$

where k_{12} and k_{13} are the rate constants of the following respective reactions:

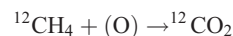


Table 4. Calculated Concentrations and Isotopic Compositions of Dissolved CH₄ and ΣCO_2 of End-Member Low-Temperature Hydrothermal Fluid

| Site | Sample | CH ₄ (μmol/kg) | $\delta^{13}\text{C}(\text{CH}_4)$ (‰PDB) | ΣCO_2 (mmol/kg) | $\delta^{13}\text{C}(\text{CO}_2)$ (‰PDB) |
|------------|-----------------|---------------------------|-------------------------------------------|-------------------------------|-------------------------------------------|
| APSK04 | HY11 T-4 ~ -12 | 270 ± 60 | -4.8 ± 0.3 | 36 ± 8 | -1.2 ± 0.4 |
| Marker 300 | D1293 T-4 ~ -8 | 150 ± 310 | -4.6 ± 0.6 | - | - |
| APSK07 | D1297 T-1 ~ -12 | 280 ± 50 | -4.9 ± 0.3 | 46 ± 7 | -0.4 ± 0.1 |
| APSK03 | D1312 T-3 ~ -12 | 210 ± 30 | -4.7 ± 0.2 | 42 ± 5 | +4.5 ± 0.5 |

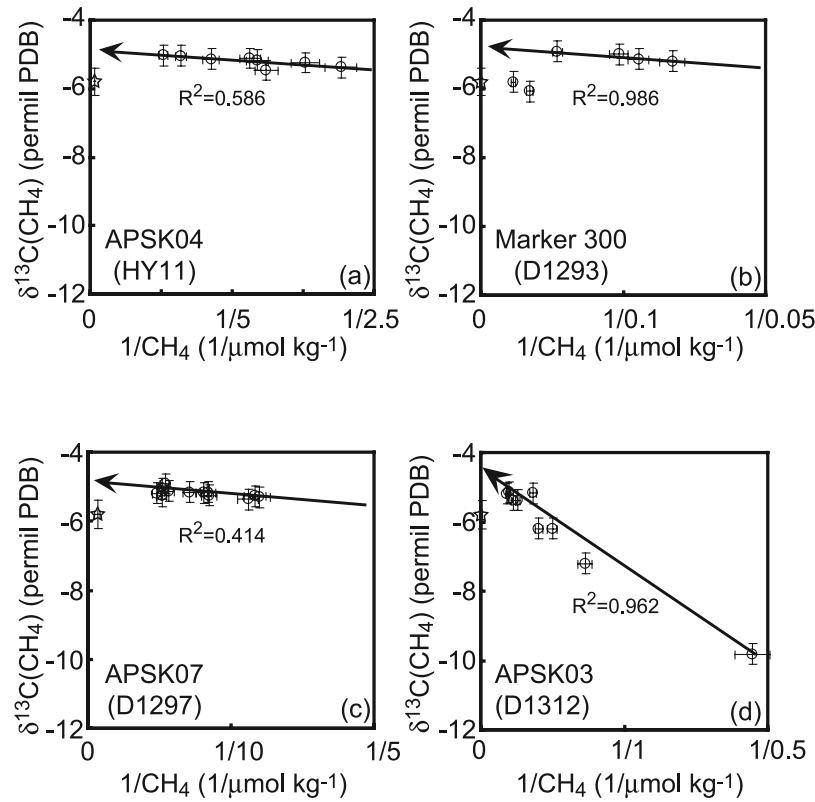
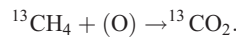


Figure 5. The $\delta^{13}\text{C}$ versus $1/(\text{CH}_4 \text{ concentration})$ for the low-temperature samples after Fluid Accumulation Sampler deployment for (a) HY11, (b) D1293, (c) D1297, and (d) D1312, with the end-member value of the pure Suiyo Seamount hydrothermal fluid recovered by “WHATS” (star). The solid arrow indicates a hypothetical mixing line between the supposed hydrothermal fluid end-member and the ambient seawater in the caldera, together with the correlation coefficient (R^2).

and



[36] The reported KIE values are $\alpha = 1.002\text{--}1.039$ for aerobic methane oxidation [Zyakun *et al.*, 1979; Barker and Fritz, 1981; Coleman *et al.*, 1981; Tsunogai *et al.*, 2000; Grant and Whiticar, 2002; Cowen *et al.*, 2002; Utsumi *et al.*, 2004; Keir *et al.*, 2005; Templeton *et al.*, 2006] and $\alpha = 1.008\text{--}1.014$ for anaerobic methane oxidation [Whiticar and Faber, 1986; Alperin *et al.*, 1988; Martens *et al.*, 1999; Tsunogai *et al.*, 2002]. In the present study, we use 1.004 as the value for α based on prior measurements at Suiyo Seamount [Utsumi *et al.*, 2004].

[37] Application of the Rayleigh fractionation model has a limitation, in that one of the controlling factors is the ratio of the cell density to CH_4 concentration [Templeton *et al.*, 2006]. Assuming that the methane production and oxidation

processes occur under conditions of a closed, homogeneous, well-mixed system, and appropriate cell density, the change in the $\delta^{13}\text{C}$ of the remaining methane during microbial oxidation should be described by the Rayleigh equation connecting the kinetic isotopic fraction factor (α) and isotopic composition as follows [e.g., Coleman *et al.*, 1981]:

$$\delta^{13}\text{C}_t - \delta^{13}\text{C}_0 = 1000(1/\alpha - 1) \ln(M_t/M_0),$$

where $\delta^{13}\text{C}_0$ and $\delta^{13}\text{C}_t$ are the carbon isotopic compositions in the original methane and residual methane at time t , and M_0 and M_t are the original and residual concentrations of methane in the system, respectively.

[38] When we calculate the methane concentrations derived from production and oxidation during hydrothermal circulation using the concentrations and isotopic compositions of methane and ΣCO_2 derived based on this supposition, the values surpass 30 mmol/kg. Since these

Table 5. Calculated Methane Production and Oxidation Observed in Low-Temperature Hydrothermal Fluid^a

| Site | Sample | Production (C_p) ($\mu\text{mol/kg}$) | Oxidation (C_o) ($\mu\text{mol/kg}$) | C_p/C_o | T_{max} ($^{\circ}\text{C}$) |
|------------|--------|---------------------------------------------|--------------------------------------------|---------------|-----------------------------------------|
| APSK04 | HY11 | 230 ± 180 | 110 ± 80 | 2.1 ± 3.1 | 11.4 |
| Marker 300 | D1293 | - | - | - | 3.6 |
| APSK07 | D1297 | 250 ± 160 | 120 ± 70 | 2.1 ± 2.6 | 20 ^b |
| APSK03 | D1312 | 120 ± 130 | 60 ± 70 | 2.0 ± 4.0 | 7.0 |

^aThe ratio of methane production to oxidation is also shown.

^bThis value is estimated from Si concentration because temperature recording was failed during D1297.

calculated methane concentrations exceed all the measured values, the value chosen for KIE is unrealistic. If these high methane concentrations were actually reached, competition between methane producers and oxidizers would very likely lead to variable ΣCO_2 concentrations, which we did not observe (Table 4).

[39] The putative microbial process occurs under the seafloor in the hydrothermal system, when the hydrothermal fluid is not diluted to a great extent by seawater. Under this condition, there is an excess of CH₄ in the fluid, while O₂ is limited, such that fractionation should approach the maximum value [Templeton *et al.*, 2006]. Therefore, we adopted the KIE value of 1.039, which is the maximum reported value [Templeton *et al.*, 2006]. On the basis of this assumption, each methane concentration was calculated for production and oxidation using our acquired data (Table 5). These values are far lower than the ΣCO_2 concentrations measured in the samples, which is consistent with the fact that we did not detect any ΣCO_2 deficit.

[40] We also observed a tendency for the estimated concentrations of produced and oxidized methane to correspond to the observed maximum temperature in the bell jar, as shown in Table 5, which suggests that the microbes are more active at higher temperatures. However, as mentioned above, the microbial activity in high-temperature fluids (290 \pm 20°C) is negligible. The microbes are active at moderate temperatures, i.e., within their optimal temperature range. Archaeal clones derived from the Suiyo Seamount subvent grow at 49°C to 89°C [Jehanthon *et al.*, 1998; Jones *et al.*, 1983], which is consistent with this result.

6.3. Life Cycles of Microbes in the Hydrothermal System

[41] Methane-oxidizing microbes use oxygen in an oxidative environment and sulfate in marine sediments. In contrast, methane producers live in a strongly reductive environment. Our conclusion that both methane oxidation and production occur during circulation prompts the question: “What environment do the microbes live in”?

[42] Despite the acquisition of samples from different sites in the Suiyo Seamount area, we obtained similar isotopic values for methane (-4.8 ± 0.3) and similar ratios of methane production to oxidation (2.1 ± 3.2). This suggests that the set of processes does not occur in a sequence of reductive and oxidative steps in the fluid flow path but occurs in a simultaneous fashion and involves a consortium. If anaerobic oxidation occurs in the reductive part of the fluid flow path and aerobic oxidation occurs in the shallower, oxidative part of the fluid flow path, the methane isotope concentrations and the ratios of methane production to oxidation should vary depending on the length and width of the path, and consequently, the residence and reaction time through the path. We hypothesize that the combination of microbial processes is mediated by a consortium of methanogenic and methane-oxidizing microbes using either oxygen or sulfate. The similar ratio of methane production to oxidation probably corresponds to the similar ratio of the rate of methane production to oxidation for the microbes within the consortium. This consortium may represent a mechanism by which microbes live at the boundary between oxidized and reduced environments. An example of this is the consortium of methane-

producing archaea and sulfate-reducing bacteria observed during anaerobic oxidation of methane in marine sediments [Boetius *et al.*, 2000].

[43] To test this hypothesis experimentally, it will be necessary to conduct isotopic analyses of these cells and to acquire microscopic evidence of the consortium using fluorescence in situ hybridization with specific 16S rRNA-targeted oligonucleotide probes.

7. Conclusion

[44] The chemical and isotopic compositions of volatile species (ΣCO_2 and CH₄) dissolved in hydrothermal fluids collected from the Suiyo Seamount hydrothermal field were investigated using newly developed sampling devices. The end-member compositions estimated from HTHFs did not differ across the four sampling sites, in terms of both ΣCO_2 and CH₄ concentrations and isotopic compositions. These results suggest that the original hydrothermal aquifer of the Suiyo Seamount hydrothermal fluid mediates chemical homogeneity within a radius of 100–200 m, as demonstrated by the composition of the major elements of the hydrothermal fluid.

[45] In contrast, the LTHFs showed diversity in terms of the concentration and carbon isotopic composition of CH₄. The end-member compositions estimated for the LTHFs from three sites showed higher CH₄ concentrations and ¹³C-enriched isotopic composition than the end-member compositions determined for the HTHFs. Since the addition of ¹³C-enriched methane to the low-temperature hydrothermal fluid cannot be explained by a single-step process, such as phase separation or the abiogenic Fischer-Tropsch reaction, the difference between the LTHFs and HTHFs is attributed to a combination of methane production and methane oxidation by microbes after formation of the LTHFs. Of the three sites from which LTHFs were collected, the site with the highest temperature showed the highest CH₄ end-member concentration, which supports the notion of microbial influence on the CH₄ concentration of the LTHFs.

[46] Using appropriate isotopic fractionation factors during microbial processes, we estimated the ratio of methane production to methane oxidation as 2.1 ± 3.2 . The similarity of the ratios of methane production to oxidation at different sites suggests that the combinatory process is mediated by a consortium of methanogens and methanotrophs in the boundary between oxidized and reduced environments that have approximately the same ratio of methane production to oxidation.

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References

- Alperin, M. J., et al. (1988), Carbon and hydrogen isotope fractionation resulting from anaerobic methane oxidation, *Global Biogeochem. Cycles*, 2, 279–288, doi:10.1029/GB002i003p00279.

- Barker, J. F., and P. Fritz (1981), Carbon isotope fractionation during microbial methane oxidation, *Nature*, 293, 289–291, doi:10.1038/293289a0.
- Bischoff, J. L., and R. J. Rosenbauer (1988), Liquid-vapor relations in the critical region of the system NaCl-H₂O from 380 to 415°C: A refined determination of the critical point and two-phase boundary of seawater, *Geochim. Cosmochim. Acta*, 52, 2121–2126, doi:10.1016/0016-7037(88)90192-5.
- Boetius, A., et al. (2000), A marine microbial consortium apparently mediating anaerobic oxidation of methane, *Nature*, 407, 623–626, doi:10.1038/35036572.
- Butterfield, D. A., et al. (1990), The geochemistry of hydrothermal fluids from the ASHES vent field, Axial Seamount, Juan de Fuca ridge: Sub-seafloor boiling and subsequent fluid–rock interaction, *J. Geophys. Res.*, 95, 12,895–12,922, doi:10.1029/JB095iB08p12895.
- Charlou, J. L., et al. (1998), Fluides hydrothermaux en contexte de roches ultrabasiques: Exemples de Logatchev (14°45'N) et Rainbow (36°14'N) sur la dorsale médio-Atlantique, paper presented at Réunion des Sciences de la Terre RST 98, Brest, France.
- Charlou, J. L., et al. (2002), Geochemistry of high H₂ and CH₄ vent fluids issuing from ultramafic rocks at the Rainbow hydrothermal field (36°14'N, MAR), *Chem. Geol.*, 191, 345–359, doi:10.1016/S0009-2541(02)00134-1.
- Coleman, D. D., et al. (1981), Fractionation of carbon and hydrogen isotopes by methane-oxidizing bacteria, *Geochim. Cosmochim. Acta*, 45, 1033–1037, doi:10.1016/0016-7037(81)90129-0.
- Corliss, J. B., et al. (1979), Submarine thermal springs on the Galapagos Rift, *Science*, 203, 1073–1083, doi:10.1126/science.203.4385.1073.
- Cowen, J. P., et al. (2002), Methane in aging hydrothermal plumes, *Geochim. Cosmochim. Acta*, 66, 3563–3571, doi:10.1016/S0016-7037(02)00975-4.
- Deming, J. W., and J. A. Baross (1993), Deep-sea smokers: Windows to a subsurface biosphere?, *Geochim. Cosmochim. Acta*, 57, 3219–3230, doi:10.1016/0016-7037(93)90535-5.
- Edmond, J. M., et al. (1979a), On the formation of metal-rich deposits at ridge crests, *Earth Planet. Sci. Lett.*, 46, 19–30, doi:10.1016/0012-821X(79)90062-1.
- Edmond, J. M., et al. (1979b), Ridge crest hydrothermal activity and the balances of the major and minor elements in the ocean: The Galapagos data, *Earth Planet. Sci. Lett.*, 46, 1–18, doi:10.1016/0012-821X(79)90061-X.
- Elsaied, H., et al. (2007), Composition of archaeal, bacterial, and eukaryal RuBisCO genotypes in three Western Pacific arc hydrothermal vent systems, *Extremophiles*, 11, 191–202, doi:10.1007/s00792-006-0025-2.
- Evans, W. C., et al. (1988), Geochemistry of some gases in hydrothermal fluids from the Southern Juan de Fuca Ridge, *J. Geophys. Res.*, 93, 15,305–15,313, doi:10.1029/JB093iB12p15305.
- Grant, N. J., and M. J. Whiticar (2002), Stable carbon isotope evidence for methane oxidation in plumes above Hydrate Ridge, Cascadia Oregon Margin, *Global Biogeochem. Cycles*, 16(4), 1124, doi:10.1029/2001GB001851.
- Hara, K., et al. (2005), Analysis of the archaeal sub-seafloor community at Suiyo Seamount on the Izu-Bonin Arc, *Adv. Space Res.*, 35, 1634–1642, doi:10.1016/j.asr.2005.04.111.
- Higashi, Y., et al. (2004), Microbial diversity in hydrothermal surface to subsurface environments of Suiyo Seamount, Izu-Bonin Arc, using a catheter-type in situ growth chamber, *FEMS Microbiol. Ecol.*, 47, 327–336, doi:10.1016/S0168-6496(04)00004-2.
- Holm, N. G., and E. M. Andersson (1998), Hydrothermal systems, in *The Molecular Origins of Life; Assembling Pieces of the Puzzle*, edited by A. Brack, pp. 86–99, Cambridge Univ. Press, Cambridge, U. K.
- Horita, J. (2001), Carbon isotope exchange in the system CO₂-CH₄ at elevated temperatures, *Geochim. Cosmochim. Acta*, 65, 1907–1919, doi:10.1016/S0016-7037(01)00570-1.
- Horita, J., and M. E. Berndt (1999), Abiogenic methane formation and isotopic fractionation under hydrothermal conditions, *Science*, 285, 1055–1057, doi:10.1126/science.285.5430.1055.
- House, C. H., et al. (2003), Carbon isotopic fractionation by Archaeans and other thermophilic prokaryotes, *Org. Geochem.*, 34, 345–356, doi:10.1016/S0146-6380(02)00237-1.
- Ijiri, A., et al. (2003), A simple method for oxygen-18 determination of milligram quantities of water using NaHCO₃, *Rapid Commun. Mass Spectrom.*, 17, 1472–1478, doi:10.1002/rcm.1081.
- Ishibashi, J., and T. Urabe (1995), Hydrothermal activity related to arc-backarc magmatism in the western Pacific, in *Backarc Basins: Tectonics and Magmatism*, edited by B. Taylor, pp. 451–495, Plenum, New York.
- Ishibashi, J., et al. (2003), Hydrothermal interaction with volcanoclastic sediment beneath the Suiyo Seamount submarine caldera, Izu-Bonin Arc, *Geochim. Cosmochim. Acta*, 67, A174.
- Jannasch, H. W., and M. J. Mottl (1985), Geomicrobiology of deep-sea hydrothermal vents, *Science*, 229, 717–725, doi:10.1126/science.229.4715.717.
- Jeanthon, C., et al. (1998), *Methanococcus infernus* sp. nov., a novel hyperthermophilic lithotrophic methanogen isolated from a deep-sea hydrothermal vent, *Int. J. Syst. Bacteriol.*, 48, 913–919.
- Jones, W. J., et al. (1983), *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent, *Arch. Microbiol.*, 136, 254–261, doi:10.1007/BF00425213.
- Kakegawa, T., et al. (2005), Geological structure of the subsurface region at the Suiyo Seamount and its relationship to sulfur isotope compositions of sulfides (in Japanese with English abstract), *J. Oceanogr.*, 14, 221–235.
- Kasai, H., et al. (2003), Archaeal community in the hydrothermal system at Suiyo Seamount on the Izu-Ogasawara Arc, *Geochim. Cosmochim. Acta*, 67, A202.
- Kasuga, S., and Y. Kato (1992), Discover of hydrothermal ore deposits in the crater of the Suiyo Smt. on the Izu-Ogasawara Arc (in Japanese with English abstract), *Proc. JAMSTEC Symp. Deep Sea Res.*, 8, 249–255.
- Keir, R. S., et al. (2005), Methane and methane carbon isotope ratios in the Northeast Atlantic including the Mid-Atlantic Ridge (50°N), *Deep Sea Res., Part I*, 52, 1043–1070, doi:10.1016/j.dsr.2004.12.006.
- Kelley, D. S., et al. (2005), A serpentinite-hosted ecosystem: The Lost City hydrothermal field, *Science*, 307, 1428–1434, doi:10.1126/science.1102556.
- Kinoshita, M., et al. (2006), Recharge/discharge interface of a secondary hydrothermal circulation in the Suiyo Seamount of the Izu-Bonin Arc, identified by submersible-operated heat flow measurements, *Earth Planet. Sci. Lett.*, 245, 498–508, doi:10.1016/j.epsl.2006.02.006.
- Kishida, K., et al. (2004), Tungsten enriched in submarine hydrothermal fluids, *Earth Planet. Sci. Lett.*, 222, 819–827, doi:10.1016/j.epsl.2004.03.034.
- Kroopnick, P. M. (1971), Oxygen and carbon in the oceans and atmosphere: Stable isotopes as tracers for consumption, production and circulation models, Ph.D. thesis, Univ. of Calif., San Diego.
- Kuwabara, T., et al. (2005), *Thermococcus coalescens* sp. nov., a cell-fusing hyperthermophilic archaeon from Suiyo Seamount, *Int. J. Syst. Evol. Microbiol.*, 55, 2507–2514, doi:10.1099/ijs.0.63432-0.
- Kuwabara, T., et al. (2007), *Thermococcus celericrescens* sp. nov., a fast-growing and cell-fusing hyperthermophilic archaeon from a deep-sea hydrothermal vent, *Int. J. Syst. Evol. Microbiol.*, 57, 437–443, doi:10.1099/ijs.0.64597-0.
- Lancet, M. S., and E. Anders (1970), Carbon isotope fractionation in the Fischer-Tropsch synthesis and in meteorites, *Science*, 170, 980–982, doi:10.1126/science.170.3961.980.
- Martens, C. S., et al. (1999), Stable isotope tracing of anaerobic methane oxidation in the gassy sediments of Eckernförde Bay, German Baltic Sea, *Am. J. Sci.*, 299, 589–610, doi:10.2475/ajs.299.7-9.589.
- Marumo, K., et al. (2005), Shallow drilling of submarine hydrothermal systems using benthic multi-coring system (in Japanese with English abstract), *J. Oceanogr.*, 14, 203–220.
- Massoth, G. J., et al. (1989), Submarine venting of phase-separated hydrothermal fluids at Axial Volcano, Juan de Fuca Ridge, *Nature*, 340, 702–705, doi:10.1038/340702a0.
- Matsumoto, K., and S. Sarata (1996), Development of deep-sea boring machine system (in Japanese with English abstract), *Shigen to Sozai*, 112, 1015–1020.
- Mori, K., et al. (2004), *Oceanithermus desulfurans* sp. nov., a novel thermophilic, sulfur-reducing bacterium isolated from a sulfide chimney in Suiyo Seamount, *Int. J. Syst. Evol. Microbiol.*, 54, 1561–1566, doi:10.1099/ijs.0.02962-0.
- Nakagawa, S., et al. (2003), *Persephonella hydrogeniphila* sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium from a deep-sea hydrothermal vent chimney, *Int. J. Syst. Evol. Microbiol.*, 53, 863–869, doi:10.1099/ijs.0.02505-0.
- Nakagawa, S., et al. (2004a), *Aeropyrum camini* sp. nov., a strictly aerobic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney, *Int. J. Syst. Evol. Microbiol.*, 54, 329–335, doi:10.1099/ijs.0.02826-0.
- Nakagawa, T., et al. (2004b), Analysis of dissimilatory sulfite reductase and 16S rRNA gene fragments from deep-sea hydrothermal sites of the Suiyo Seamount, Izu-Bonin Arc, Western Pacific, *Appl. Environ. Microbiol.*, 70, 393–403, doi:10.1128/AEM.70.1.393-403.2004.
- Pace, N. R. (1991), Origin of life-facing up to the physical setting, *Cell*, 65, 531–533, doi:10.1016/0092-8674(91)90082-A.
- Proskurowski, G., et al. (2008a), Stable isotopic evidence in support of active microbial methane cycling in low-temperature diffuse flow vents at 9°50'N East Pacific Rise, *Geochim. Cosmochim. Acta*, 72, 2005–2023, doi:10.1016/j.gca.2008.01.025.
- Proskurowski, G., et al. (2008b), Abiogenic hydrocarbon production at Lost City hydrothermal field, *Science*, 319, 604–607, doi:10.1126/science.1151194.

- Saegusa, S., et al. (2006), Development of a multibottle gas-tight fluid sampler WHATS II for Japanese submersibles/ROVs, *Geofluids*, 6, 234–240.
- Sako, Y., et al. (2003), *Marinithermus hydrothermalis* gen. nov., sp. nov., a strictly aerobic, thermophilic bacterium from a deep-sea hydrothermal vent chimney, *Int. J. Syst. Evol. Microbiol.*, 53, 59–65, doi:10.1099/ijs.0.02364-0.
- Shock, E. L., and M. D. Schlute (1998), Organic synthesis during fluid mixing in hydrothermal systems, *J. Geophys. Res.*, 103, 28,513–28,527, doi:10.1029/98JE02142.
- Simoneit, B. R. T., et al. (2004), Composition and origin of hydrothermal petroleum and associated lipids in the sulfide deposits of the Rainbow field (Mid-Atlantic Ridge at 36°N), *Geochim. Cosmochim. Acta*, 68, 2275–2294, doi:10.1016/j.gca.2003.11.025.
- Sunamura, M., et al. (2004), Two bacteria phylotypes are predominant in the Suiyo Seamount hydrothermal plume, *Appl. Environ. Microbiol.*, 70, 1190–1198, doi:10.1128/AEM.70.2.1190-1198.2004.
- Takai, K., and K. Horikoshi (1999), Genetic diversity of archaea in deep-sea hydrothermal vent environments, *Genetics*, 152, 1285–1297.
- Takai, K., et al. (2003a), *Deferribacter desulfuricans* sp. nov., a novel sulfur-, nitrate- and arsenate-reducing thermophile isolated from a deep-sea hydrothermal vent, *Int. J. Syst. Evol. Microbiol.*, 53, 839–846, doi:10.1099/ijs.0.02479-0.
- Takai, K., et al. (2003b), *Balnearium lithotrophicum* gen. nov., sp. nov., a novel thermophilic, strictly anaerobic, hydrogen-oxidizing chemolithoautotroph isolated from a black smoker chimney in the Suiyo Seamount hydrothermal system, *Int. J. Syst. Evol. Microbiol.*, 53, 1947–1954, doi:10.1099/ijs.0.02773-0.
- Takano, Y., et al. (2004), Amino acids in the 308°C deep-sea hydrothermal system of the Suiyo Seamount, Izu-Bonin Arc, Pacific Ocean, *Earth Planet. Sci. Lett.*, 219, 147–153, doi:10.1016/S0012-821X(03)00699-X.
- Taylor, B., et al. (1990), Multichannel seismic survey of the central Izu-Bonin Arc, *Proc. Ocean Drill. Program, Initial Rep.*, 126, 51–60.
- Templeton, A. S., et al. (2006), Variable carbon isotope fractionation expressed by aerobic CH₄-oxidizing bacteria, *Geochim. Cosmochim. Acta*, 70, 1739–1752, doi:10.1016/j.gca.2005.12.002.
- Tsunogai, U., et al. (2000), Carbon isotopic distribution of methane in deep-sea hydrothermal plume, Myojin Knoll Caldera, Izu-Bonin Arc: Implications for microbial methane oxidation in the oceans and applications to heat flux estimation, *Geochim. Cosmochim. Acta*, 64, 2439–2452, doi:10.1016/S0016-7037(00)00374-4.
- Tsunogai, U., et al. (2002), Carbon isotopic evidence of methane oxidation through sulfate reduction in sediment beneath cold seep vents on the seafloor at Nankai Trough, *Mar. Geol.*, 187, 145–160, doi:10.1016/S0025-3227(02)00263-3.
- Tsunogai, U., et al. (2003), WHATS: A new multi-bottle gas-tight sampler for sea-floor vent fluids (in Japanese with English abstract), *Chikyukagaku*, 37, 101–109.
- Tsunogai, U., et al. (2005), Stable isotopic compositions of methane and carbon monoxide in the Suiyo hydrothermal plume, Izu-Bonin Arc: Tracers for microbial consumption/production, *Earth Planet. Sci. Lett.*, 237, 326–340, doi:10.1016/j.epsl.2005.05.042.
- Urabe, T., et al. (2001), The Archaeal Park Project Update, *Int. Ridge Crest Res.: Biol. Stud.*, 10, 23–25.
- Utsumi, M., et al. (2004), Direct measurement of microbial methane oxidation at hydrothermal vent ecosystems, *Eos. Trans. AGU*, 85(47), Fall Meet. Suppl., V41B-1373.
- Van Dover, C. L. (2000), Evolution and biogeography, in *The Ecology of Deep-Sea Hydrothermal Vents*, edited by C. L. Dover, pp. 313–354, Princeton Univ., N. J.
- Von Damm, K. L., and J. L. Bischoff (1987), Chemistry of hydrothermal solutions from the southern Juan de Fuca Ridge, *J. Geophys. Res.*, 92, 11,334–11,346, doi:10.1029/JB092iB11p11334.
- Von Damm, K. L., and M. D. Lilley (2004), Diffuse flow hydrothermal fluids from 9°50'N East Pacific Rise: Origin, evolution and biogeochemical controls, in *The Subsurface Biosphere at Mid-Ocean Ridges*, edited by W. S. D. Wilcock et al., pp. 243–266, AGU, Washington, D. C.
- Von Damm, K. L., et al. (1985), Chemistry of submarine hydrothermal solutions at 21°N, East Pacific Rise, *Geochim. Cosmochim. Acta*, 49, 2197–2220, doi:10.1016/0016-7037(85)90222-4.
- Watanabe, K., and T. Kajimura (1993), Topography, geology and hydrothermal deposits at Suiyo seamount (in Japanese with English abstract), *Proc. JAMSTEC Symp. Deep Sea Res.*, 9, 77–89.
- Watanabe, K., and T. Kajimura (1994), The hydrothermal mineralization at Suiyo seamount, in the Izu-Ogasawara Arc (in Japanese with English abstract), *J. Soc. Resour. Geol.*, 44, 133–140.
- Watanabe, K., et al. (1994), Survey method about the submarine volcano and its sea-floor hydrothermal ore deposit—A example of Suiyo seamount in the Izu-Ogasawara arc with the submersible “Shinkai2000” (in Japanese with English abstract), *J. Jpn. Soc. Mar. Surv. Tech.*, 6, 29–44.
- Welhan, J. A. (1988), Origins of methane in hydrothermal systems, *Chem. Geol.*, 71, 183–198, doi:10.1016/0009-2541(88)90114-3.
- Welhan, J. A., and H. Craig (1979), Methane and hydrogen in East Pacific Rise hydrothermal fluids, *Geophys. Res. Lett.*, 6, 829–831, doi:10.1029/GL006i011p00829.
- Welhan, J. A., and H. Craig (1983), Methane, hydrogen, helium in hydrothermal fluids at 21°N on the East Pacific Rise, in *Hydrothermal Processes at Seafloor Spreading Centers*, edited by P. A. Rona et al., pp. 391–406, Plenum Press, New York.
- Whiticar, M. J., and E. Faber (1986), Methane oxidation in sediment and water column environments—Isotope evidence, *Org. Geochem.*, 10, 759–768, doi:10.1016/S0146-6380(86)80013-4.
- Yamagishi, A. (2005), Underground microbiospheres of the marine hydrothermal vent system, *J. Oceanogr.*, 14, 319–326.
- Yamanaka, T., et al. (2001), Biogeochemical study of total fatty acid in surface sediment of Suiyo hydrothermal system, paper presented at Japan Earth and Planetary Science Joint Meeting, Geod. Soc. of Jpn., Tokyo.
- Yuasa, M. (1985), Sofugan Tectonic Line, A new tectonic boundary separating Northern and southern parts of the Ogasawara (Bonin) Arc, Northwest Pacific, in *Formation of Active Ocean Margins*, edited by N. Nasu et al., pp. 483–496, Terra Sci., Tokyo.
- Zyakun, A. M., et al. (1979), Fractionation of stable carbon isotopes in methane during microbiological oxidation, *Geochem. Int.*, 16, 164–169.
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