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Origin and fate of deep-sea seeping methane bubbles at Kuroshima Knoll, Ryukyu forearc region, Japan

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Seafloor seeping methane bubbles were successfully sampled on the summit area of the Kuroshima Knoll (depth of *ca.* 640 m) using the gas-tight sampler WHATS attached to the *Shinkai 2000*. To evaluate the origin of the bubbles and verify that the dissociation of methane hydrate was actually in progress, the chemical and isotopic composition of the samples were analyzed. The major component of the gas bubbles was methane (C₁) with traces of CO₂ (67 ± 16 ppmv) and helium (11 ± 1 ppmv; ⁴He/²⁰Ne = 320) having a moderate ³He/⁴He ratio (0.44R_a). C₁ was enriched relative to other hydrocarbons (C₁/(C₂+C₃) > 3000). The δ¹³C values for C₁ (−40.1‰_VPDB), C₂ (−28.3‰_VPDB), and C₅ (−28.0‰_VPDB) were similar to those of hydrocarbons produced by thermal decomposition of organic matter. The contribution of the mantle-derived ³He-enriched component in coexisting helium also supports thermogenic generation. On the other hand, the other light hydrocarbons showed an unusual ¹³C-enrichment in C₃ (−19.1‰_VPDB), iso-C₄ (−22.4‰_VPDB), and *n*-C₄ (−19.9‰_VPDB). C₃ and C₄, had been fractionated both chemically and isotopically through subsequent microbial destruction during the long storage from Miocene in the gas reservoir. In addition, the anaerobic oxidation of CH₄ within shallow sediments removed about 20% of CH₄, until seepage into ocean water column. Regarding the contribution of gases originating from hydrate dissociation to the bubbles, observed helium amount in the bubbles suggests that methane hydrate is considered to be a minor contributor to the bubbles, at least at present. Direct leakage of gases from deep reservoirs is a more plausible for the source. Regarding the fate of the hydrocarbons in the bubbles in the water column, all rising bubbles at Kuroshima Knoll dissolved within 140 m of the seafloor. After the dissolution, the plume spreads horizontally along with the surface of equal density in the water column, while the concentrations decrease through dilution by eddy diffusion, rather than by oxidation.

Keywords: thermogenic hydrocarbons, methane hydrates, carbon isotopes, helium isotopes, Kuroshima Knoll, microbial alteration, propane

INTRODUCTION

The contribution of methane (CH₄) to global warming is estimated to be about 20% (IPCC, 2007). Enormous quantities of carbon occur in marine sediments in

the form of CH₄ (Kvenvolden, 1988) and at least some CH₄ in marine sediments seeps into the ocean and thus into the atmosphere (Etiope and Milkov, 2004; Judd, 2004; Mau *et al.*, 2007). The mechanisms and fate of this seeping as well as its quantification are poorly constrained. In particular, CH₄ emitted from deep seafloor sources has been ignored to date in estimating atmospheric CH₄ budgets (Etiope and Milkov, 2004; Judd, 2004). Indeed, most CH₄ would be oxidized within bottom water if vertical eddy diffusion in the water column is the only process for transporting CH₄ upward (De Angelis *et al.*, 1993; Tsunogai *et al.*, 2000; Valentine *et al.*, 2001). The seafloor discharges of CH₄ bubbles, however, can efficiently transport CH₄ from water depths greater than 2000 m to surface (Merewether *et al.*, 1985; Paull *et al.*, 1995; Heeschen *et al.*, 2003; Sauter *et al.*, 2006). In addition, episodic huge discharges of CH₄ due to massive decomposition of

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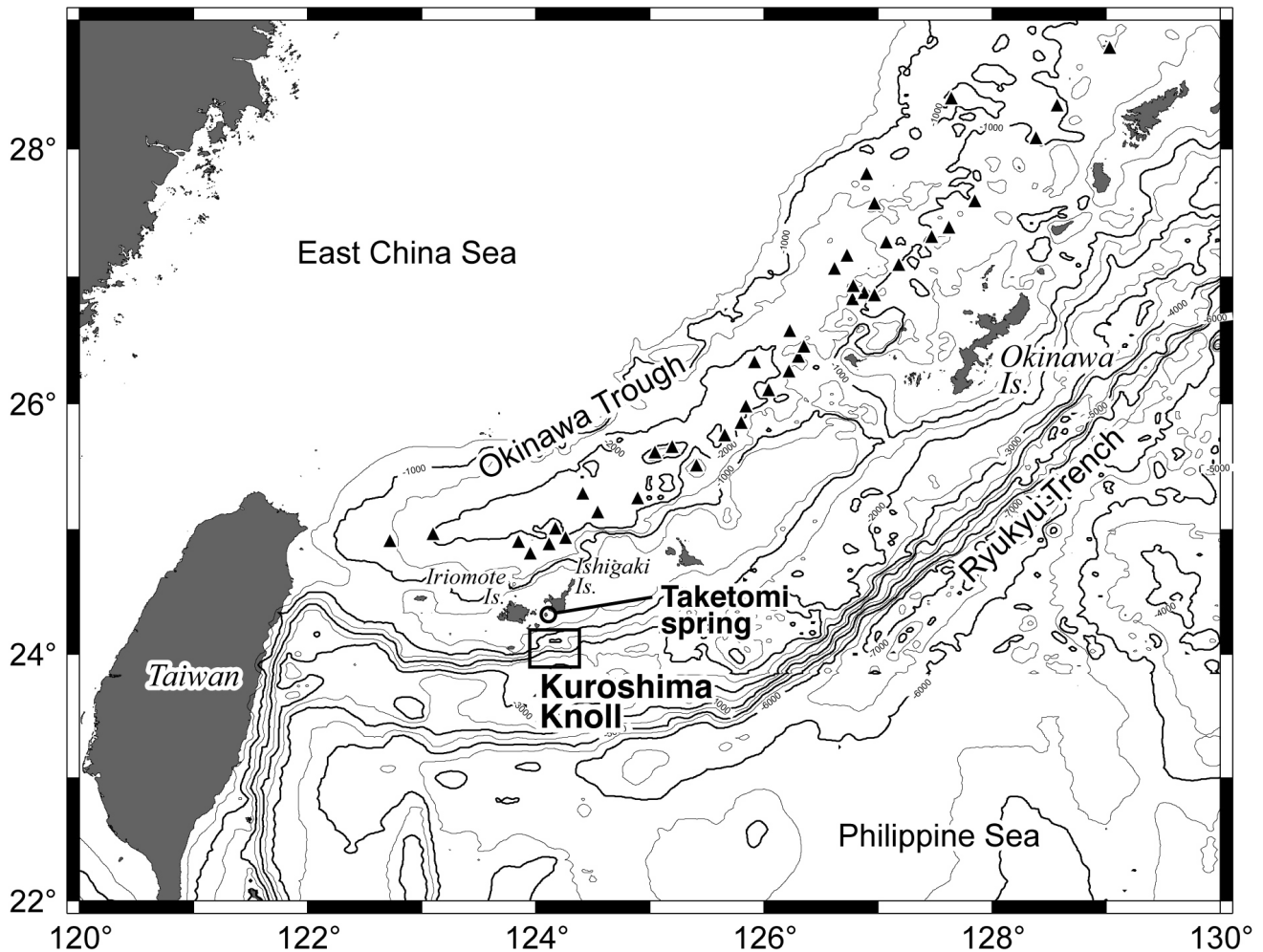


Fig. 1. Locations of Kuroshima Knoll (square), Taketomi spring (circle), and submarine Quaternary volcanic features (▲) (modified from Machiyama *et al.*, 2001). The detailed bottom topography in the square (the area around Kuroshima Knoll) is presented in Fig. 2.

seafloor methane hydrates have been suggested as possible causes of sudden climate changes in the geological past (MacDonald, 1990; Dickens *et al.*, 1997; Bains *et al.*, 1999; Svensen *et al.*, 2004). Besides to having environmental significance, seafloor CH₄ bubbles might also be a possible geohazard and have a resource significance (Sills and Wheeler, 1992).

In 1997, gas bubbling with large-scale chemosynthetic communities and carbonate crusts including chimney-like structures were found on top of Kuroshima Knoll (depth of *ca.* 640 m), located in the forearc region of the Ryukyu island arc (Matsumoto *et al.*, 1998) (Figs. 1 and 2). Subsequent studies revealed that the bubbles were composed mostly of CH₄ (Machiyama *et al.*, 2001). Based on both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the carbonate crusts, Takeuchi *et al.* (2007) concluded that methane hydrate layers had developed under the area. The CH₄-rich fluid derived from

the partial dissociation of the methane hydrate produced both the bubbles and the carbonate crusts (Takeuchi *et al.*, 2007). They assumed that subtle changes in the marine environment were responsible for the dissociation of methane hydrate. If this assumption is correct, the area is an important field for studying the dissociation process for seafloor methane hydrate and discussing future possible changes in seafloor CH₄ flux to the ocean and atmosphere. However, we had little evidence for the existence of methane hydrate beneath the seafloor of the knoll and for the progression of dissociation. In addition, the influence of CH₄ discharge on the surrounding marine environment was poorly constrained.

In this study, we collected samples of both gas bubbles and bottom fluid from the active gas seeping site of Kuroshima Knoll (Fig. 2) using the WHATS gas-tight sampler (Water and Hydrothermal-fluid *Atsuryoku* Tight

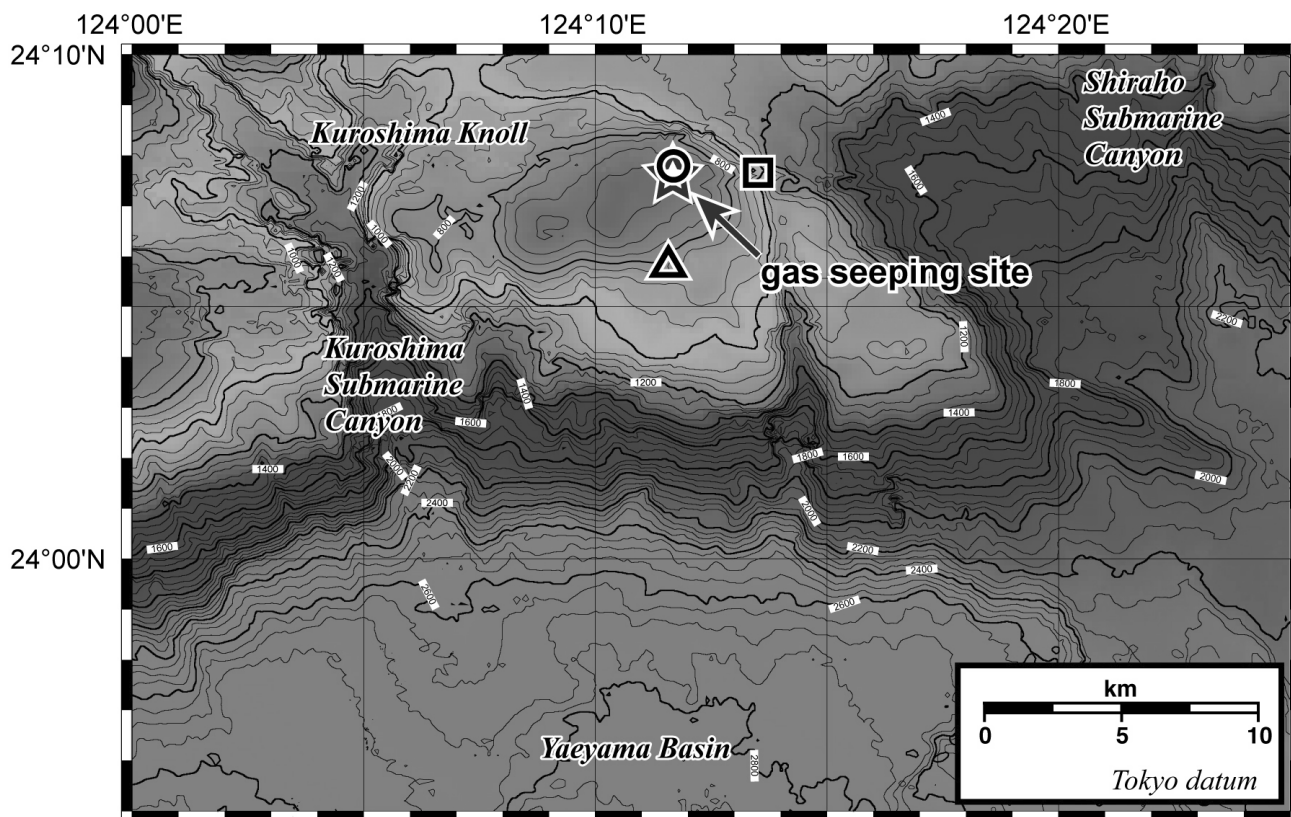


Fig. 2. The bottom topography of Kuroshima Knoll and the locations of the gas seeping site (☆) and the sampling stations for hydrocasts (○, Stn. 01-0; □, Stn. 01-1; △, Stn. 01-2) (modified from Machiyama *et al.*, 2001).

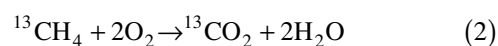
Sampler) (Tsunogai *et al.*, 2003; Saegusa *et al.*, 2006) attached to the vehicle *Shinkai 2000* to determine the origin of CH₄ and discuss the possible sub-seafloor dissociation of methane hydrate using geochemical tracers. Knowledge of the origin of CH₄ (i.e., biogenic from acetate fermentation or CO₂ reduction, thermogenic, inorganic) and the other coexisting gases would provide information on the processes and environments responsible for their generation. Thus seepage gases could be used as a tracer for sub-seafloor hydrocarbon reservoirs and of secondary processes, such as migration through geodynamic processes (Price and Schoell, 1995; Etiope *et al.*, 2009), microbial alteration (Head *et al.*, 2003; Kinnaman *et al.*, 2007), and gas hydrate formation/decomposition (Sassen *et al.*, 2001; Milkov *et al.*, 2004).

Besides the gas bubbles and seeping fluid at the seafloor, the effluent plume in the water column was sampled in and around the knoll to evaluate the distribution and the fate of the CH₄ bubbles in the ocean water column, with special emphasis on the influence of aerobic CH₄ oxidation on the fate of CH₄ in the water column. The distribution of the concentration and stable carbon isotopic composition ($\delta^{13}\text{C}$) of CH₄ in a CH₄-enriched

plume are good tracers for determining the fate of CH₄ in a water column, especially for quantitatively determining the progress of microbial oxidation (Tsunogai *et al.*, 1998, 2000, 2005; Nishimura *et al.*, 1999). Indeed, CH₄ oxidation consumes ¹²CH₄ preferentially, leaving the residual CH₄ enriched in ¹³CH₄ (Silverman and Oyama, 1968), while $\delta^{13}\text{C}_{\text{C1}}$ does not vary through eddy diffusion. In addition, it was shown that the kinetic isotope effect (KIE) α is around 1.005 at the bottom of the water column (Tsunogai *et al.*, 2000). α is defined as the ratio of the relative reaction rates of molecules containing different isotopes:

$$\alpha = k_{12} / k_{13}, \quad (1)$$

where k_{13} and k_{12} are the respective rate constants for



and

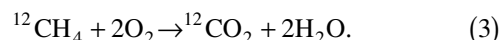


Table 1. Chemical and isotopic compositions of the gas bubble samples, together with their sampling locations

Sample ID	Sampling location		Depth m	CH ₄ %	$\delta^{13}\text{C}_{\text{C1}}$ ‰ _{V-PDB}	C ₂ H ₆ ppm	$\delta^{13}\text{C}_{\text{C2}}$ ‰ _{V-PDB}	C ₃ H ₈ ppm	$\delta^{13}\text{C}_{\text{C3}}$ ‰ _{V-PDB}	CO ₂ ppm	⁴ He ppm
	Lat. (N)	Long. (E)									
<i>Samples taken by WHATS</i>											
D1365 W1	24°07.812	124°11.539	635	105 ± 5	-39.5	294	-28.3	11	-17.6	71	11.2
D1365 W2	24°07.812	124°11.539	635	—	—	—	—	—	—	—	11.3
D1365 W3	24°07.833	124°11.536	637	104 ± 5	-40.5	279	-28.3	31	-20.7	82	13.1
D1367 W2	24°07.812	124°11.535	635	101 ± 5	-40.3	317	—	—	—	49	9.57
<i>Samples taken by M-type sampler</i>											
D1357 M2	24°07.800	124°11.536	638	19 ± 5	-41.8	725	—	—	—	151	—
D1364 M1	24°07.820	124°11.570	642	17 ± 5	-43.3	—	—	—	—	821	—

Sample ID	²⁰ Ne	³ He/ ⁴ He	iso-C ₄ H ₁₀	$\delta^{13}\text{C}_{\text{isoC4}}$	<i>n</i> -C ₄ H ₁₀	$\delta^{13}\text{C}_{\text{nC4}}$	iso-C ₅ H ₁₂	$\delta^{13}\text{C}_{\text{isoC5}}$	<i>n</i> -C ₅ H ₁₂	$\delta^{13}\text{C}_{\text{nC5}}$
	ppb	/R _a	ppm	‰ _{V-PDB}	ppm	‰ _{V-PDB}	ppb	‰ _{V-PDB}	ppb	‰ _{V-PDB}
<i>Samples taken by WHATS</i>										
D1365 W1	21	0.436	0.9	-22.3	0.6	-18.5	366	-28.3	22	-17.9
D1365 W2	32	0.449	—	—	—	—	—	—	—	—
D1365 W3	36	0.449	2.2	-22.6	1.4	-21.4	424	-28.8	19	-15.9
D1367 W2	640	0.454	—	—	—	—	—	—	—	—
<i>Samples taken by M-type sampler</i>										
D1357 M2	—	—	—	—	—	—	—	—	—	—
D1364 M1	—	—	—	—	—	—	—	—	—	—

—: not determined.

Using the distribution of $\delta^{13}\text{C}_{\text{C1}}$ in a CH₄-enriched plume and the determined KIE, we traced the progress of CH₄ oxidation in the CH₄-enriched plume and determined whether the main cause of the CH₄ decrease in the water column was eddy diffusion (dilution) or oxidation.

GEOLOGICAL BACKGROUND

The Kuroshima Knoll (Figs. 1 and 2) is about 26 km south of the island of Ishigaki in the forearc region of the southern Ryukyu arc. The knoll has a flat table for its top at a depth of 1000 to 630 m, while the foot part reaches to a depth of 2400 m. On the basis of geological observations on the mudstones, sandstones, and mud cores recovered from the knoll, Cenozoic sediments, such as the Pliocene Shimajiri Group, are assumed to cover most of its surface (Matsumoto *et al.*, 1998; Takeuchi *et al.*, 2007).

The Cenozoic of the Ryukyu arc is divided into the lower Eocene Wano and Kayo Formations, upper Eocene Miyara Group, lower Miocene Yaeyama Group, Sonai conglomerate of unknown age, middle Miocene Takarajima Group, Pliocene Shimajiri Group, middle to upper Pleistocene Ryukyu Group and Holocene coastal and terrestrial deposits (Nakagawa, 1983). The region of the southern Ryukyu arc, including the islands of Ishigaki

and Iriomote (Fig. 1), can be characterized by volcanic activity in the Miocene, such as the 21 Ma old granitic intrusions in Ishigaki (Kawano and Ueda, 1966) and volcanic breccia, tuff breccia, and lava flows along with small dikes in Iriomote (Monden, 1968; Tiba and Saito, 1974). On the other hand, the Pliocene Shimajiri Group consists mostly of fossiliferous sandstone and siltstone. Except for the backarc region (Fig. 1), no volcanic activity has been found in the southern Ryukyu forearc area from the Pliocene to the present (Nakagawa, 1983).

Large-scale colonies of *Calyptogena kawamurai* and *Bathymodiolus* spp. (both living and dead) (Okutani *et al.*, 2004), calcareous crusts, many gravels of sand chimneys, and emissions of gas bubbles have been found on the seafloor in diving surveys (Matsumoto *et al.*, 1998; Machiyama *et al.*, 2001). Active microbial oxidation of CH₄ (both aerobic and anaerobic) has been found in sediments just beneath the seafloor, especially in areas that can be characterized by slow, diffusive seeping of CH₄ gas or CH₄-enriched fluid (Inagaki *et al.*, 2004).

On the basis of the ¹³C-depleted carbon isotopic ratios (from -4.1 to -43.2‰_{PDB}) and the ¹⁸O-enrichment (from +2.5 to +7.9‰_{PDB}) of the carbonates, Takeuchi *et al.* (2007) proposed that methane hydrate layers could have developed under Kuroshima Knoll. The CH₄-rich,

Table 2. Contents and stable carbon isotopic compositions of CH₄ in the bottom fluid samples, together with their sampling locations, depths, and heights from the seafloor

Sample ID	Latitude (N)	Longitude (E)	Depth* (m)	Height** (m)	CH ₄ (μmol/kg)	δ ¹³ C _{Cl} (‰ _{V-PDB})
<i>Samples taken by WHATS</i>						
D1355W2	24°07.798'	124°11.547'	638	<0.3	52	-33.3
D1355W4	24°07.798'	124°11.565'	641	<0.3	19	-37.0
D1356W2	24°07.805'	124°11.540'	637	<0.3	2.0 × 10 ⁴	-33.0
D1356W3	24°07.831'	124°11.553'	638	<0.3	1.5 × 10 ⁴	-37.5
D1357W1	24°07.800'	124°11.536'	638	<0.3	662	-35.0
D1357W3	24°07.800'	124°11.536'	638	<0.3	1.4 × 10 ⁴	-37.8
D1357W4	24°07.810'	124°11.571'	641	<0.3	2.3 × 10 ³	-38.3
D1360W2	24°07.811'	124°11.134'	643	<0.3	1.1	-39.8
D1363W2	24°07.805'	124°11.535'	636	<0.3	601	-33.7
D1363W4	24°07.809'	124°11.532'	635	<0.3	55	-39.8
D1364W2	24°07.825'	124°11.561'	642	<0.3	13	-31.3
D1364W4	24°07.794'	124°11.129'	642	<0.3	1.5	-40.4
D1366W2	24°07.814'	124°11.346'	635	<0.3	3.3 × 10 ³	-32.2
D1368W4	24°07.815'	124°11.352'	640	<0.3	165	-40.3
<i>Samples taken by Niskin</i>						
D1355N2	24°07.798'	124°11.547'	638	1.5	12	-38.2
D1356N1	24°07.797'	124°11.553'	638	1.5	1.3	-37.4
D1360N1	24°07.772'	124°11.527'	637	1.5	0.9	-38.7
D1360N2	24°07.814'	124°11.535'	636	1.5	0.9	-38.7
D1363N2	24°07.805'	124°11.535'	636	1.5	63	-36.1

*The seafloor depth.

**Approximate sampling height from the seafloor.

¹⁸O-enriched fluids likely derived from the partial dissociation of the methane hydrate produced both the bubbles and carbonates on the seafloor (Takeuchi *et al.*, 2007). Because methane hydrate is theoretically stable in the top 70 m layer of the knoll, Takeuchi *et al.* (2007) assumed some subtle changes in the marine environment as possible cause of the dissociation of methane hydrate. Other than the δ¹⁸O values of the carbonates, however, there is little evidence for the existence of methane hydrate beneath the seafloor of the knoll.

SAMPLING

Gas bubbles and bottom fluid (seawater taken just above the seafloor of the gas seepage site) were collected by the human occupied vehicle *Shinkai 2000* of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) during the NT02-08 cruise in June 2002, using the WHATS (Tsunogai *et al.*, 2003; Saegusa *et al.*, 2006). The sampler comprised four 150 cm³ stainless steel sample cylinders, eight ball valves, a motor-driven arm, a rail, a peristaltic pump, and a flexible Teflon tube connected to the inlet. The motor-driven arm on the rail is

used to open one of the four cylinders. By pumping out seawater that had filled the cylinder, we introduced sample fluid/gas from the inlet into the cylinder. After completely replacing the seawater with sample, the valves at both ends of the cylinder are tightly closed by moving the motor-driven arm again. At this stage, the next cylinder is ready for sampling. The entire operation was conducted inside the submersible cabin.

In dives for sampling bottom fluid, a titanium inlet tube was used for the sample inlet, while a specially designed funnel-shaped inlet was used for sampling gas bubbles (Saegusa *et al.*, 2006). Because we could not recognize fluid seeping from Kuroshima Knoll, we took seawater less than 30 cm above the seafloor, in areas where active fluid seeping was anticipated, such as areas covered by dense *Bathymodiolus* mussels. A temperature probe was attached to the titanium inlet tube to measure the temperature of the incoming bottom fluid. The bottom fluid samples, however, showed temperatures close to that of the ambient seawater (about 8.0°C), so that we could not clarify the difference from the ambient seawater. In addition to samples taken by the WHATS, samples of gas bubbles were collected by non-gas-tight, cylinder

samplers made of acrylic resin (noted as M-type samplers; Sakai *et al.*, 1990; Gamo *et al.*, 1991) and samples of bottom fluid were collected by Niskin samplers. The Niskin samplers were attached to the *Shinkai 2000* and operated at the heights of 1.5 m from the seafloor. All the samples of gas bubbles and bottom fluid are listed in Tables 1 and 2, respectively.

The collected samples were immediately subsampled into several kinds of bottles in the onboard laboratory of the mother ship *Natsushima*. For the determinations of the major components in the gas bubbles (N_2 , O_2 , H_2S , CH_4 , CO_2 , CO , and non-methane hydrocarbons), each sample in a stainless steel cylinder was expanded into a container (50 cm^3) made of stainless steel with a ball valve at each end (Xu *et al.*, 1995) and stored under inner pressures higher than atmospheric pressure. In addition, for the determination of noble gases in the bubbles, an aliquot of gas sample was expanded into a pre-evacuated line and subsampled in a lead glass bottle (50 cm^3) under a pressure slightly higher than atmospheric pressure.

For measurements of dissolved gas components, especially CH_4 , in the samples of bottom fluid, each fluid sample taken in a stainless steel cylinder was first transferred into a pre-evacuated line (*ca.* $1,500\text{ cm}^3$ volume) and mixed with both mercury chloride (to remove dissolved H_2S as HgS) and phosphoric acid (to reduce the pH of the fluid to less than 1). After waiting for gas exchange equilibrium between the gas and the liquid phase within the line, the pressure was measured for the gas phase and part of it was subsampled into a container (50 cm^3) made of stainless steel with a bellows valve at each end.

Each sample of gas bubbles collected by the M-type bottom sampler was subsampled into a glass bottle (200 cm^3) with a stopcock at each end through the downward displacement of pure water. Each sample of ambient bottom seawater collected by the Niskin sampler was slowly transferred into 65 cm^3 glass vials, for the determination of the concentration and $\delta^{13}C$ of CH_4 (Tsunogai *et al.*, 2000). After approximately 3-fold volume overflow to prevent air contamination, 0.5 cm^3 of saturated $HgCl_2$ solution (6 wt%) was slowly added to each vial as a preservative. The vial was then sealed with a gray butyl rubber stopper and stored in the dark at $4^\circ C$ until analysis.

Samples of effluent plume water supplied from the gas seeping field were taken in around the knoll on 26 Oct. 2005 at stations 01-0, 01-1, and 01-2 (Fig. 2) by the R/V *Tansei-maru* (JAMSTEC). While recording serial hydrographic data using a CTD system (Falmouth Scientific, Inc.), the samples of effluent plume were collected with a 24-port carousel multisampler with 5 L Niskin bottles (General Oceanic Inc.). To analyze the concentration and $\delta^{13}C$ of CH_4 , a water sample was slowly transferred into a 120 cm^3 glass vial. After approximately 3-

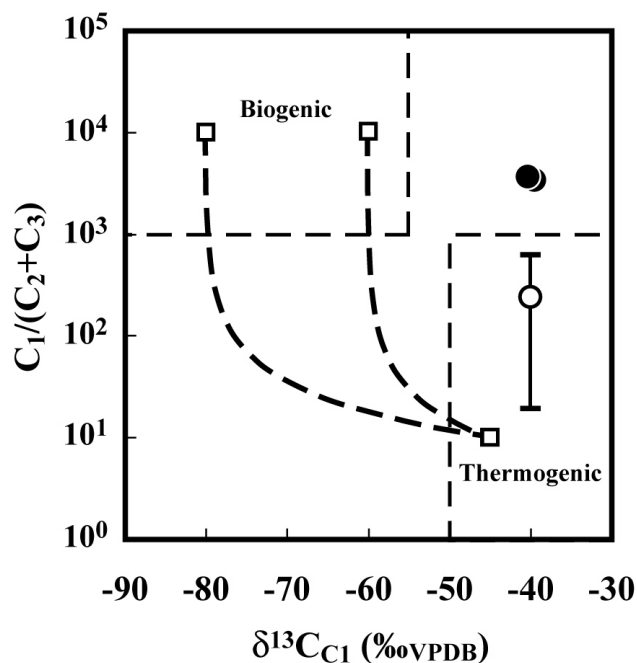


Fig. 3. The $C_1/(C_2+C_3)$ ratios of the Kuroshima bubble samples (●) plotted as a function of their carbon isotopic compositions of methane ($\delta^{13}C_{C_1}$), together with those prior to the microbial alteration during storage in gas reservoir estimated in this study (○; see the main text for details). Approximate source regions of thermogenic hydrocarbons and biogenic hydrocarbons are also plotted, together with a line for hypothetical mixing line between them with representative compositions (see the main text for details) (modified from Bernard *et al.*, 1977).

fold volume overflow to prevent air contamination, 1 cm^3 of saturated $HgCl_2$ solution (6 wt%) was slowly added as a preservative. To minimize air contamination with the addition of $HgCl_2$ solution, the solution was degassed immediately prior to its addition. The vial was then sealed with a butyl rubber stopper and stored in the dark at $4^\circ C$ until analysis. The actual sampling depth of each Niskin bottle was verified by analyzing the salinity and dissolved oxygen concentration for each bottle using standard methods (Carpenter, 1965).

ANALYTICAL METHODS

The concentrations and $\delta^{13}C$ values of CO_2 , CH_4 , CO and non-methane hydrocarbons in the gas samples (including the gas phase samples extracted from the bottom fluid samples) were determined by continuous-flow isotope ratio mass spectrometry (CF-IRMS) at Hokkaido University (Tsunogai *et al.*, 1999, 2000, 2002b; Ijiri *et al.*, 2003; Komatsu *et al.*, 2005). The concentrations of N_2 , O_2 , H_2 , and H_2S in the gas samples were determined

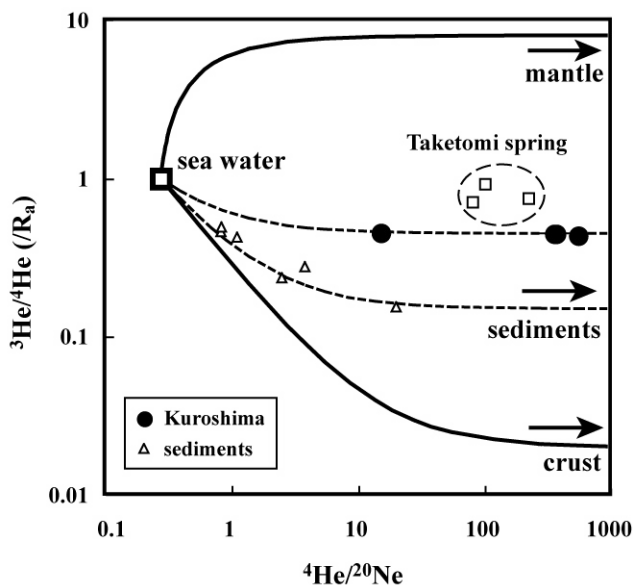


Fig. 4. The helium isotopic compositions of the bubble samples plotted as a function of their $^4\text{He}/^{20}\text{Ne}$ ratios (●), together with those of the mantle (Lupton, 1983), crust (Lupton, 1983; Mamyrin and Tolstikhin, 1984), pore gases in deep sediments taken around the Japan islands during the Deep Sea Drilling Project Leg 87 (Sano and Wakita, 1987), and seafloor seeping gases at the Taketomi spring (Oomori *et al.*, 1993). Hypothetical mixing lines between the seawater and each end-member (mantle, crust, sediments, and Kuroshima bubbles) are also plotted as well (see the main text for details).

through conventional methods (Sakai *et al.*, 1976). The helium and neon contents and $^3\text{He}/^4\text{He}$ ratio in the gas samples were measured with a modified-VG5400 (MS-III) noble gas mass spectrometer in the University of Tokyo with an accuracy better than 4%, following the procedure described by Aka *et al.* (2001), except for using the interlaboratory helium standard (HESJ, Matsuda *et al.*, 2002) for the standard material of the $^3\text{He}/^4\text{He}$ ratio.

RESULTS

Gas bubbles

Analytical results for the bubble samples are listed in Table 1 and presented in Figs. 3, 4, and 5. The samples of gas bubbles taken by the WHATS (D1365W1, D1365W2, W1365W3, and D1367W2 in Table 1) were composed mostly of CH_4 (C_1) having uniform $\delta^{13}\text{C}$ values around -40‰ while the concentrations of N_2 , O_2 , and H_2S were small, less than 1 vol%. Only trace amounts of CO_2 and CO were detected; less than 100 ppmv and less than 3 ppmv respectively. Non-methane hydrocarbons were also present in trace amounts, having concentrations of 300 ± 20 ppmv for ethane (C_2), 21 ± 14 ppmv for pro-

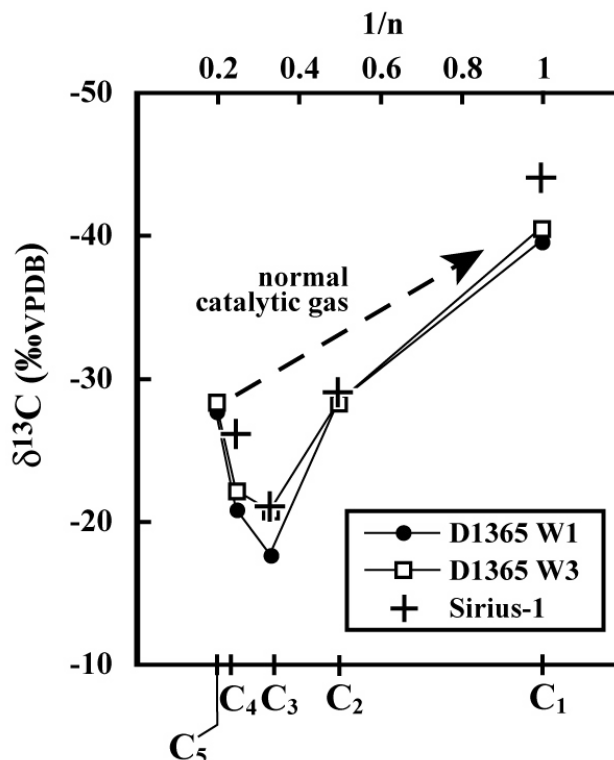


Fig. 5. The carbon isotopic compositions of hydrocarbons in the bubble samples taken at Kuroshima Knoll (●: D1365W1; □: D1367W2) plotted as functions of the reciprocal of carbon number, together with compositions for the Sirius-1 Exmouth Plateau, offshore Australia, where anomalous carbon isotopic compositions have been reported (James and Burns, 1984).

pane (C_3), 1.6 ± 0.9 ppmv for isobutane (iso- C_4), 1.0 ± 0.5 ppmv for *n*-butane (*n*- C_4), 0.4 ± 0.04 ppmv for isopentane (iso- C_5), and 0.02 ± 0.002 ppmv for *n*-pentane (*n*- C_5). The $\text{C}_1/(\text{C}_2+\text{C}_3)$ ratio is greater than 3400 (Fig. 3) and the C_2/C_3 ratio is greater than 9. The $\delta^{13}\text{C}$ values of the non-methane hydrocarbons were characterized by ^{13}C -enrichment relative to C_1 , which gave -28.3‰ for C_2 , $-19.1 \pm 2.2\text{‰}$ for C_3 , $-22.4 \pm 0.2\text{‰}$ for *n*- C_4 , $-19.9 \pm 2.0\text{‰}$ for iso- C_4 , $-28.5 \pm 0.4\text{‰}$ for *n*- C_5 , and $-16.9 \pm 1.5\text{‰}$ for iso- C_5 (Fig. 5). Noble gases in the samples were characterized by both helium enrichment (9.6 to 13.1 ppmv) and high $^4\text{He}/^{20}\text{Ne}$ ratios (from 15 to 545) in comparison with values for air, indicating that no air contamination or fractionation affected bubble samples taken by the WHATS. Thus the concentration and isotopic composition of gases represented those of the bubbles on the seafloor. On the other hand, the gas samples taken by the M-type sampler (D1357M2 and D1364M1 in Table 1) were rather different from those taken by the WHATS, having concentrations for C_1 , N_2 , and O_2 of 20 vol%, 55

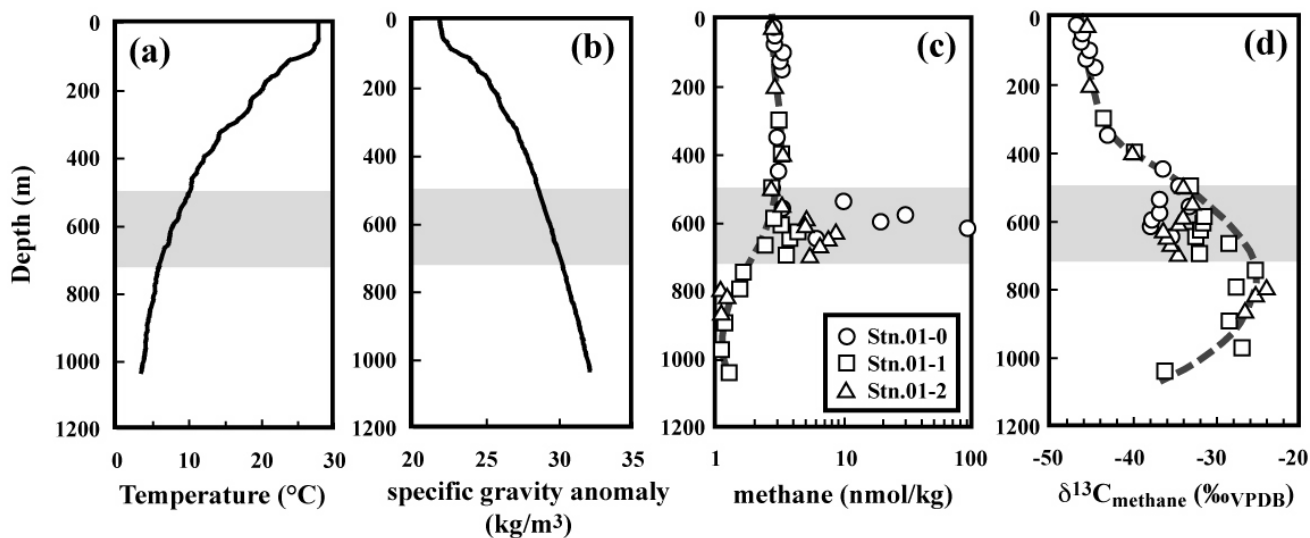


Fig. 6. Depth profiles of (a) temperature, (b) potential density (the specific gravity anomaly), (c) methane concentration, and (d) carbon isotopic composition of methane ($\delta^{13}C_{CI}$) in the water columns around the Kuroshima Knoll. The symbols in (c) and (d) are the same as those in Fig. 2. The shaded zone corresponds to the depths where the effluent plume had been detected. The dotted lines in (c) and (d) denote the background profiles.

vol% and 25 vol%, respectively. We concluded that air had significantly contaminated the samples, presumably during the transportation from the seafloor to the surface. Thus, we do not consider them in the discussions that follow. To determine the concentration and isotopic composition of a gas sample taken from a deep seafloor, the sample should be thus kept gas-tight during transportation.

Bottom fluid

Analytical results for the bottom fluid samples, which might represent a mixture of bottom seawater and seeping material in the knoll, are presented in Table 2. They show significant enrichment in CH_4 , especially for samples taken just above the seafloor (with sampling heights less than 0.3 m from the seafloor) by the WHATS. The observed concentration of CH_4 almost corresponds to the concentration under a saturation condition of about $10^5 \mu\text{mol/kg}$ for the pressure and the temperature at the seafloor depth (65 bar and 8°C , respectively). This was consistent with the seafloor observations of the gas bubbles, which consisted mostly of CH_4 .

Except for CH_4 , however, the major dissolved components (Na^+ , K^+ , Mg^{2+} , Cl^- , and SO_4^{2-}) in the bottom fluid samples showed neither enrichment nor depletion in comparison with seawater. If the site would be an active seeping site of some liquid material from deep sub-seafloor, it is difficult to assume such liquid having little anomalies in the major chemical components compared to seawater. The seeping material seems to be limited to

gas bubbles and liquid a few amount in the knoll. That is, simple upward migration of the gases from sub-seafloor gas reservoir and mixing into bottom seawater and/or shallow pore water might be more probable for the mechanism to explain the CH_4 -enrichment in the bottom fluid samples.

Effluent plume

We found clear enrichment of CH_4 in the water column at depths between 536 m and 644 m, the potential densities (expressed as specific gravity anomalies) being almost uniform at $28\text{--}30 \text{ kg/m}^3$ (Fig. 6). On the other hand, we did not find enrichment of CH_4 at depths less than 500 m. The maximum enrichment was 82.0 nmol/kg at a depth of 615 m for station 01-0 (bottom depth of 640 m), located just above the gas seeping site (Fig. 2), while the background level was around 1 nmol/kg at that depth (Fig. 6). In addition, we found smaller but clear anomalies at depths of 600–630 m for stations 01-1 and 01-2, both of which were about 3 km horizontally from the gas seeping site.

The depths of the seafloor at stations 01-1 and 01-2 were *ca.* 1050 and 870 m, respectively and thus it is unlikely that the vertical transport of CH_4 (or CH_4 -enriched fluid) emitted from adjacent seafloor was the cause of CH_4 enrichment at stations 01-1 and 01-2. The lateral transport of the CH_4 plume from station 01-0 (gas seeping site) is more plausible.

The vertical transport of CH_4 in station 01-0 from the adjacent gas seeping site was limited to heights of less

than 140 m from the seafloor, probably owing to complete dissolution of the gas bubbles to that height. After dissolution, the plume spread horizontally along with the surface of equal density of the plume, while the concentrations reduced through either dilution or oxidation in the oxic, low-CH₄ water column.

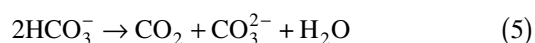
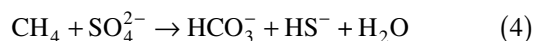
DISCUSSION

Initial generation of hydrocarbons in the bubbles

Both chemical and isotopic compositions of gaseous alkanes (C₁–C₅) are useful for inferring the origin of hydrocarbons. Specifically, we can differentiate thermogenic hydrocarbons (generated by thermal degradation of organic matter) from biogenic hydrocarbons (generated by microbial degradation of organic matter) by plotting the stable carbon isotopic compositions of CH₄ (δ¹³C_{C1}) vs. the light gas composition (C₁/(C₂+C₃)) on a widely used conventional diagram (Bernard *et al.*, 1977). The application of this diagram in the identification of surface gas seepage, however, provides misleading information for gases that underwent later alteration, such as oxidation of biogenic gas or molecular separation during migration (Tsunogai *et al.*, 2002a).

In Fig. 3, the C₁/(C₂+C₃) ratio of the Kuroshima bubble samples is plotted as a function of δ¹³C for CH₄ (δ¹³C_{C1}). Approximate source regions of both thermogenic and biogenic hydrocarbons are also plotted together with a hypothetical mixing line between them having respective representative compositions: δ¹³C_{C1} = –45‰_{VPDB} and C₁/(C₂+C₃) = 10 for thermogenic hydrocarbons and δ¹³C_{C1} = –80‰_{VPDB} or –60‰_{VPDB} and C₁/(C₂+C₃) = 10⁴ for biogenic hydrocarbons (Bernard *et al.*, 1977). While the high C₁/(C₂+C₃) ratios suggest microbial generation, the ¹³C-enriched δ¹³C values of CH₄ (–40.1 ± 0.5‰_{VPDB}) suggest thermogenesis. It is difficult to explain this contradiction through mixing between microbial and thermogenic hydrocarbons, as schematically shown by the theoretical mixing line. Rather, there was significant secondary alteration subsequent to generation.

One possible secondary process is the δ¹³C isotopic fractionation due to microbial oxidation of CH₄ under an aerobic/anaerobic condition. Tsunogai *et al.* (2002a) reported up to 50‰ ¹³C-enrichment in residual CH₄ through anaerobic microbial oxidation within surface marine sediments. The carbonate crusts including chimney-like structures found in the knoll also suggest anaerobic oxidation of CH₄ and subsequent carbonate precipitation following the reactions.



Anaerobic oxidation of CH₄, however, results in the reduction of the C₁/(C₂+C₃) ratio in most cases (Whiticar *et al.*, 1986), and thus it is difficult to explain the observed values. Furthermore, the ¹³C-enrichment up to 20‰ in δ¹³C₁ requires more than 90% oxidation of CH₄. In case of anaerobic oxidation of CH₄, while half of oxidized carbon precipitate as CaCO₃, the rest of oxidized carbon should remain in bubbles as CO₂ through the reaction (5), under steady state condition. Because the bubbles contain little CO₂ relative to CH₄ (Table 1), such enormous oxidation in the bubbles is unlikely.

An alternative possible process is the molecular fractionation of hydrocarbons generated originally through the thermogenic process. That is, the removal of heavier hydrocarbons from thermogenic hydrocarbons could explain the observed contradiction. Post-genesis CH₄ enrichment through molecular fractionation is ubiquitous for natural hydrocarbons (Nagy, 1960; Price and Schoell, 1995; Mango, 2001; Snowdon, 2001). There is almost one order of magnitude less C₃ than C₂ (C₂/C₃ > 9) in the bubbles which suggests some molecular fractionation is responsible for the C₁-enrichment in the hydrocarbons. Furthermore, the ¹³C-enrichment of C₃ (–19.1‰_{VPDB}), iso-C₄ (–22.4‰_{VPDB}), and n-C₄ (–19.9‰_{VPDB}) in comparison with C₂ (–28.3‰_{VPDB}) and C₅ (n-C₅ + iso-C₅) (–28.0‰_{VPDB}) suggests significant secondary alteration for the hydrocarbons. Regarding thermogenic hydrocarbons, the δ¹³C value for each hydrocarbon reflects the integrated kinetic isotope effect during the cleavage of chemical bonds from parental material. This begins with long-chain organic matter and thus smaller carbon number molecules tend to exhibit lower δ¹³C, with there being a reverse correlation with the reciprocal of the carbon number (Chung *et al.*, 1988; Mango and Elrod, 1999; Du *et al.*, 2003) as shown by the dotted arrow in Fig. 5. The δ¹³C values of C₃ and n-C₄ in the bubbles are larger than the values expected for C₃ and n-C₄ produced from sedimentary organic matter (–20 to –30‰_{VPDB}) and also the value for coexisting C₅ (–28.0‰_{VPDB}) through thermogenic decomposition (Chung *et al.*, 1988; Mango and Elrod, 1999; Du *et al.*, 2003) or even microbial processes (Oremland *et al.*, 1988; Taylor *et al.*, 2000; Nakagawa *et al.*, 2003). Hence, removal processes for the heavier hydrocarbons accompanying substantial isotopic fractionation must be highly probable for the C₁-enrichment in the hydrocarbons in the bubbles.

One possible molecular and isotopic fractionation process is fractional crystallization during hydrate formation. Gas hydrate is an ice-like crystalline mineral in which hydrocarbons are held within rigid cages of water molecules. While the structure I is usually pure CH₄

(Kvenvolden, 1995), structure II and structure H include C₂–C₄ hydrocarbons as significant components (Sloan, 1998). The molecular distribution of the bubbles could be consistent with those subtracting the hydrocarbons forming the structure II/H hydrate from the original thermogenic hydrocarbons enriched in C₂–C₄ hydrocarbons, as a consequence of active gas hydrate crystallization during migration in the sub-seafloor. If such hydrate crystallization is significant in the knoll and accompanied by significant carbon isotopic fractionation, the residual bubbles would be CH₄-enriched relative to the C₂–C₄ hydrocarbons and the C₂–C₄ hydrocarbons would be ¹³C-enriched relative to the initial hydrocarbons. In previous field observations, however, little carbon isotopic fractionation was found during crystallization of gas hydrate, including C₂–C₄ hydrocarbons (Brooks *et al.*, 1986; Kennicutt *et al.*, 1988). It is difficult to explain the observed ¹³C-enrichment for C₃ and *n*-C₄ in the bubbles as a consequence of the fractional crystallization during hydrate formation, and thus we should assume alternative processes.

An alternative process for explaining both molecular and isotopic fractionations is the fractional decomposition of hydrocarbons through some sub-seafloor microbial activity. James and Burns (1984) found that C₃ and C₄ alkanes were significantly ¹³C-enriched compared with other hydrocarbons in deep subsurface hydrocarbon reservoirs, which they attributed to selective microbial consumption of C₃ and C₄ alkanes. In Fig. 5, ^δ¹³C values for the hydrocarbons in the bubbles are plotted as a function of the reciprocal of each carbon number, together with values for the Sirus-1 Exmouth Plateau, offshore Australia, for which significant secondary alteration has been suggested (James and Burns, 1984). The alteration would probably be due to anaerobic microorganisms (Perry, 1980; Stephens and Dalton, 1986; Ashraf *et al.*, 1994; Head *et al.*, 2003). The two sets of results coincide strikingly well. Regarding the microbial processes for these deep subsurface hydrocarbons, preferential degradation of C₃ followed by that of C₄ and then that of C₂ has also been noted (Boreham *et al.*, 2001) with particularly high levels of biodegradation in lower-temperature (<60°C) reservoirs (Wenger *et al.*, 2002). In addition, Strapoc *et al.* (2007) found kinetic isotope fractionation in propane presumably produced through anaerobic microbial consumption. The kinetic isotope fractionation during aerobic microbial consumption of C₂–C₄ hydrocarbons has been quantified in laboratory incubation experiments as well (Kinnaman *et al.*, 2007; Bouchard *et al.*, 2008). Thus, microbial consumption of C₃ (significantly), C₄ (moderately), and C₂ (partly) in thermogenic hydrocarbons could explain the observed molecular and isotopic fractionations of hydrocarbons.

To verify this hypothesis quantitatively, we estimated

the initial C₁/(C₂+C₃) (the C₁/(C₂+C₃) prior to the microbial alteration) by correcting the microbial alteration in the concentrations and ^δ¹³C values of C₂–C₄ hydrocarbons, assuming (1) the microbial alteration was limited to C₂–C₄ hydrocarbons, so that the observed concentrations and the ^δ¹³C values in the bubbles corresponds to those of the initial for methane (103 ± 3 vol% and –40.1‰) and C₅ (0.4 ppmv and –28.0‰), (2) the initial ^δ¹³C values of C₂, C₃, and C₄, were –33.0‰, –30.5‰, and –29.0‰, respectively, showing a reverse correlation with the reciprocal of carbon number in the initial C₁–C₅ hydrocarbons, (3) the KIE (–1000(1/α – 1)) in carbon isotopes of C₂ during the microbial alteration was 3 ± 1, which corresponds to the KIE during the atmospheric oxidation of C₂ (Tsunogai *et al.*, 1999), (4) the KIEs in carbon isotopes of C₃ and C₄ during the microbial alteration were 2 ± 0.7 and 1.5 ± 0.5, respectively, which corresponds to 2/3 and 2/4 of the KIE of C₂, due to the dilution effect of KIEs by unreacted carbon in a molecule (Tsunogai *et al.*, 1999). The assumed KIE for C₃ corresponds well to that determined by the field observation (Strapoc *et al.*, 2007).

Under these assumptions, we estimated the initial C₁/(C₂+C₃) from the concentrations and ^δ¹³C values of C₁–C₅ hydrocarbons in the bubbles, by correcting the microbial alteration applying the Rayleigh equation (7) (Coleman *et al.*, 1982) to each hydrocarbon.

$$\delta^{13}C_f - \delta^{13}C_0 = 1000(1/\alpha - 1)\ln(C_f/C_0), \quad (7)$$

where C₀ and C_f were the initial and final concentrations, respectively, and ^δ¹³C₀ and ^δ¹³C_f were the initial and final ^δ¹³C values, respectively. The estimated initial concentrations of C₂–C₄ hydrocarbons corresponds to the initial C₁/(C₂+C₃) of 235 (median), 19 (minimum) and 612 (maximum). All the initial C₁/(C₂+C₃) ratios correspond to that of the thermogenic hydrocarbons (Fig. 3).

In conclusion, microbial consumption of C₃ (significantly), C₄ (moderately), and C₂ (partly) in thermogenic hydrocarbons can explain the observed molecular and isotopic fractionations of hydrocarbons, by increasing the C₁/(C₂+C₃) and ^δ¹³C values for C₃ (significantly), C₄ (moderately), and C₂ (partly) of residual hydrocarbons.

Evidence for the contribution of geothermal heat

The thermogenic origin of the hydrocarbons is also supported by the ³He/⁴He ratio of coexisting helium. The contribution of primordial, ³He-enriched helium has been widely recognized in thermogenic natural gases in subduction zones, reflecting the contribution of mantle heat in converting organic matter to hydrocarbons thermogenically (Poreda *et al.*, 1988).

In Fig. 4, the measured ³He/⁴He ratios of helium in the bubble samples are plotted as a function of ⁴He/²⁰Ne

ratios (solid circles) together with those for the mantle ($8R_a$, where R_a denotes the $^3\text{He}/^4\text{He}$ ratio of air of 1.4×10^{-6}) (Lupton, 1983), continental crust (about $0.02R_a$) (Lupton, 1983; Mamyrin and Tolstikhin, 1984) and sediments (the pore gases in the sedimentary layer) around Japanese islands (Deep Sea Drilling Project sites 583D, 583F, and 584; Sano and Wakita, 1987) (open triangles). To exclude samples contaminated by air to a significant extent, the data for sediments are limited to sediments having $^4\text{He}/^{20}\text{Ne}$ ratios exceeding 0.5. In addition, bold lines for the theoretical mixing with seawater (air saturated seawater; $1R_a$) are presented for the mantle and crust. Similarly, the mixing with seawater is presented for both the sedimentary pore gases and Kuroshima bubbles by dotted lines. By extrapolating the dotted mixing lines to the region with higher $^4\text{He}/^{20}\text{Ne}$ ratios, we can obtain the endmember $^3\text{He}/^4\text{He}$ ratios for the bubbles and sediments as $0.44R_a$ and $0.15R_a$, respectively, through removing seawater contaminants from the samples, assuming all ^{20}Ne in the samples was derived from the seawater.

While the endmember $^3\text{He}/^4\text{He}$ ratio for the bubbles is lower than the ratios for the mantle or seawater, it is much higher than the ratio for the crust or sediments. It is difficult to explain the composition of Kuroshima bubbles merely by the *in situ* contribution of ^4He -enriched radiogenic helium in the crust/sediments. Although the mixing of the seawater components with the crust or sediments could produce $^3\text{He}/^4\text{He}$ ratios similar to those for the bubbles, it is impossible to explain the high $^4\text{He}/^{20}\text{Ne}$ ratio of the bubbles through the mixing of seawater with sediments or crust as schematically shown in Fig. 4. Thus, there must be a contribution of mantle helium to the hydrocarbons in the bubbles, and thus thermogenic generation due to the contribution of geothermal heat is highly probable for the initial generation of hydrocarbons in the bubbles.

Comparison with the nearby natural gas seepages

The gas bubbles exhibit chemical and isotopic compositions that are similar to those of shallow-seafloor gas bubbles at the Taketomi spring. The Taketomi submarine spring (10–30 m depth) is 20 km northwest of the knoll and on the same forearc seafloor of the southern Ryukyu arc (Fig. 1). Gas bubbles composed mostly of CH_4 (70–80%) are discharged together with hot water having a temperature of up to 40–60°C (Kaneshima *et al.*, 1983; Oomori, 1987; Oomori *et al.*, 1993; Hirayama *et al.*, 2007). The $\delta^{13}\text{C}$ values of C_1 (–30 to –40‰ c_{VPDB}), the $\text{C}_1/(\text{C}_2+\text{C}_3)$ ratios of 3000–5000, the C_2/C_3 ratios of 10, the $^3\text{He}/^4\text{He}$ ratios of 0.7–0.9 R_a , and the $^4\text{He}/^{20}\text{Ne}$ ratios of 80–200 (Oomori *et al.*, 1993) of the gas bubbles are comparable to values for Kuroshima Knoll. The Late Miocene Yaeyama Group, which includes coal and volcanic products, is assumed to be the source rock of the hydrocar-

bons in the Taketomi spring (Kaneshima *et al.*, 1983). Similar ^3He -enriched helium have been reported for subaerial natural gas seepages in western Taiwan (Sano *et al.*, 1986; Poreda *et al.*, 1988; Yang *et al.*, 2003) accompanying thermogenic hydrocarbons (Lu and Lin, 1986; Yang *et al.*, 2004; You *et al.*, 2004), most of which originated from the Miocene Mushan and Wuchihshan Formations (Lu and Lin, 1986). The contribution of mantle heat and generation of hydrocarbons through the thermocatalytic decomposition of organic matter is ubiquitous in the Miocene age of the area.

The surface of Kuroshima Knoll is covered mostly by the sedimentary rocks of the Pliocene Shimajiri Group (Matsumoto *et al.*, 1998; Takeuchi *et al.*, 2007). The sedimentary rocks of Miocene Yaeyama Group, however, usually underlies the Shimajiri Group in the southern Ryukyu islands (Tsuburaya and Sato, 1985). Although we could not find the Miocene Yaeyama Group on the surface, we can reasonably assume the Miocene Yaeyama Group exists in a deeper layer beneath the seafloor and it is highly probable that this group is the source rock of the gas bubbles in Kuroshima Knoll. Because the southern Ryukyu forearc area can be characterized by active volcanic activity in the Miocene (Kawano and Ueda, 1966; Monden, 1968; Tiba and Saito, 1974), both the initial generation in the Miocene and post-genesis storage until recent times in the group are consistent with our geochemical findings for the hydrocarbons of Kuroshima Knoll, such as (1) the contribution of geothermal heat to the initial generation so as to accumulate ^3He -enriched mantle helium and (2) long storage in a deep crustal reservoir so as to reduce C_2 – C_4 hydrocarbons, as presented in the previous sections.

Sub-seafloor anaerobic oxidation of methane

In comparison with the $\delta^{13}\text{C}$ values of CH_4 in the bubbles (–40.1 ± 0.5‰ c_{VPDB}), the values for bottom fluid samples show a substantial enrichment in ^{13}C ; +4‰ enrichment on average and +8‰ enrichment at maximum (Table 2). In addition, the lowermost $\delta^{13}\text{C}$ value (–40.4‰ c_{VPDB}) of CH_4 in the bottom fluid samples corresponds to values for the bubbles. It is unlikely there is a contribution of CH_4 from ambient water as for the cause of the $\delta^{13}\text{C}$ variation because the concentrations of CH_4 in the bottom fluid (Table 2) were 10^2 – 10^7 times those in ambient bottom water (Fig. 6). We conclude that kinetic microbial oxidation of CH_4 must be responsible for the deviation: that is, CH_4 in the bottom fluid samples is the residue of CH_4 after the oxidation.

If the oxidation occurred only in the aerobic water column after venting into the water column, there should be ^{13}C -enrichment in accordance with the depletion in the concentration. However, we could not find a clear correlation between the concentrations and $\delta^{13}\text{C}$ values

of CH_4 in the bottom fluid samples (Table 2). Furthermore, while the samples D1356W2, D1356W3, and D1357W3 had concentrations 10^7 times that of ambient seawater (almost to saturation), the $\delta^{13}\text{C}$ values indicated a clear ^{13}C -enrichment in comparison with the bubbles. It is unlikely such rapid oxidation in the water column had progressed in the fluid before dilution with the surrounding ambient water. We conclude that the majority of the oxidation progressed under the seafloor. This is in agreement with the observation by Inagaki *et al.* (2004), who observed ^{13}C -enrichment of CH_4 up to 20‰ within pore water of the seeping site. The CH_4 in the liquid samples contained the residual CH_4 oxidized anaerobically during diffusive migration in the sedimentary layer through the reaction (4) (Kulm *et al.*, 1986; Tsunogai *et al.*, 2002a).

Because the reaction requires the mixing of seawater SO_4^{2-} with CH_4 , it might be limited to a thin surface layer, probably less than 1 meter thick. By reducing the alkalinity of the mixture, carbonates have precipitated in the knoll as observed at another cold seepage site (Kulm and Suess, 1990). On the other hand, the observed homogeneous $\delta^{13}\text{C}$ values of CH_4 in the bubbles suggest that they had not undergone anaerobic oxidation, probably owing to the rapid, advective migration of the bubbles in the sedimentary layer. The trace concentration of CO_2 in the bubbles also supports that there was minimum oxidation of the bubbles prior to seepage.

Contribution of gas hydrate dissociation

As already discussed, the contribution of gases derived from the dissociation of sub-seafloor methane hydrate is not required to explain the chemical and isotopic compositions of hydrocarbons in the bubbles seeping from Kuroshima Knoll. In addition, the chemical and isotopic compositions of noble gases suggest that the dissociation of methane hydrate under the seafloor is not a realistic source of the bubbles.

Because the $^3\text{He}/^4\text{He}$ ratios and $^4\text{He}/^{20}\text{Ne}$ ratios of the bubbles highly deviate from the ratios for seawater, it is unlikely that there is a helium source either in the seawater column or in the shallow sedimentary layer where the dissociation of hydrate has been assumed. If we assume the dissociation of methane hydrate is the only source of the bubbles in the knoll, the helium in the bubbles must have been trapped in the methane hydrate as well, together with CH_4 in the bubbles. However, it is difficult to explain the helium enrichment in the bubbles derived from hydrate because helium is rarely incorporated into the structure of natural methane hydrate (Dickens and Kennedy, 2000; Winckler *et al.*, 2002). In the case of the pure natural gas hydrates taken from Hydrate Ridge, Cascadia Margin, for instance, the $^4\text{He}/\text{CH}_4$ ratios were from 0.0003 to 0.009 ppmv (Winckler *et al.*, 2002), while

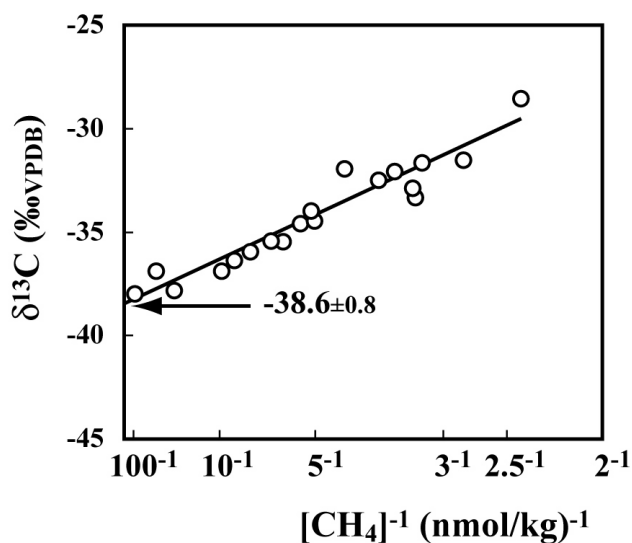


Fig. 7. Relationship between $\delta^{13}\text{C}$ for CH_4 and the reciprocal of the CH_4 concentration in the effluent plume (column water samples taken from 500 to 700 m depths). The solid line is the least-squares fitting of the data.

the ratios for the Kuroshima bubbles were more than 10 ppmv. The dissociation of methane hydrate cannot be the only source of the bubbles in the knoll. Thus, if we persist with the contribution of methane hydrate, we must assume additional He-enriched gases for the source of the bubbles, which seems unrealistic for the area. Because the $^4\text{He}/\text{CH}_4$ ratios in the Kuroshima bubbles can be classified into the representative $^4\text{He}/\text{CH}_4$ ratios of thermogenic natural gases in and around Japan islands (Wakita and Sano, 1983; Urabe *et al.*, 1985; Poreda *et al.*, 1988), direct leakage of hydrocarbons from sub-seafloor gas reservoirs to seafloor is the most probable process to explain the origin of the bubbles in Kuroshima Knoll.

Submarine massive hydrate is ubiquitous in the deep ocean floor around the world (Brooks *et al.*, 1986; Suess *et al.*, 1999). Because hydrate decomposes when the temperature rises, decomposition of sub-seafloor methane hydrate has been assumed as the source of seafloor discharges of CH_4 bubbles at many sites of the world ocean, as presented for the case of the Kuroshima Knoll in this study. Both the present progression of global climate change and the findings of the massive deposits of methane hydrate on the seafloor at active CH_4 seeping sites seem to support the assumption. Most of the submarine massive hydrates, however, seems to be stable at present (Sassen *et al.*, 2001). In addition, methane hydrate can be produced as a result of natural CH_4 seeping activity on the seafloor. Regarding the origin of the seafloor seeping bubbles, we also need to take into account other pos-

sibilities, such as leakage of hydrocarbons from sub-seafloor gas reservoirs, as presented in this study.

Distribution and fate of methane in the water column

We observed a clear enrichment of CH₄ in the water column at depths between 536 and 694 m (Fig. 6). On the other hand, we did not find any CH₄ enrichment shallower than 500 m at the stations. All the rising bubbles in Kuroshima Knoll have dissolved within 140 m of the seafloor. This is consistent with observations of other bubbles seeping from seafloor (Suess *et al.*, 1999; Valentine *et al.*, 2001). Because the depth is substantially deeper than the maximum mixed layer depth (*ca.* 100 m) in the area, direct input into atmosphere is negligible for the gas seeping from Kuroshima Knoll.

To clarify whether the CH₄ decrease in the plume can be explained by simple dilution by the background seawater, the measured $\delta^{13}\text{C}$ values of CH₄ in the plume are plotted against the reciprocal of the CH₄ concentration for the samples taken within the plumes (column water samples taken from 500 to 700 m depths) (Fig. 7). The least-squares fitted line for the plume samples is also shown in the figure. The linear correlation suggests that simple mixing between the seeping CH₄ and CH₄ in background seawater is the main process determining both the concentration and $\delta^{13}\text{C}$ of CH₄ in the plume, and the $\delta^{13}\text{C}$ value of seeping CH₄ is uniform during the mixing (Tsunogai *et al.*, 1998, 2000, 2005). That is, aerobic microbial oxidation in the oxic water column is negligible for CH₄ in the plume, and thus CH₄ is diluted rapidly by eddy diffusion rather than oxidation in the water column. In conclusion, all the rising bubbles at Kuroshima Knoll have dissolved within 140 m of the seafloor. After dissolution, the plume spreads horizontally along with the surface of equal density of the plume, while the concentration is reduced through dilution by eddy diffusion, rather than by oxidation.

Quantification of sub-seafloor anaerobic oxidation

The intercept of the least-squares fitted line for the plume samples in Fig. 7 corresponds to the end-member $\delta^{13}\text{C}$ value of CH₄ seeping from the knoll (Tsunogai *et al.*, 1998), which can be considered the weighted average $\delta^{13}\text{C}$ value of CH₄ emitted from the knoll to the water column. Assuming the $\delta^{13}\text{C}$ values of the bubbles ($-40.1\text{‰}_{\text{VPDB}}$) represent the initial $\delta^{13}\text{C}$ of CH₄ prior to sub-seafloor anaerobic oxidation, we can evaluate the extent of the sub-seafloor anaerobic microbial oxidation quantitatively using the discrepancy between the weighted average $\delta^{13}\text{C}$ value ($-38.6\text{‰}_{\text{VPDB}}$) and the $\delta^{13}\text{C}$ values of the bubbles because the discrepancy ($+2.5\text{‰}_{\text{VPDB}}$) corresponds to the average extent of the progress of the sub-seafloor anaerobic oxidation of CH₄ at Kuroshima Knoll.

Assuming the kinetic isotope effect due to the anaero-

bic microbial CH₄ oxidation α (k_{12}/k_{13}) to be 1.008 (Tsunogai *et al.*, 2002a), the fraction of residual CH₄ can be calculated from the $\delta^{13}\text{C}$ variation using the Rayleigh equation (7). Using this equation, we estimated the average C_f/C_0 to be 0.82. Thus, about 20% of CH₄ has been oxidized before seeping into the water column.

CONCLUSIONS

Most portions of the hydrocarbons in the Kuroshima bubbles were supplied directly from deep gas reservoirs produced by thermogenic processes in the Miocene. The hydrocarbons, especially C₃- and C₄-, had been fractionated both chemically and isotopically through microbial destruction during the storage in the gas reservoir. In addition, the anaerobic oxidation of CH₄ within shallow sediments just beneath the seafloor removes about 20% of CH₄ on average, until seepage into ocean water column. After the seepage, all rising bubbles dissolved into the ocean water column within 140 m of the seafloor. After the dissolution, the plume spreads horizontally along with the surface of equal density in the water column, while the concentrations decrease through dilution by eddy diffusion, rather than by oxidation.

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