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Carbon isotope biogeochemistry of acetate in sub-seafloor sediments in the Sea of Okhotsk near Sakhalin Island, Russia

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Abstract

Biogeochemical processes involving acetate in sub-seafloor sediments were investigated by examining the stable carbon isotopic relationships of acetate and other relevant carbon-bearing materials (i.e., total organic carbon (TOC) and ΣCO_2) in five piston cores retrieved from the Sea of Okhotsk off Sakhalin Island, Russia. The cores were classified into two types on the basis of SO_4^{2-} content: (I) those with sulfate-reducing sediments in which the depletion of pore-water SO₄²⁻ with increasing depth was slight, and (II) those with methanogenic sediments in which SO_4^{2-} concentrations were less than 2 mM more than 3 m below the seafloor. Acetate was detected in all cores. The acetate content in methanogenic sediments $(2.6-23.0\,\mu M)$ was relatively higher than in the sulfate-reducing sediment (2.8–8.8 μ M). In the sulfate-reducing sediments, the depth profiles of $\delta^{13}C_{acetate}$ approximately parallel those of $\delta^{13}C_{TOC}$, with the $\delta^{13}C_{acetate}$ values (-39% to -33%) depleted by about 12% relative to $\delta^{13}C_{TOC}$ (-24.5%) to -22.3%). These approximately parallel depth profiles suggest that the principal acetate production process in the sulfate-reducing sediments is fermentation of dissolved organic compounds. The fermentation products, however, tend to be similar or slightly enriched in ¹³C compared to their substrates. Therefore, the ¹³C depletion of acetate relative to TOC in the sulfate reduction zone suggests that some portion of the acetate was synthesized by acetogenesis in which the synthesized acetate is depleted in ¹³C compared with its precursor. Given the large contribution of land-derived organic matter in the studied sediments, organoautotrophic acetogenesis using the lignin-derived syringate monomer, which originates from land plants, is likely. In the methanogenic sediments, the δ^{13} Cacetate values in sediments throughout the cores (-39\%) to -25\%) were depleted compared to δ^{13} CTOC (-25.5\%) to -21.4%). This suggests some acetogenic contribution to the total acetate production. The depth profiles of $\delta^{13}C_{acetate}$ in methanogenic sediments did not parallel those of either $\delta^{13}C_{TOC}$ or $\delta^{13}C_{\Sigma CO2}$, probably because of the mixed isotopic effect from some production and consumption processes; namely fermentation, acetogenesis, and acetoclastic methanogenesis.

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1. Introduction

Acetate is a central intermediate of anaerobic metabolism in modern aquatic environments such as marine sediments (e.g., Sørensen et al., 1981; Brysch et al., 1987; Valentine, 2002). It is an end product of hydrolysis and microbial fermentation of carbohydrates (Iannotti et al., 1973) and long chain fatty acids (King and Klug, 1982), and of acetogenesis, wherein acetate is synthesized by an acetogen using the acetyl-CoA pathway for the reduction of CO₂ (e.g., Balch et al., 1977; Jones and Simon, 1985; Drake et al., 2002). It also serves as a substrate for both methanogenic and sulfate-reducing bacteria (e.g., Jeris and McCarty, 1965; Smith and Mah, 1966; Sørensen et al., 1981; Widdle and Pfenning, 1981).

Conceptually, the stable carbon isotopic composition (δ^{13} C) of acetate is presumed to be sensitive to the specific biogeochemical processes involved in its production and consumption. $\delta^{13}C$ of acetate is thus expected to be a useful indicator for quantifying biochemical pathways mediated by acetate. In fact, isotopic fractionation has been observed during acetate production and consumption in pure microbial cultures (Blair et al., 1985; Krzycki et al., 1987; Gelwicks et al., 1989; Preuß et al., 1989; Gelwicks et al., 1994; Londry and Des Maris, 2003; Valentine et al., 2004; Penning and Conrad, 2006; Goevert and Conrad, 2008). However, because of the generally low environmental levels of acetate, there are few carbon isotopic measurements of naturally occurring acetate in the literature and even fewer of naturally occurring acetate in the marine environment (Blair et al., 1987; Blair and Carter, 1992).

Recently, Heuer et al. (2006) developed a sensitive analytical method to measure δ^{13} C of acetate in marine sediment pore water. Heuer et al. (2009) subsequently used this method to measure δ^{13} C of acetate in deep subseafloor sediments in the northern Cascadia Margin, demonstrating that the isotopic relationship between acetate and dissolved organic carbon is a useful indicator of acetate sources and sinks, which can vary with depth in the sediment. Lever et al. (2010) also used this analytical method to measure δ^{13} C of acetate in deep subsurface sediments from the Juan de Fuca Ridge flank, and reported that acetate depleted in ¹³C relative to sedimentary organic matter indicates an acetogenic contribution to total acetate production. Investigations such as these in various types of marine sediments are helpful for improving our understanding of microbial activity and organic matter degradation via an acetate intermediate in marine sediments.

In this study, we investigated biogeochemical processes involving acetate in coastal sediments from the Sea of Okhotsk off Sakhalin Island north of Japan by examining the stable carbon isotopic composition of acetate in pore water and relevant carbon-bearing materials, specifically, total organic carbon (TOC) in the sediment and ΣCO_2 in pore water. The Sea of Okhotsk has an effective transport system that entrains particles from the bottom on the northwestern shelf into the adjacent deeper basin. This is associated with the formation of sea ice and dense shelf water (DSW) over the northwestern shelf region (Nakatsuka et al., 2002, 2004). The DSW contains large amounts of resuspended particles because of strong tidal mixing on the shelf (Kowalik and Polyakov, 1998). This resuspended matter from the shallower northwestern shelf is effectively transported to the southern deeper basin through the intermediate layers. A large amount of terrestrial organic matter supplied by Amur River to the northwestern area of the sea is further transported to the southern region by the intermediate water flow along east coast of Sakhalin Island (Seki et al. 2006). Therefore, our objectives were to determine the effects of terrestrial organic matter input to coastal sediments in this region on sub-seafloor microbial activity and the degradation of organic matter via acetate.

2. Materials and methods

We analyzed five piston cores retrieved from the western part of the Okhotsk Sea off Sakhalin in August-September 2007 during cruise XP07 of R/V *Professor Khromov* (Fig. 1): core B6 (collected at 49°29.918'N, 145°0.596'E; water depth 267 m; core length 575 cm), C6 (52°14.543'N, 145°59.952'E; water depth 850 m; core length 660 cm), C9 (52°14.965'N, 146°00.281'E; water depth 1431 m; core length 680 cm), D1 (53°59.558'N,



Fig. 1. Map of the study area showing the core sites and bottom topography. Contour depths are in meters.

144°50.098′E; water depth 1400 m; core length 712 cm), and D3 (53°59.854′N, 144°10.515′E; water depth 900 m; core length 725 cm).

For core C9 only, an age model was determined by Harada et al. (submitted) from the radiocarbon ages of planktonic foraminifera and the sediment color reflectance variable b* (Hunter Associates Laboratory, Inc., 2008) by comparison with the δ^{18} O curve of the Greenland ice core labeled with Dansgaard-Oeschger cycle interstadial numbers (Shackleton et al., 2004). This is possible because b* in the Okhotsk Sea sensitively reflects the millennial-scale climate changes recorded in the δ^{18} O curve of the ice core from the Greenland ice core project (GRIP) (Harada et al., submitted). On the basis of the age model, we estimated that the sediments in core C9 were deposited from 98 ka to the present. We could not correlate C9 sediment ages with those in the other cores on the basis of b*. As for the reason, we believe that the sediments of cores B6, C6, D1 and D3 is deposited during 10 kyr, because changes in b* of core C9 and δ^{18} O of the GRIP since 10 ka is small and cannot be correlated with each other over this time interval. The probable high sedimentation rates of cores B6, C6, D1, and D3 might be caused by the high accumulation rate

of terrestrial materials. Such differences in sedimentation rates should affect microbial activity in sediments, because the sedimentation rate affects the accumulation rate of organic matter, which is a controlling factor for the sulfate reduction rate in marine sediments (Westrich and Berner, 1984).

Cores were cut into meter-long sections and 50 cm³ of wet sediment was immediately taken from the bottom of each section for pore water sampling (i.e., 1-m sampling intervals). The pore water samples were extracted onboard by using a stainless-steel squeezer (Manheim and Sayles, 1974). Extracted pore water was filtered through $0.45-\mu m$ polytetrafluoroethylene (PTFE) filters (Millipore Co.). Subsamples of the pore water (about 5 mL) were stored frozen at -20° C in precombusted 10-mL glass vials sealed with a Teflon-coated septum and screw cap for later measurement of the acetate content and stable carbon isotopic composition. About 2 mL of each pore water sample was transferred to 3-mL glass vials with butyl septa, poisoned with HgCl₂, and stored at room temperature until measurement of the ΣCO_2 content and stable carbon isotopic composition. The remaining pore water was stored in polypropylene bottles under refrigeration at 3°C until measurement of the major dissolved components (Cl⁻, SO₄²⁻, etc.). After the pore water was extracted, the squeezed sediment samples were refrigerated at -20°C in plastic bags for analysis of sediment organic carbon.

The SO_4^{2-} content of pore water samples was analyzed using an ion chromatograph at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC). Analytical precision for SO_4^{2-} was estimated to be within 0.4% by repeated measurements of the same samples.

The pore water acetate content and carbon isotopic composition ($\delta^{13}C_{acetate}$) were determined at JAMSTEC by isotope-ratio-monitoring liquid chromatography/ mass spectrometry (irm-LC/MS) (Delta Plus XP isotope-ratio mass spectrometer connected to LC IsoLink (ThermoFinnigan, Bremen, Germany)) as described by Heuer et al., (2006), except that their chromatography protocol was modified. We used a TSK gel ODS-120A column (5- μ m particle size, 250 mm length, 4.6 mm i.d.; Tosoh Corp., Tokyo, Japan) equipped with a guard

column (TSKguardgel ODS-120A; 15 mm length; Tosoh Corp.). The column was kept at room temperature. For sample injection we adopted a 100- μ l sample loop, which is twice the size of that described by Heuer et al. (2006) to improve the detection limits for acetate. The quantitative determination of acetate is based on the linear correlation between the peak area of the m/z 44 signal recorded by irm-LC/MS and the amount of carbon injected (Heuer et al., 2006). The sensitivity of our irm-LC/MS system was around 0.40 Vs ng⁻¹ C on average. Standard deviations for carbon isotope measurements obtained by repeated analysis of the same sample were <0.6‰. The detection limit for carbon isotope analysis of acetate, confirmed by using a laboratory standard sodium acetate solution in artificial seawater, was $3 \mu M$. Examples of irm-LC/MS chromatograms for standard solutions of formate and acetate, and of the pore water sample from 2.7 meters below the sea floor (mbsf) in core D1 (acetate concentration: 13.6μ M) are shown in Figure 2.

The Σ CO₂ content and stable carbon isotopic composition (δ^{13} C_{2CO2}) in the pore water samples were measured at JAMSTEC with a ThermoFinnigan Delta Plus XP isotope-ratio mass spectrometer (IRMS) connected to a Flash EA 1112 Automatic Elemental Analyzer via a ConFlo III interface similar to the method described by Miyajima et al. (1995) and Toki et al. (2004). A portion



Fig. 2. irm-LC/MS chromatograms of standard solutions of formate and acetate (a), and of a pore water sample from 2.7 meters below the sea floor (mbsf) in core D1 (acetate concentration: $13.6 \,\mu$ M).

of the headspace gas in each sample vial was injected through a septum into the gas chromatograph in the elemental analyzer. The standard deviation obtained by repeated carbon isotope analysis of the laboratory standard NaHCO₃ solution was <0.2%.

The total nitrogen content and the TOC content and carbon isotopic composition ($\delta^{13}C_{TOC}$) of the sediment samples were also determined at JAMSTEC, after removal of carbonates with HCl, by using the ThermoFinnigan Delta plus XP IRMS connected to the Flash EA 1112 Automatic Elemental Analyzer via the ConFlo III interface (Ogawa et al., in press). Analytical error (1 σ) was estimated to be within 0.1‰ for $\delta^{13}C_{TOC}$ by repeated measurements of natural samples and aboratory standards.

3. Results and discussion

3.1. δ^{13} CTOC and C/N atomic ratios

Values for $\delta^{13}C_{TOC}$ ranged from -22.0% to -25.5%. The C/N atomic ratios ranged from 7 to 11. A plot of $\delta^{13}C_{TOC}$ versus the C/N ratio reveals their linear relationship (Fig. 3). Bulk organic δ^{13} C and C/N ratios have been widely used to estimate the contribution from terrestrial organic matter to total organic matter in coastal sediments because organic matter originating with marine phytoplankton is often isotopically heavier and has a lower C/N ratio than organic matter of terrestrial origin (e.g. Sackett and Thompson, 1963; Hunt, 1968; Shultz and Calder, 1976; Matson and Brinson, 1990). The linear relationship between $\delta^{13}C_{TOC}$ and C/N atomic ratios of the studied sediments (Fig. 3) clearly indicates the mixing of terrestrial and marine organic matter. Seki et al. (submitted) reported the contribution of higher terrestrial plants throughout the time interval covered by core C9 sediments based on the molecular distributions of long-chain n-alkanes. $\delta^{13}C_{TOC}$ values and C/N ratios in core C9 sediments (-23.4‰ to -22.4‰ and 6.4-7.4, respectively) were comparable to those in cores C6 and D3 (-22.4‰ to -21.4‰ and 6.7-7.6, respectively), and were enriched in ¹³C and lower than those in cores B6 and D1 (-25.5% to -23.3% and 8.4-9.8, respectively). We therefore infer that the contribution from terrestrial



Fig. 3. Relationship between $\delta^{13}C_{TOC}$ and the C/N atomic ratio in core sediments. Hollow symbols represent data from cores with sulfate reducing-sediments; solid symbols represent data from cores with methanogenic sediments.

organic matter in these cores is the same or greater than that in core C9 sediments, in which a substantial contribution from higher terrestrial plants was observed.

3.2. Classification of cores based on TOC and SO_4^2 content and $\delta^{13}C_{\Sigma CO2}$

The depth profiles of pore water SO₄^{2–} concentrations show depletion with increasing depth in all cores (Fig. 4a and a'). The SO42- concentrations in cores C6, D1, and D3 decreased substantially with increasing depth, and were less than 2 mM at depths greater than 3 mbsf (Fig. 4a'). In comparison, the decrease of pore water SO_{4²⁻} concentration in cores B6 and C9 was slight, and the concentrations at the bottom of the cores were greater than 5 mM (Fig. 4a). The average TOC content exceeded 1% by dry weight in the SO42--depleted cores C6, D1, and D3 (Fig. 4b'). Because the accumulation rate of organic matter is a primary controlling factor for the sulfate reduction rate in marine sediments (Westrich and Berner, 1984), the rapid consumption of SO_{4²⁻} in the upper 3 m of cores C6, D1, and D3 is probably attributable to both the high sedimentation rate and the high TOC content (>1%) in the cores.

 $\delta^{13}C_{\Sigma CO2}$ values in cores B6 and C9 decreased with increasing depth because of mixing of seawater ΣCO_2

and ΣCO_2 derived from organic matter oxidation accompanying sulfate reduction (Fig. 4e). In cores C6, D1, and D3, $\delta^{13}C_{\Sigma CO2}$ increased below the depth at which SO4²⁻ content was depleted (Fig. 4e') because of the ¹³C-enrichment of residual ΣCO_2 caused by microbial reduction of CO₂ to produce ¹²C-enriched methane.

On the basis of these chemical characteristics of the pore water, we classified the cores into two types: those containing sulfate-reducing sediments (cores B6 and C9), which encompass only the sulfate reduction zone, and those with methanogenic sediments (C6, D1, and D3), which include both the sulfate reduction zone and the methanogenesis zone.

3.3. Acetate contents and $\delta^{13}C_{acetate}$ in sulfate-reducing sediments

Acetate concentrations in the pore water of sulfatereducing sediments ranged from $2.8 \,\mu$ M to $8.8 \,\mu$ M (Fig. 4d, d'). These values are comparable to those previously reported for the sulfate reduction zone of marine sediments (<15 μ M) (e.g., Wellsbury and Parkes, 1995; Parkes et al., 2007; Heuer et al., 2009; Lever et al., 2010). According to these studies, the acetate content in the sulfate reduction zone is generally kept very low by rapid bacterial consumption because acetate is the principal substrate for sulfate reduction in marine sediments (e.g., Sørensen et al., 1981; Winfrey and Ward, 1983; Parkes et al., 1989; Wellsbury and Parkes, 1995).

The $\delta^{13}C_{acetate}$ in sulfate-reducing sediments ranged from -39% to -33% (Fig. 4e). The depth profiles of $\delta^{13}C_{acetate}$ approximately parallel those of $\delta^{13}C_{TOC}$, with the $\delta^{13}C_{acetate}$ values depleted by about 12‰ relative to $\delta^{13}C_{TOC}$ (Fig. 4e). These features, along with its depletion relative to $\delta^{13}C_{\Sigma CO2}$, are the most prominent characteristics of $\delta^{13}C_{acetate}$ in the sulfate-reducing sediments.

The approximately parallel depth profiles of $\delta^{13}C_{acetate}$ and $\delta^{13}C_{TOC}$ suggest that the principal process producing acetate in the sulfate-reducing sediments is fermentation of dissolved organic compounds. The processes that consume acetate, that is, the consumption of acetate during sulfate reduction, could not be identified on the basis of only $\delta^{13}C_{acetate}$ values. In the sulfate reduction zone, the $\delta^{13}C_{acetate}$ values should mainly reflect isotopic



Fig. 4. Depth profiles of pore-water SO₄²⁻ concentration in sulfate-reducing sediments (a) and methanogenic sediments (a'), and the same for TOC content (% dry wt) (b, b'), pore-water Σ CO₂ (c, c'), pore-water acetate concentration (d, d'), and the carbon isotopic compositions of Σ CO₂, TOC and acetate (e, e').

fractionation during acetate production, because there is only minor kinetic isotopic fractionation of carbon in acetate by sulfate reduction (Londry and Des Maris, 2003; Goevert and Conrad, 2008). However, the ¹³C depletion of acetate cannot be explained by the fermentative process alone, because fermentation products tend to be similar or slightly enriched in ¹³C with respect to their substrates (Penning and Conrad, 2006; Heuer et al., 2010). Thus, some production process other than fermentation must produce ¹³C-depleted acetate.

The ¹³C-depletion of acetate relative to TOC in the sulfate reduction zone is probably attributable to the proportion of the total acetate production that comes from acetogens, as previously reported (Lever et al., 2010, Ijiri et al., submitted). Acetate synthesized via the acetyl-CoA pathway during acetogenesis by an acetogen is depleted in ¹³C compared with its precursor (House et al., 2003). As a result of strong ¹³C-isotopic

fractionation during CO₂ reduction via the reductive acetyl-CoA pathway (Gelwicks et al., 1989; House et al., 2003), the end products, including acetate, are isotopically depleted relative to their carbon source.

There are various possible acetogenesis, such as lithoautotrophy, organoautotrophy, and organoheterotrophy (Drake et al., 2002; Lever et al., 2010). In general, sulfate-reducing bacteria are thought to outcompete acetogens for the shared H₂ substrate (Hoehler et al., 1999). Thus, the co-occurrence of acetogenesis using H₂ and sulfate reduction might occur only under certain conditions. However, Lever et al. (2010) inferred the co-occurrence of acetogenesis and sulfate reduction on basis of the observed ¹³C depletion of acetate relative to TOC in deep subsurface sediments of the Juan de Fuca Ridge flank. They reported that in situ energy yields of organoautotrophic acetogenesis with the lignin-derived monomer syringate and H₂ might even exceed that of sulfate reduction, thus allowing acetogens to successfully compete for H₂.

Lignin, which consists of methoxylated aromatic monomers (Sarkanen and Ludwig 1971), is a major component of terrestrial plant residues (King, 1986; Meyer-Reil, 1987, 1991; Hoppe, 1991; Kiem and Kögel-Knabner, 2003), and acetogenesis using methoxylated aromatic acids and syringate has been observed in culture experiments (Daniel et al., 1988; Hsu et al., 1990; Daniel et al., 1991). Although the lignin content of the studied sediments is unknown, given the inferred large contribution of land-derived organic matter in the studied area, organoautotrophic acetogenesis using the lignin-derived syringate monomer and H₂ can be considered likely. In addition, organoautotrophic acetogenesis using the lignin-derived monomer (syringate and vanillate) and pore water CO₂ can also be considered likely, because the depth profiles of δ^{13} Cacetate approximately parallel not only those of $\delta^{13}C_{TOC}$ but also those of $\delta^{13}C_{\Sigma CO2}$, with the $\delta^{13}C_{\text{acetate}}$ values depleted by about 18% relative to $\delta^{13}C_{\Sigma CO2}$ (Fig. 4e). Lever et al. (2010) also reported high energy yields from organoautotrophic acetogenesis using lignin monomers and pore water CO₂. For either case, we cannot estimate the relative contribution of the acetogenic process to total acetate production because the carbon isotopic fractionation during organoautotrophic acetogenesis has not been determined.

3.4. Acetate content and $\delta^{13}C_{acetate}$ in methanogenic sediments

The acetate concentration in pore water of methanogenic sediments ranged from 2.6μ M to 23.0μ M (Fig. 4d'). The acetate content in cores C6 and D3 increased with increasing depth. The concentrations in cores D1 and D3 were relatively higher than in the cores with sulfate-reducing sediment.

The $\delta^{13}C_{acetate}$ in methanogenic sediments ranged from -39% to -25% (Fig. 4e'). Because the $\delta^{13}C_{acetate}$ values were depleted relative to $\delta^{13}C_{TOC}$ in these sediments throughout the cores (Fig. 4e'), we infer some acetogenic contribution to the total acetate production. However, the depth profiles of $\delta^{13}C_{acetate}$ in methanogenic sediments are

more complex than those in sulfate-reducing sediments. The difference between $\delta^{13}C_{acetate}$ and $\delta^{13}C_{TOC}$ drastically changes with depth in each core. To explain the complex depth profile of $\delta^{13}C_{acetate}$, we infer mixed isotopic effects from some production and consumption processes. In the methanogenic sediments, isotopic fractionation is a consideration not only for the acetate production processes but also for the acetate consumption process, that is, acetoclastic methanogenesis.

During acetoclastic methanogenesis, the preferential consumption of acetate results in the enrichment of ¹³C in the residual pore water acetate pool (Krzycki et al., 1987; Gelwicks et al., 1994; Valentine et al., 2004; Penning et al., 2006). The relatively enriched δ^{13} Cacetate value in core D1 (up to -25‰) might reflect the contribution of acetoclastic methanogenesis. Furthermore, acetogenesis involving the reduction of pore-water CO₂ in the methanogenic zone just below the sulfate reduction zone (Heuer et al., 2009; Ijiri et al., submitted) should also be considered. In the depth range where CO₂ is used for acetogenesis, changes in $\delta^{13}C_{acetate}$ reflect $\delta^{13}C_{\Sigma CO2}$ changes (Heuer et al., 2009; Ijiri et al. submitted). Indeed, the increase of $\delta^{13}C_{acetate}$ with increasing depth below 2 mbsf in core D3 appears coincident with the observed rapid increase with depth in $\delta^{13}C_{\Sigma CO2}$ (Fig. 4e'). This suggests the contribution of acetogenesis, in which pore-water CO2 is used, to the total acetate production in core D3. Note that the effect of CO₂ reduction by acetogenesis on the increase in $\delta^{13}C_{\Sigma CO2}$ below 2 mbsf in core D3 was minor, because the acetate content is minor relative to the ΣCO_2 content (<0.01%); instead, the increase in $\delta^{13}C_{\Sigma CO2}$ should be attributed to CO₂ reduction during methanogenesis. In contrast, the depth profiles of $\delta^{13}C_{acetate}$ in cores C6 and D1 do not seem to be related to those of $\delta^{13}C_{\Sigma CO2}$.

Although further discussion is limited by the low sampling resolution for the drastically varied depth profiles of $\delta^{13}C_{acetate}$, our results show that comparison between the carbon isotopic composition of acetate and those of other relevant carbon-bearing compounds is a useful indicator for the biogeochemical processes involving acetate in the methanogenic zone.

4. Summary and conclusion

We investigated biogeochemical processes involving acetate by examining the stable carbon isotopic relationships of acetate and other relevant carbon-bearing materials (TOC and ΣCO_2) in samples from piston cores retrieved from the Sea of Okhotsk off Sakhalin Island.

On the basis of SO₄^{2–} content and $\delta^{13}C_{\Sigma CO2}$, the cores were classified into those containing sulfate-reducing sediments (B6 and C9) and those with methanogenic sediments (C6, D1, and D3).

In the cores with sulfate-reducing sediments, the depth profiles of $\delta^{13}C_{acetate}$ approximately parallel those of $\delta^{13}C_{TOC}$, with $\delta^{13}C_{acetate}$ values depleted by about 12‰ compared to $\delta^{13}C_{TOC}$. The approximately parallel depth profiles suggest that the principal acetate production process in the sulfate-reducing sediments is fermentation of dissolved organic compounds. However, the most likely explanation for the ¹³C depletion of acetate relative to TOC in the sulfate reduction zone is an acetogenic contribution to total acetate production, because acetate synthesized by an acetogen via the acetyl-CoA pathway is depleted in ¹³C relative to its precursor. Given the large contribution of land-derived organic matter to the studied area, organoautotrophic acetogenesis from the lignin-derived syringate monomer, which originates from land plants, is considered likely.

In the methanogenic sediments, the $\delta^{13}C_{acetate}$ values were depleted relative to $\delta^{13}C_{TOC}$ throughout the cores; thus, we infer some acetogenic contribution to the total acetate production as in the sulfate-reducing sediments. However, the depth profiles of $\delta^{13}C_{acetate}$ in methanogenic sediments are more complex than those in sulfate-reducing sediments; that is, the relationship between $\delta^{13}C$ of acetate and of other carbon-bearing compounds changes drastically with depth. As an explanation, we infer mixed isotopic effects from some production and consumption processes: fermentation, acetogenesis using various precursors, and acetoclastic methanogenesis.

Acknowledgments

We acknowledge the officers, crew, and scientific party aboard R/V *Professor Khromov*, operated by the Far Eastern Regional Hydrometeorological Research Institute, Russia, for their valuable collaboration. We gratefully acknowledge reviewers Dr. H. Naraoka and Dr. M. Kaneko for their careful reading and valuable comments for revision of this paper. Funding for this research was provided by the Research Joint Project between Japan and Russia No. 06037711-000164 from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Exploratory Research No. 21654072, and the Kurita Water and Environment Foundation.

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