Research Article

Improvements in Drill-Core Headspace Gas Analysis for Samples from Microbially Active Depths

Kazuya Miyakawa and Fumiaki Okumura

1Horonobe Underground Research Center, Japan Atomic Energy Agency, Hokushin 432-2, Horonobe-cho, Hokkaido 098-3224, Japan
2JAPEX Research Center, Japan Petroleum Exploration Co., Ltd., 1-2-1 Hamada, Mihama-ku, Chiba 261-0025, Japan

Correspondence should be addressed to Kazuya Miyakawa; miyakawa.kazuya@d.nagoya-u.jp

Received 11 April 2018; Accepted 29 July 2018; Published 1 October 2018

1. Introduction

Investigation of the origin of deep hydrocarbons is an important aspect of resource exploration and may lead to an improved understanding of geological environments. In this investigation, gases adsorbed on rock fragments or bore cores were studied by headspace gas analysis (e.g., [1–5]), which provides information on the generation and migration of light hydrocarbons and gases. The IsoJar™ (Isotech Laboratories and Humble Instruments, USA) container, which is widely used in such analyses (e.g., [6, 7]) comprises a plastic container of ~600 ml volume with an aluminum screw cap on which there is a rubber septum through which headspace gas can be taken by syringe. The analysis procedure (e.g., [8]) normally involves storage of wet cuttings or cores in the jar with water and an air headspace for several days or weeks, the addition of a microbicidal such as benzalkonium chloride (BKC) to minimize bacterial activity, the partitioning of gas into the headspace during storage, and analysis of these gases (e.g., [9–11]). The use of distilled or tap water avoids contamination from dissolved gases. The $\delta^{13}C_{CH_4}$ values of gases from depths of <1000 m, in the biogenic region, are usually in the range of $-70$‰ to $-60$‰, with isotopic compositions becoming heavier as depth increases towards the thermogenic region (e.g., [12, 13]). Large variations in carbon isotopic ratios in CH$_4$ and CO$_2$ are often reported for depths of <1000 m, with $\delta^{13}C_{CH_4}$ values sometimes reaching $-20$‰ (e.g., [14–17]). These variations are associated with the effects of microbial activity on methane production or oxidation in underground environments [18]. There are a number of factors that control the rate of methanogenesis [19], including temperature [20], groundwater salinity [21], pH [22], and pore space [23]. Peak microbial activity occurs at 35–45°C, which corresponds to depths of <1000 m [19, 24].
At greater depths, microbial action decreases as thermogenic production increases with the onset of catagenesis (subsequent to diagenesis at shallower depths [8]). More importantly, pore diameters of at least 1 μm are necessary for in situ methanogenesis, as microbes are in the 1–10 μm size range [25], which suggests that active methane production occurs at depths of <1500 m [24]. At shallow depths (less than several meters) below the ocean floor, where the concentration of dissolved gas is relatively low, considerable care was taken to avoid contamination and microbial activity (e.g., [26]). Hachikubo et al. [27] adjusted the concentration of BKC in samples to ~2.5% using 25 ml vials to obtain precise depth profiles of gases relative to hydrates. While the concentration and/or amount of microbicide normally added to IsoJar™ vessels are often omitted in reports, it is considered that the final concentration in IsoJar™ containers should be of the order of 0.01%, which is two orders of magnitude less than that reported by Hachikubo et al. [27]. It is speculated that another possible cause of variations in carbon isotopic composition may be microbial activity in the headspace after sampling, as the amount of microbicide commonly used with samples from microbially active depths might be insufficient to suppress microbial activity.

In a previous study, gas samples from two boreholes (PB-V01 and SAB-1, both ~500 m deep) in the Horonobe area, Hokkaido, were processed using IsoJar™ containers [14]. In that study, cores were stored in IsoJar™ containers with water and a few drops of BKC solution [14] for up to three months before headspace analysis. Because sampling date of cores and analysis date of gases, which are necessary for the calculation of the storage period, have not been presented in Funaki et al. [14], these unpublished information are summarized in Tables S1a and S1b. Concentration of the BKC solution and amounts of cores and water also have not been reported in Funaki et al. [14]. In the conventional way of using IsoJar™ headspace gas analysis, it is considered that the concentration of BKC solution is lower than 10%, in which case concentrations of BKC in jars are in order of 0.01%. In the construction of the Horonobe Underground Research Laboratory (URL), including two boreholes (PB-V01 and SAB-1), water was collected from groundwater at ~50 m depth, and this was used by Funaki et al. [14] as filling water for the IsoJar™ containers. Gases dissolved in deep groundwater from the URL were also analyzed, using an evacuated-vial (EV) method [28]. Measured δ13C values for CH4 and CO2 from both sets of analyses are plotted against each other in Figure 1. Large variations in δ13CCH4 values from the IsoJar™ measurements (Figure 1) were attributed to methane-oxidizing bacterial activity using sulfate ions in the deep underground environment or to isotopic fractionation during gas migration through fractures [14]. However, these possible causes are considered unlikely because (a) geochemical studies in the Horonobe area indicate that reducing conditions are maintained deep underground and sulfate ions are either absent or present at very low concentrations [29–32]; (b) studies of iodine enrichment [33] indicates that any traces of methane oxidation by sulfate in pore waters of sediments would have been eroded during upward fluid flow due to compaction during burial; and (c) there is no evidence in the study area of isotopic fractionation in gases during migration [34, 35]. The δ13CCH4 and δ13CCO2 values obtained by the EV method (Figure 1) show little scatter plotting in the carbonate reduction field.

Differences between these data sets could be attributed to factors such as aerobic microbial oxidation of methane in the containers after sampling and/or using groundwater from the different depths of core samples. Possible causes were investigated in the present study to improve the methodology of headspace gas analysis using IsoJar™. Gases from the Wakkani Formation in the Horonobe area were sampled using the methods of Funaki et al. [14] and Miyakawa et al. [28]. The effects of the sampling method (storage period, water type, and additives) on carbon isotopic ratios in CH4 and CO2 were investigated, and improvements in headspace gas analysis techniques are suggested.

2. Geological Setting

The Horonobe area is located in northwestern Hokkaido, in a Neogene–Quaternary sedimentary basin (Figure 2). Since August 2006, the Japan Atomic Energy Agency (JAEA) has been excavating the URL for a research associated with the development of technologies related to the geological disposal of high-level radioactive waste. Geologically, the URL area comprises marine sediments of the Wakkani Formation (Neogene siliceous mudstone containing opal-CT) and Koetoi Formation (Neogene–Quaternary diatomaceous mudstone containing opal-A). Burial and subsidence of these formations occurred throughout the Neogene and Quaternary, when they underwent early diagenetic thermal alteration at temperatures of <60°C [36]. The URL and surrounding geology are depicted in Figure 3. The high-pressure boreholes have steel casings, with valves allowing the sampling of groundwater from multiple depths or zones.
The δD vs. δ18O plots for groundwater from the Wakkanai and Koetoi Formations indicate that it is a mixture of local meteoric water and altered seawater [38, 39]. Methanogenic and methane-oxidizing microbial communities have played an important role in these formations [40–42], where secondary microbial gas with 13C-enriched isotopic values (δ13CCH4, −74‰ to −28‰; δ13CCO2, −7‰ to +31‰) formed through CO2 reduction after uplift of the area [28].

3. Sampling and Analytical Methods

3.1. IsoJar™ Samples

3.1.1. Effect of Storage Period. Core samples including in situ pore water (300–400 g), crushed to pieces roughly 30–50 mm in diameter, were placed in IsoJar™ containers with 250–300 g of “filling” water. HgCl2-saturated or 10% BKC aqueous solution (10 drops (~0.5 ml) of either) was added to suppress microbial activity, and the jars were sealed with an air headspace. Final concentrations of both microbicides in the jars were about 0.01%–0.02%. The jars were kept in the dark at room temperature for 5–92 d before analysis. Details of each experiment are summarized in Table 1 and Table S2.

The cores were obtained immediately after drilling of borehole 350-Fz-01 from the bottom of the east shaft (Figure 3). Although distilled or tap water is usually used in the IsoJar™ method, groundwater from depths of 53.5–64.5 m and 350 m in borehole 13-350-C01 (drilled in the 350 m gallery; Figure 3) was used to match the 50 m groundwater used by Funaki et al. [14]. The priority in this study was to evaluate the effects of sampling method on the carbon isotopic ratios of CH4 and CO2, rather than to obtain accurate in situ values. A large portion of CH4 dissolved in groundwater around the URL had already escaped due to the pressure decrease associated with excavation, so it was expected that only small amounts of gas would remain in cores. Groundwater, rather than tap water, was used to compensate for this (with water from borehole 13-350-C01 being used because water was not available from borehole 350-Fz-01). Core samples IJ1–IJ25 were from depths of 384–416 m (Table S2). Core samples IJ26–IJ28 were from a depth of 470 m (Table S2) and kept in vacuum storage for one month after drilling. At these depths, the C isotopic ratios for CH4 and CO2 had similar values to those from 350 m depth [28].

3.1.2. Effect of Additives. The effective amount of additives was investigated as follows. IsoJar™ samples were prepared
as described in Section 3.1.1, with up to 20 ml of BKC and HgCl₂ solutions per jar (Table 2 and Table S3). All core samples were from a depth of 480 m in 350-Fz-01. Fresh cores taken immediately after drilling were not available for analysis, and the samples used in this study had been kept in vacuum storage for about six months. Groundwater from borehole 13-350-C01 was used as filling water. Headspace gas compositions were determined after storage in IsoJar™ containers were desorbed into the headspace by ultrasonic shaking. Concentrations of O₂, N₂, CO₂, CH₄, C₂H₆, and C₃H₈ in headspace gas were determined by gas chromatography (GC) using a GC7890A Valve System (Agilent Technologies, USA). Carbon isotopic values (δ¹³CCH₄ and δ¹³CCO₂) were determined by GC combustion isotope-ratio mass spectrometry (GC-C-IRMS), using an IsoPrime GC-MS system (GV Instruments, UK), and are expressed in the usual VPDB δ notation. The lower limit of determination of carbon isotope ratios (δ¹³CCH₄ and δ¹³CCO₂) requires concentrations of 0.01%. Details of GC and GC-C-IRMS procedures can be found in Waseda and Iwano [43].

3.3. Analytical Procedure. Gases adsorbed on rock fragments in the IsoJar™ containers were desorbed into the headspace by ultrasonic shaking. Concentrations of O₂, N₂, CO₂, CH₄, C₂H₆, and C₃H₈ in headspace gas were determined by gas chromatography (GC) using a GC7890A Valve System (Agilent Technologies, USA). Carbon isotopic values (δ¹³CCH₄ and δ¹³CCO₂) were determined by GC combustion isotope-ratio mass spectrometry (GC-C-IRMS), using an IsoPrime GC-MS system (GV Instruments, UK), and are expressed in the usual VPDB δ notation. The lower limit of determination of carbon isotope ratios (δ¹³CCH₄ and δ¹³CCO₂) requires concentrations of 0.01%. Details of GC and GC-C-IRMS procedures can be found in Waseda and Iwano [43].

4. Results

Headspace concentrations of the gases analyzed and δ¹³CCH₄ and δ¹³CCO₂ values for the IsoJar™ samples prepared as described in Section 3.1.1 are listed in Table 1 and Table S2, and the results for the EV samples are listed in Table 3 and Table S4.

Sample contamination by air does not affect the present discussion of isotopic ratios because concentrations of CH₄ and CO₂ in air are much lower than those in the free gas from groundwater (where CH₄ = 74%–100%; CO₂ = 1%–20%; [28, 34]).

CO₂ in the free gas undergoes isotopic fractionation during exchange with dissolved inorganic carbon (e.g., CO₂(aq), HCO₃⁻, and CO₃²⁻), with HCO₃⁻ being the dominant aqueous species at around neutral pH. To compare δ¹³CCO₂ values of the IsoJar™ samples with those of the EV samples, a fractionation correction (intrinsic isotopic fractionation factor) of +7.9‰ between CO₂ gas and HCO₃⁻ at 25°C [44] was added to δ¹³CCO₂ values measured for the IsoJar™ samples. The range of room temperatures was 20°C–25°C, giving an error of up to -0.6‰.

Variations in δ¹³CCO₂ and δ¹³CCH₄ values with storage time are shown in Figures 4(a) and 4(b), respectively. IsoJar™ samples with groundwater (350 m) stored for less than one week give values similar to the EV samples. Storage periods of ≤7 d and ≥20 d were considered, as no data are available for the interval of 7–20 d.

The δ¹³CCO₂ and δ¹³CCH₄ ratios for the EV samples are relatively constant, although δ¹³CCH₄ values show a small variation (<4‰) after 98 d (Figure 4(b)), which is consistent with a previous study [28]. It is possible that some microbes are not removed by 0.22 μm groundwater filtration (e.g., [45]), and this might have caused the slight variation in δ¹³CCH₄ values. Differences between carbon isotopic values...
of CH₄ and CO₂ (δ¹³C₀₂ – δ¹³CCH₄ = isotopic separation factor; [46]) in the Horonobe area decrease slightly (<1‰) with increasing depth and temperature according to isotopic equilibrium values, with values at depths of 250 m and 350 m being similar to each other within the natural variation of ~2‰ (1σ) [28]. Therefore, δ¹³C₀₂ and δ¹³CCH₄ values of 250 m groundwater obtained by the EV method (Table 3) were used as reference values for the IsoJar™ samples with 350 m groundwater.

The δ¹³C₀₂ values for IsoJar™ samples show two separate trends for 350 m and 50 m groundwater, with both showing a slight decrease over time, from +15‰ at 5 d to +13‰ at 92 d and from +5‰ at 17 d to ~1‰ at ~3‰ at 79 d, respectively (Figure 4(a)). The patterns are the same for both BKC and HgCl₂ additives. The two trends suggest that the δ¹³C₀₂ ratios represent dissolved gases from different depths. The concentration of CO₂ in the groundwater, as HCO₃⁻, is relatively high even when degassed at atmospheric pressure (30–50 mmol kg⁻¹; [47]), compared with that of adsorbed CO₂, so carbon isotopic values should be largely dependent on dissolved CO₂. The δ¹³C₀₂ values of samples with 50 m groundwater (dashed line in Figure 4(a)) are distinct from those of samples with 350 m groundwater, indicating that the former samples are strongly contaminated.

The δ¹³CCH₄ values for IsoJar™ samples (Figure 4(b)) increase markedly with time regardless of additives and fluctuate by more than 30‰ after 80 d; separate trends for different depths are not evident. The concentration of CH₄ remaining in the groundwater at atmospheric pressure after sampling is relatively low (~3 mmol kg⁻¹; [47]), and δ¹³CCH₄ values for the IsoJar™ samples mainly represent gases adsorbed on cores. Therefore, while the relatively large amounts of dissolved CO₂ reduced the effects of isotopic fractionation on δ¹³C₀₂ values, δ¹³CCH₄ values were strongly affected.

The results of the effect of additives as described in Section 3.1.2 are shown and discussed in Section 5.2.

5. Discussion

5.1. Methane Oxidation. During methane oxidation, decreasing CH₄ and increasing CO₂ concentrations are associated
with carbon isotopic fractionation, resulting in enrichment of $^{13}$C in unreacted CH$_4$ and depletion in CO$_2$ produced. However, there is no clear relationship between headspace gas concentrations and storage period, possibly because of the variability of adsorbed gas levels in natural samples. An apparent carbon isotopic fractionation factor, $\alpha$, defined as $\alpha = (\delta^{13}C_{CO2} + 1000)/(\delta^{13}C_{CH4} + 1000)$, was calculated using the $\delta^{13}C_{CO2}$ and $\delta^{13}C_{CH4}$ values in Table 1 (Figure 5). Initial values of $\alpha$ in the Horonobe samples, calculated using the results of the EV method, were around 1.06–1.08, in good agreement with those determined by Miyakawa et al. [28]. The values of $\alpha$ for the IsoJar™ samples decreased from 1.02 in the methane oxidation zone [48].

A bivariate plot of $\delta^{13}C_{CH4}$ vs. $\delta^{13}C_{CO2}$ (Figure 6) indicates two trends for the IsoJar™ samples (Figure 6(b)), as in Figure 4(a). Thus, data plotted in Figures 4(b) and 5 indicate that the variations can be explained by the mixing of the two trends (Figure 6(b)). It seems, therefore, that the characteristics of both methane oxidation and contamination of 50 m groundwater in the IsoJar™ container after sampling.

5.2. Effect of Additives. Significant isotopic fractionation occurred in the IsoJar™ samples, strongly affecting $\delta^{13}C_{CH4}$ values despite the addition of BKC or HgCl$_2$ (Figure 4), suggesting that the amounts of additives used were insufficient to suppress microbial activity. Results of effect of additives as
described in Section 3.1.2 are listed in Table 2 and shown in Figure 7. With < 10 ml BKC solution or < 0.3% BKC concentration, δ₁³CCH₄ values fluctuated significantly, and isotopic compositions became lighter. This is opposite to the effect of microbial carbonate reduction and may be due to low CH₄ concentrations (Table 2) resulting from storage of the cores for about six months in a vacuum container (to avoid oxidation by the air and drying), possibly with significant removal of adsorbed gases. An instrumental mass bias of carbon isotope ratio was reported in a very low concentration of hydrocarbons with respect to mass spectrometry [49]. In this study, all the data of CH₄ concentrations were above the lower limit of determination of 0.01% (Table 2) indicating that large fluctuations of δ₁³CCH₄ values (Figure 7) are not due to instrumental mass bias. The mechanism of any reaction opposing fractionation is not clear. In a low CH₄ concentration, the carbon isotope ratio may be easily disturbed by complex microbial metabolism (e.g., methane oxidation and carbonate reduction). With > 10 ml BKC solution or > 0.3% BKC concentration, the δ₁³CCH₄ values were relatively constant at about −56‰ (Figure 7), which is in good agreement with the values for EV samples.

Although the δ₁³CCH₄ values for IsoJar™ samples with HgCl₂ solution are relatively constant (Figure 7), they are consistently 5‰–6‰ lower than those with BKC solution. Concentrations of CO₂ in the headspace of samples IJ47 and IJ48, to which 20 ml HgCl₂ solution was added, are considerably higher than those in the other samples (Table 2). A possible cause of the decrease in δ₁³CCH₄ values may be isotopic fractionation associated with mercuric reactions with methane, which generate HCl and lead to CO₂ outgassing. Although this mechanism is not clear, considerable care should be required using HgCl₂ as a microbicide with respect to carbon isotope fractionation.

### 6. Conclusions

This study investigated the possible causes of carbon isotopic variations (in δ₁³CCH₄ and δ₁³CCO₂ values) in borehole drillings to 500 m depth, as reported by Funaki et al. [14]. The results have led to improvements in the IsoJar™ method for the determination of carbon isotopic ratios in CH₄ and
CO₂ adsorbed on bore cores. It was found that with air in the IsoJar™ headspace, microbes oxidize CH₄ to CO₂ during storage, accompanied by isotopic fractionation, especially for samples from depths of < 1000 m where microbes are more active. Isotopic fractionation resulted in δ¹³CCH₄ and δ¹³CCO₂ values reaching >30‰ and >2‰ after 80 d storage, respectively, while samples analyzed within a week of sampling showed no such effect. The significant isotopic fractionation in CH₄ was due to its low concentration in the sampling container, while the weaker fractionation in CO₂ was due to its relatively high concentration. The conventional amount of BKC additive (~0.5 ml of 10% solution) was insufficient to suppress microbial activity at least when using in situ groundwater as filling water. The large variations in isotopic compositions reported by Funaki et al. [14] thus appear to have been caused by microbial methane oxidation in the IsoJar™ containers after sampling and contamination with groundwater from different depths. Important technique improvements are summarized as follows: (1) if long-term sample storage is necessary, >10 ml of 10% BKC solution
should be used or >0.3% BKC concentration is required; (2) analysis within a week of sampling is strongly recommended; and (3) for CO₂ analysis, groundwater from different depths should not be used.

**Data Availability**

The all data used to support the findings of this study are included within the article and Supplementary information file.

**Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**Acknowledgments**

The authors are grateful to Hironori Funaki (JAEA) and Taiki Ishikawa (Mitsubishi Materials Techno (MMT)) for providing helpful information regarding sampling methods. Amane Waseda (IAPEX) provided valuable comments that greatly improved the manuscript. The authors would also like to thank Yusuke Kitagawa (MMT) and Eiichi Ishii (JAEA) for the help with sampling. Hiroshi Sasamoto (JAEA) and Aaron Stallard (Stallard Scientific Editing) are gratefully acknowledged for improving the manuscript.

**Supplementary Materials**

Sampling date, analysis date, sampling depth of cores, and carbon isotope ratios of CH₄ and CO₂ of headspace gases in IsoJar™ samples of Funaki et al. [14] (Tables S1a and S1b). Sampling date, analysis date, sampling depth of cores, and chemical compositions of headspace gases in IsoJar™ samples and EV samples of this study (Tables S2, S3, and S4). *(Supplementary Materials)*

**References**


vician black shales of southern Quebec (Canada) and their significance for naturally occurring hydrocarbons in shallow groundwater,” *International Journal of Coal Geology*, vol. 158, pp. 44–64, 2016.

[12] M. Schoell, *Recent advances in petroleum isotope geochemis-


[19] B. J. Katz, “Microbial processes and natural gas accumula-


[22] A. Visser, Y. Gao, and G. Lettinga, “Effects of pH on methano-

genesis and sulphate reduction in thermophilic (55°C) UASB


