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Stable carbon and hydrogen isotope measurements on Black Sea water-column methane

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Abstract

We report measurements of δ^{13}C-CH$_4$ and δ^2H-CH$_4$ at a central station in the Black Sea. We considered the Black Sea as a "biogeochemical bucket" and the single station as a basin-wide integrator of processes affecting methane. Considering the rapid (3.6–18 yr) turnover of methane and the similarity of these stable isotope distributions to the methane concentration and oxidation rate profiles [Reeburgh, Ward, Whalen, Sandbeck, Kilpatrick, Kerkhof, 1991. Black Sea methane geochemistry. Deep-Sea Research 38, S1189–S1210], it appears that methane is being added approximately as fast as it is being oxidized. Methane can be thought of as "running in place" in the Black Sea water column.

Recent reports of extensive vents on the northern side of the Black Sea suggest that they might be a methane source capable of effectively balancing the Black Sea methane budget. Unfortunately, we have limited information on basin-wide seep fluxes and cannot identify them with stable isotope measurements. Methane oxidation (and accompanying isotope fractionation) is so extensive that the water-column stable isotope measurements provide little information on methane sources. Future measurements of ^{14}C-CH$_4$ should permit partitioning Black Sea methane sources into fossil and recent components.
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1. Introduction

Methane geochemistry was studied during the 1988 US–Turkey Black Sea Expedition (Reeburgh et al., 1991). Shipboard operations during that expedition were restricted to Turkish and international waters, so that work focused on the water-column at a single central deep station (41°05′ N, 34°00′ E, 2200 m depth, BSK-2) and analyses on two cores, one on the shelf (41°35.5′ N, 28°46.92′ E, 170 m, BS5-1), and the other at the deep station. Detailed measurements of water-column methane concentration were performed at sea using a gas chromatograph equipped with a stripping-trapping manifold. These measurements were precise to 0.8% over most of the concentrations encountered. The water-column methane profile agreed reasonably well with previous measurements on stored samples by Scranton (1977) and Atkinson and Richards (1967). A major goal of our 1988 work was to determine the role of the anaerobic oxidation of

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methane in the Black Sea water-column. Methane oxidation rates were measured using two independent tracer techniques: tracer-level additions of ^{3}H-CH_4 and non-tracer additions of ^{14}C-CH_4. Results from the two techniques were indistinguishable, with an overall average deep Black Sea methane oxidation rate for both techniques of ~ 2.3 nM day^{-1}. Sediment CH_4 concentration profiles were measured at the shelf station and the central basin station. Sediment methane concentration and oxidation rate measurements were made at the central station (BSK-2) only. These results are summarized in Reeburgh et al. (1991). Samples for stable isotope analysis, the subject of this paper, were collected during the 1988 expedition at the central deep station.

1.1. Methane budget

The measurements referred to above were used to construct a steady-state methane budget for the Black Sea based on loss terms or sinks (Reeburgh et al., 1991). The sinks considered were: measured water-column methane oxidation rates, measured sediment methane oxidation rates, calculated gas evasion rates, and export by Bosporus outflow. The largest sink term, water-column anaerobic methane oxidation, was about 70-fold larger than the next largest term, evasion at the air:sea interface. Large-scale water-column methanogenesis was excluded on thermodynamic grounds. Methanogenesis has been reported in zooplankton guts and fecal pellet microenvironments (Marty, 1993; de Angelis and Lee, 1994; Karl and Tilbrook, 1994), but thermodynamic arguments (Hoehler et al., 1994) indicate that so long as sulfate reduction is occurring, there can be no large-scale water-column methanogenesis. Black Sea water-column sulfate reduction rates at nM day^{-1} have been reported by Albert et al. (1995).

Ivanov et al. (2002) used radioisotope tracers (^{14}CO$_2$, ^{14}C-acetate) to measure the rates of methanogenesis by CO$_2$ reduction and acetate fermentation, respectively, and subtracted measured rates of methane oxidation (^{14}C-CH$_4$ tracer) from total methanogenesis to obtain a value for net consumption. Their values were similar in magnitude to the Reeburgh et al. (1991) oxidation rates. The Ivanov et al. (2002) experiments involve tracers with specific activities that are too low, and were conducted on samples with pool sizes that were both too large (CO$_2$) and too small (acetate, CH$_4$) to provide meaningful results, so we consider their net consumption rate estimate to be fortuitous.

Since methane is not produced in anoxic marine water-columns, the sink terms must be balanced by a sediment source. Sediment distributions were measured in two cores, one from the shelf (BS5-1) and one from the deep basin (BSK-2). A concave-up methane distribution in the shelf core showed that anaerobic methane oxidation (Reeburgh, 1980) was occurring, and a small methane concentration gradient in the portion of the core above the anaerobic methane oxidation zone permitted calculation of a methane flux of 0.2 mol m^{-2} yr^{-1}. Methane concentrations lower than adjacent overlying water concentrations observed in the deep basin core, as well as methane oxidation rate measurements using ^{14}C-CH$_4$ showed that the deep sediments must be consuming methane from the adjacent overlying waters. Reeburgh et al. (1991) assumed that sediments within the depth interval from 100 to 1500 m were the methane source to the water-column. Only 13% of the methane flux needed to balance the budget could be accounted for if the flux was 0.2 mol m^{-2} yr^{-1}; a sediment methane flux of 1.5 mol m^{-2} yr^{-1} was needed to balance the budget. Clearly, an additional methane source is needed.

Although the Reeburgh et al. (1991) methane budget is a straightforward summation of methane sinks, it is difficult to physically reconcile a sediment methane source over the 100–1500 m depth interval. The budget is dominated by water-column oxidation, which occurs throughout the water-column. The water-column methane concentration and methane oxidation rate distributions are uniform at depths greater than 500 m. An unexpected short turnover time for methane (3.6–18 yr) can be determined from the methane concentrations and oxidation rates. There is no obvious way to maintain these uniform distributions by mixing. Curvature in the T–S diagram makes vertical advection-diffusion models (Spencer and Brewer, 1971; Reeburgh, 1976) inapplicable in the deep Black Sea. A stable water-column that may mix by heat-flow-driven convection in the bottom 400 m or by double-diffusion (Murray et al., 1991) provides no obvious way to mix and maintain these uniform depth distributions. The stable isotope measurements reported here were undertaken to provide information on the depth distribution of other possible Black Sea methane sources as well as the extent of methane oxidation.

2. Methods

2.1. Sampling, stripping, and trapping

These samples were collected at the central deep station (BSK-2) during the 1988 US–Turkey Black Sea Expedition. Methane for isotope analysis was stripped from samples of Black Sea water and was collected on traps containing a Molecular Sieve for later oxidation and isotope analysis. Water samples from approximately 100-m depth intervals were collected with 30-l Niskin bottles and were transferred to evacuated 20-l glass carboys. Methane was stripped from the carboys using techniques similar to those used for collection of ^{222}Rn (Mathieu et al., 1988).

The glass carboys were fitted with a one-hole butyl rubber stopper containing a headpiece consisting of a modified Swagelok female run tee. The headpiece contained a gas inlet that extended well below the water surface and permitted stripping, a gas outlet, and shut-off valves. After filling, the carboys were connected to Lamont radon stripping boards (Mathieu et al., 1988) with heavy wall (1/2″ OD × 1/8″ wall) Tygon tubing and polyethylene quick-connect fittings. The stripping boards were modified with a three-valve bypass to accommodate traps for cryogenic collection of methane (Fig. 1). The circulating gas stream was passed through a Drierite/Ascarite trap to remove the bulk of the water vapor and CO_2 before trapping methane on liquid nitrogen-cooled U-traps containing a molecular sieve sorbent. The pressure in the complete stripping system was adjusted to just below ambient with He, and the samples were stripped for 1.5 h. The U-traps were immersed in liquid nitrogen during stripping and were sealed while immersed in liquid nitrogen with refrigeration pinch-off clamps (Imperial Eastman Corporation No. 105FF Pinch-Off Clamps, Weiss, 1968).

2.2. Methane traps

The methane sample traps were made of 31″ lengths of 3/8″ OD × 0.315″ wall aluminum tubing. The tubing was bent into a "U" at its midpoint and
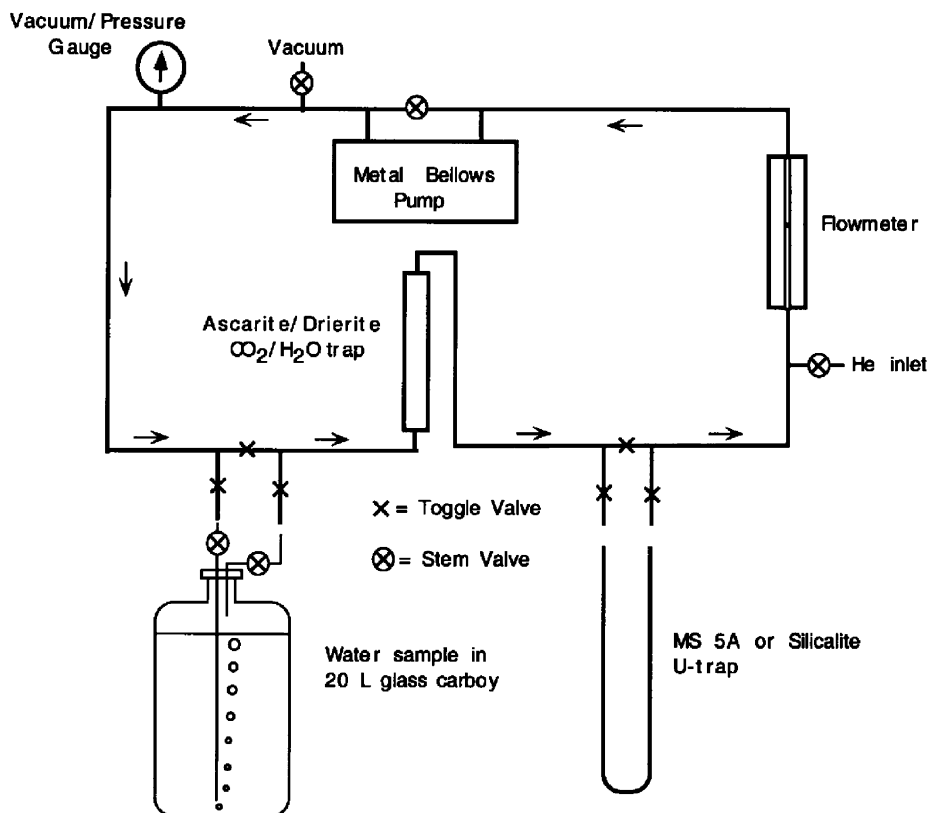

Fig. 1. Schematic diagram of the stripping system used to collect water-column methane samples for stable isotope analysis. A Lamont radon stripping board (Mathieu et al., 1988) was modified with a three-valve bypass to accommodate the U-traps.

filled with the packing material to within 6″ of the stub ends of the U. The traps were filled with either silicalite (Flanigen et al., 1978) or molecular sieve 5A. Silicalite (now available from UOP Molsiv Adsorbents as HISIV 3000) is a hydrophobic crystalline silica molecular sieve that has been reported to absorb hydrocarbons and low molecular weight organic compounds from water-saturated gas streams at room temperature. There has been much more experience with molecular sieve 5A as a trapping material, but silicalite was reported to be a more effective sorbent at higher temperatures. Trapping of methane is quantitative with liquid nitrogen. Both sorbents were obtained as 1/16″ diameter wire-form, which permitted high flow rates through the traps. The packing was capped with a plug of Pyrex glass wool, and each leg of the U-trap was flattened slightly above the glass wool plug to prevent movement of the packing during stripping. Both sorbents were activated by baking at 300 °C for 2 h with a helium flow of ∼50 cc/min. Copper tubing is preferred for pinch-off sealing, but a CuO scale formed on the outsides of the U-traps during activation. This compromised the tube fitting seals in some cases, so we elected to use aluminum tubing and to back up the pinch-off seals with Swagelok caps. The aluminum tubing work-hardened and became brittle at the pinch-off site. Although the pinch-off seals in Al tubing were difficult to open, we were successful in obtaining 20 samples for oxidation and isotope analysis.

2.3. Oxidation and mass spectrometry

The methane isotope samples were oxidized at the University of Alaska, Fairbanks, in 1992. A low-blank circulating combustion line (Alperin, 1988) was modified to accept methane samples baked off of the U-traps. The pinch seals were opened by re-rounding the tubing with the refrigeration clamp and the contents of each trap were baked off at 300 °C into the combustion line. Prior to oxidizing the methane over CuO at 800 °C, CO_2 and H_2O were removed cryogenically, and CO was removed with Schutze reagent followed by a cryogenic CO_2 trap. Following oxidation, the resulting carbon dioxide was expanded into a known volume for quantification and was frozen into multiple glass tubes, which were flame sealed. Water resulting from the methane combustion also was collected and frozen into a single glass tube. The measurements of $\delta^{13}C$-CH_4 and δ^2H-CH_4 were performed in

1997 at the University of California, Irvine, using a Finnigan-MAT 252 isotope ratio mass spectrometer equipped with a tube cracker inlet system (Tyler et al., 1997). The CO_2 samples intended for ^{13}C analysis were run directly. The combustion water intended for the ^2H analyses was frozen directly into glass tubes. The preferred procedure for ^2H analysis (Coleman, et al., 1982) is to transfer the water directly to tubes filled with activated zinc for reduction of H_2O to H_2. These samples were stored over 5 years before transfer to tubes with activated zinc, so there was concern that errors due to isotope exchange could result. The samples were large and condensed water was visible, so they were analyzed with the expectation that errors resulting from storage would be small. We did not adhere strictly to Demény's (1995) recommendation that H_2O:Zn ratios be held constant to avoid sorption and fractionation of H isotopes.

The CO_2 and H_2O resulting from the original CH_4 samples were analyzed for $^{13}C/^{12}C$ and $^2H/^1H$ ratios by comparison to isotope reference gases of CO_2 and H_2 made by Oztech Trading Corporation. (Dallas, TX). These have been calibrated to within 0.01‰ and 1.0‰ against NIST standard reference materials NBS-19 and SMOW. Values for $\delta^{13}C$ and δ^2H are reported on the V-PDB and V-SMOW scales. The accuracy of individual sample measurements, based on reproducibility when all possible errors associated with sampling, processing, and measuring are included, is ±0.10‰ for $\delta^{13}C$ and ±30‰ for δ^2H.

3. Results and discussion

The depth distributions of δ^2H-CH_4 and $\delta^{13}C$-CH_4 at the central Black Sea station are shown in Figs. 2 and 3. Methane concentrations obtained from the Reeburgh et al. (1991) study are also plotted for the respective sample depths. It is evident that molecular sieve 5A and silicalite are equivalent as methane-trapping media.

3.1. δ^2H-CH_4

The δ^2H-CH_4-depth distribution in Fig. 2 shows considerable scatter, which must result from isotope fractionation during some stage of the analysis. Hydrogen isotope measurements are difficult and are notorious for showing large fractionation effects, and it appears that a number of our operations could have contributed to the scatter.
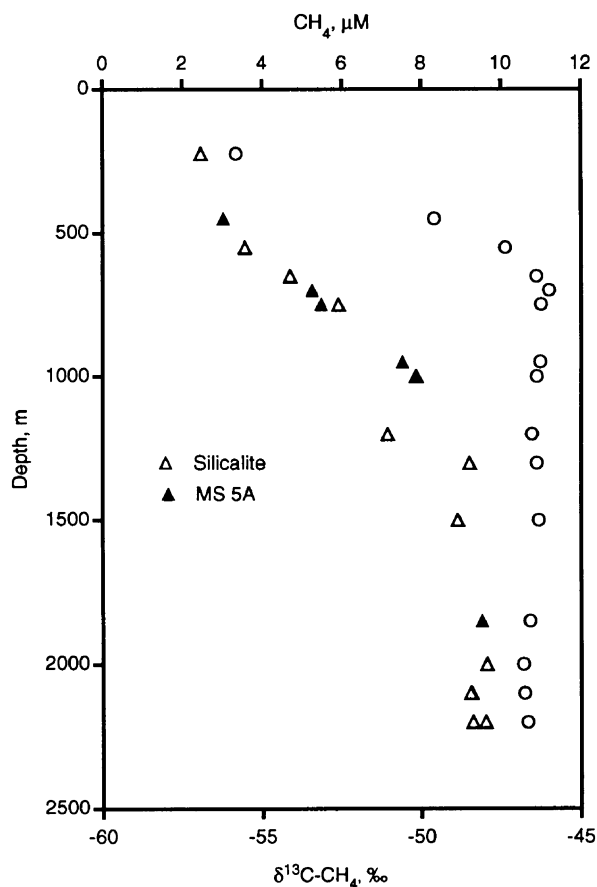
Fig. 2. Depth distribution of δ^2H-CH$_4$ at station BSK-2 in the Black Sea. These measurements were performed on the water resulting from combustion of extracted methane. Samples at 650 m (−319.1 ‰) and 2100 m (−435.04‰) are not plotted. The distribution shows scatter that we cannot explain, but since these are the first δ^2H-CH$_4$ measurements for an anoxic water-column, we show the data in Fig. 2 for comparison with future measurements. Excluding the unplotted points and considering measurements below 1000 m, the average water-column δ^2H-CH$_4$ is −127 ± 39‰.
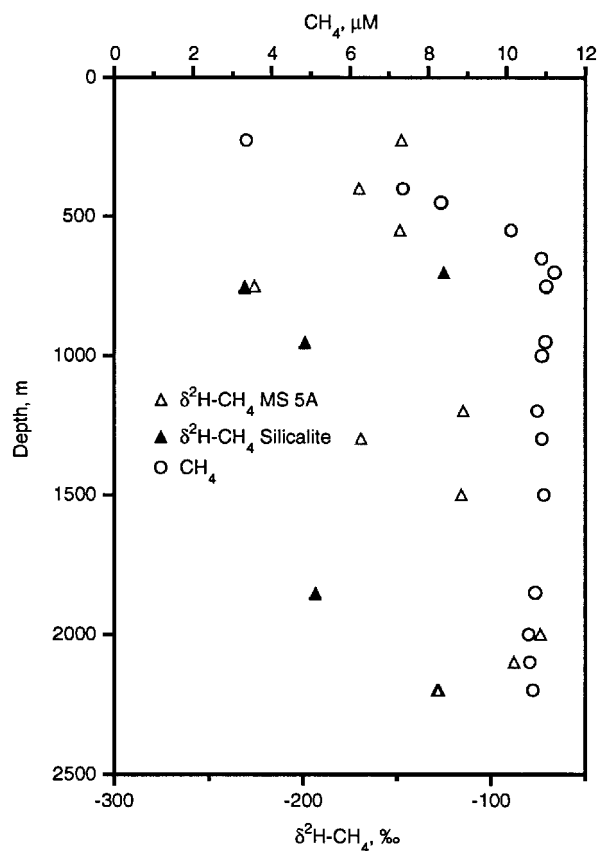

Fig. 3. Depth distribution of δ^{13}C-CH$_4$ at station BSK-2 in the Black Sea. Samples collected in Silicalite and Molecular Sieve 5A traps show that both media are equivalent. Methane concentrations are from equivalent depths in Reeburgh et al. (1991).

These samples were large and condensed water was visible, so they were analyzed with the expectation that errors resulting from storage would be small. We have no way to assess the effect of storage, but suspect it is small. As indicated above, the combustion water samples for δ^2H-CH$_4$ analysis were frozen directly into glass tubes, flame sealed, and stored for 5 years before transfer to zinc and analysis, contrary to the recommendations of Coleman et al. (1982). Further, we did not adhere strictly to Demeny's (1995) recommendation that H$_2$O:Zn ratios be held constant to avoid sorption and fractionation of H isotopes. We expect that

fractionation introduced by the non-constant H$_2$O:Zn ratio might be on the order of 20‰ (John Kessler, pers. commun.). We feel that incomplete methane oxidation is the most realistic explanation for the scatter. A body of isotope studies on atmospheric methane shows that incomplete oxidation has an almost indistinguishable effect on carbon isotopes, but large effects on hydrogen isotopes, so variations in carbon isotope give no indication of problems with hydrogen isotopes. Since these are the first δ^2H-CH$_4$ measurements for an anoxic water-column, we show the data in Fig. 2 for comparison with future measurements. The average water-column δ^2H-CH$_4$ below 1000 m is −127 ± 39‰.

3.2. δ^{13}C–CH$_4$

Distributions of δ^{13}C-CH$_4$ are relatively constant below 1000 m with a δ^{13}C of −48‰, and become

isotopically lighter from ~1000 m toward the surface, with a value of −57‰ at 200 m. The similarity of the δ^{13}C-CH$_4$ to the methane concentration and oxidation rate profiles raises the same questions discussed earlier. All are uniform at water depths greater than 600 m, and all are difficult to explain in the absence of a large sediment source or mixing mechanism.

Are there other possible methane sources in the Black Sea? Only one report of a non-diagenetic methane source for the Black Sea existed before the 1988 Black Sea Expedition. Yefremova and Zhizhchenko (1974) reported the presence of methane hydrates in the Black Sea, but reported no locations and gave no indication that they were unstable and might be an important water-column source. Hunt and Whelan (1978) reported surface sediment δ^{13}C-CH$_4$ values ranging from −63‰ to −70‰.

Since publication of Reeburgh et al. (1991) a number of reports of bubbling vents, methane clathrate hydrates, and mud volcanoes, all in the northern half of the Black Sea, have been published. Luth et al. (1999) reported studies on a seep area southwest of the Crimean Peninsula. Methane seeps in this area range in depth from 35 to 785 m and extend through the oxic/anoxic interface, which is located between 130 and 180 m. Luth et al. (1999) reported bacterial mats and abundant carbonate structures, ranging from small, flat structures in oxic waters to tall chimneys in the anoxic areas. The presence of carbonates is compelling evidence of extensive anaerobic methane oxidation, but no information was given on methane fluxes to the water-column. Michaelis et al. (2002) used a manned submersible to study a seep area located at the shelf edge west of the Crimean peninsula (44°46′ N, 31°60′ E, ~230 m) and observed microbial structures up to 4 m high. These structures consist of microbial mats with methane vents throughout their structure that are internally stabilized by carbonate precipitates. Tracer experiments using ^{14}C-CH$_4$ and ^{35}SO$_4^{2-}$ indicated active anaerobic oxidation of methane. Stable carbon isotope analysis of the carbonates indicated that a major part of the carbonate originated from anaerobic oxidation of methane. Analyses of methane from nearby seeps gave δ^{13}C values of −62.4‰ to −68.3‰.

Seismic evidence of seeps, mud volcanoes, and methane clathrate hydrates has been reported off the Crimean coast (Bouriak and Akhmetjanov, 1998; Ivanov et al., 1998). The seawater methane hydrate stability work of Dickens and Quinby-Hunt

(1994) indicates that methane hydrates in the deep Black Sea (9 °C) should be stable below 750 m. While the marine gas hydrate stability zone creates an effective geochemical barrier to upward-migrating methane, an unknown quantity of methane is able to transit the barrier (Ginsburg, 1998). Although the top clathrate boundary lies within the hydrate P–T stability field, lower methane concentrations here render this surface unstable (i.e. the chemical potential is lower). Thus, methane clathrate hydrates are a dynamic methane reservoir (Dickens, 2003), so hydrates within the P–T stability field are unstable and will dissociate at an unknown rate due to the difference of the chemical potential of methane in the hydrate phase and the overlying dissolved interstitial methane. Decomposing methane hydrates could serve as a methane source to the deep Black Sea water-column over the 750–2200 m depth interval. Radiocarbon measurements on clathrate methane are few, but methane clathrate hydrates collected at Hydrate Ridge, Cascadia Margin, are devoid of ^{14}C (Winckler, et al., 2002), indicating that there is no contribution of a recent (^{14}C-active) organic carbon reservoir to the hydrate pool.

If methane with a δ^{13}C of −63‰ to −70 ‰ is the source, then considerable quantities of methane must be oxidized to produce the observed water-column values of −48 ‰. This follows from the Reeburgh et al. (1991) observation that anaerobic methane oxidation is the largest sink term. The δ^{13}C-CH$_4$ of water-column methane is so strongly modified by oxidation and isotope fractionation that it cannot be a clear indicator of methane sources. If we treat the Black Sea water-column as an open system that is in concentration and isotopic steady-state, the following equation can be used to estimate the isotope fraction factor (α) associated with methane oxidation (John Kessler, pers. commun.):

$$\alpha = \frac{\delta^{13}C\text{-}CH_4(p) + 1000}{\delta^{13}C\text{-}CH_4(s) + 1000},$$

where δ^{13}C-CH$_4$(s) is the source methane (−63‰ to −70‰) and δ^{13}C-CH$_4$(p) represents the product, or water-column (−48‰) methane. The result, $\alpha = 1.016$–1.024, is higher, but close to the fractionation factor for anaerobic methane oxidation determined in sediments by Alperin et al. (1988) (1.0088 ± 0.0013) and Martens et al. (1999) (1.012 ± 0.001). The sediments in which these fractionation factors were determined were relatively closed

systems, with methane concentrations and oxidation rates that were at least 10^3 higher. These differences could account for the small difference in fractionation factor. This result suggests that our assumptions of concentration and isotopic steady state, as well as our suggestion that methane is being added to the Black Sea water-column approximately as fast as it is being oxidized, are realistic. The changes in methane concentration and δ^{13}C-CH$_4$ from the surface to about 1000 m result from a combination of factors: large numbers of vents that can exchange directly or more actively with the atmosphere, lower methane oxidation rates, less complete oxidation, and as a result, less isotope fractionation.

Restricted circulation in the Black Sea water-column allows using the Black Sea as a "biogeochemical bucket" to integrate the cumulative effects of isotope fractionation resulting from microbial oxidation of methane. Methane appears to be added to the Black Sea water-column approximately as fast as it is being oxidized, so it can be thought of as "running in place". Methane oxidation (and accompanying isotope fractionation) is so extensive that the water-column stable isotope measurements provide little information on methane sources. Measurements of natural ^{14}C-CH$_4$, which might be expected to be radiocarbon-dead, offer a possible means of discriminating between diagenetic methane formed from recently deposited organic carbon and fossil methane from hydrates and thermogenic sources.

Acknowledgements

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