Geochemical Journal, Vol. 47, pp. 567 to 571, 2013 doi:10.2343/geochemj.2.0272

NOTE

Increase in acetate concentrations during sediment sample onboard storage: a caution for pore-water geochemical analyses

AKIRA IJIRI,[1,2]* YOKO OHTOMO,[1] YUKI MORONO,[1,2] MINORU IKEHARA[3] and FUMIO INAGAKI[1,2]

[1]Geomicrobiology Group, Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Nankoku, Kochi 783-8502, Japan
[2]Geobio-Engineering and Technology Group, Submarine Resources Research Project, JAMSTEC, Nankoku, Kochi 783-8502, Japan
[3]Center for Advanced Marine Core Research, Kochi University, Nankoku, Kochi 783-8502, Japan

(*Received March 29, 2013; Accepted July 8, 2013*)

Microbial activity in marine sediment plays an important biogeochemical role in cycling of carbon and other elements. Acetate is a key intermediate of various microbial metabolic pathways. In this study, we measured concentrations and stable carbon isotopic compositions ($\delta^{13}C$) of acetate in pore water of two core samples stored at near *in-situ* temperatures (4°C) within 3 hours and at room temperatures (25°C) for 19 hours after sample recovery. Acetate concentrations at 4°C were less than 3 μM throughout the sediment column, whereas they increased up to 13 μM in the samples stored at 25°C and corresponding $\delta^{13}C$ values of acetate were enriched up to 8.7‰ relative to those of total organic carbon. Our results indicate that acetate-mediated microbial activity is rapidly changed with temperature increase and possible air contamination, and also suggest that sample processing at near *in-situ* temperatures in the short-term is required for accurate pore-water geochemical analysis.

Keywords: acetogen, microbial community, pore water, marine sediment, storage condition

INTRODUCTION

Shipboard squeezing of pore water from marine sediment is a common procedure for the study of pore-water geochemistry. When a sediment sample is recovered from the deep seabed or subseafloor, an increase in ambient temperature of the sediment sample, in addition to a decrease in total pressure, is unavoidable during the sample processing and storage. Pore-water, therefore, has to be extracted immediately after sample retrieval to minimize possible changes of pore-water chemical constituents. However, it often takes several hours on the ship until a sample is available for pore-water extraction. The effects of sediment condition on major elements in pore-water have been previously assessed (Masuzawa *et al*., 1980; De Lange *et al*., 1992). Since new analytical methods have recently been developed to measure various chemical components in pore water, it is worthwhile to further address the artifacts caused by sample processing and storage prior to the analysis.

The concentration and carbon isotopic composition ($\delta^{13}C$) of acetate in marine sediment are such recent additions to the pore-water geochemical analyses, following the technological advancement of a sensitive analytical tool (Heuer *et al*., 2006), which was developed to understand subseafloor biogeochemical processes via acetate. It has been pointed out that acetate in sediment could be sensitive to a temperature increase, resulting in increased acetate concentrations after incubation of sediment at high temperature (Wellsbury *et al*., 1997). In this brief note, we simulated two possible cases of shipboard sample processing; i) short-term storage of the corer with rubber stoppers in a refrigerator at near *in-situ* temperature (4°C) until pore water extraction to minimize air contamination and temperature change represents an ordinarily procedure on board, even though a small temperature change from the absolute *in-situ* temperature to 4°C and air contamination is unavoidable, ii) storage at room temperature for 19 hours represents a worst possible condition. Such a worst case occasionally occur to cut core samples which have to be warmed to room temperature for non-destructive measurement of physical properties using a multi-sensor core logger (MSCL) for 12–17 hours before pore-water extraction (Ijiri *et al*., 2012). Those two samples have been provided to mea-

*Corresponding author (e-mail: ijiri@jamstec.go.jp)

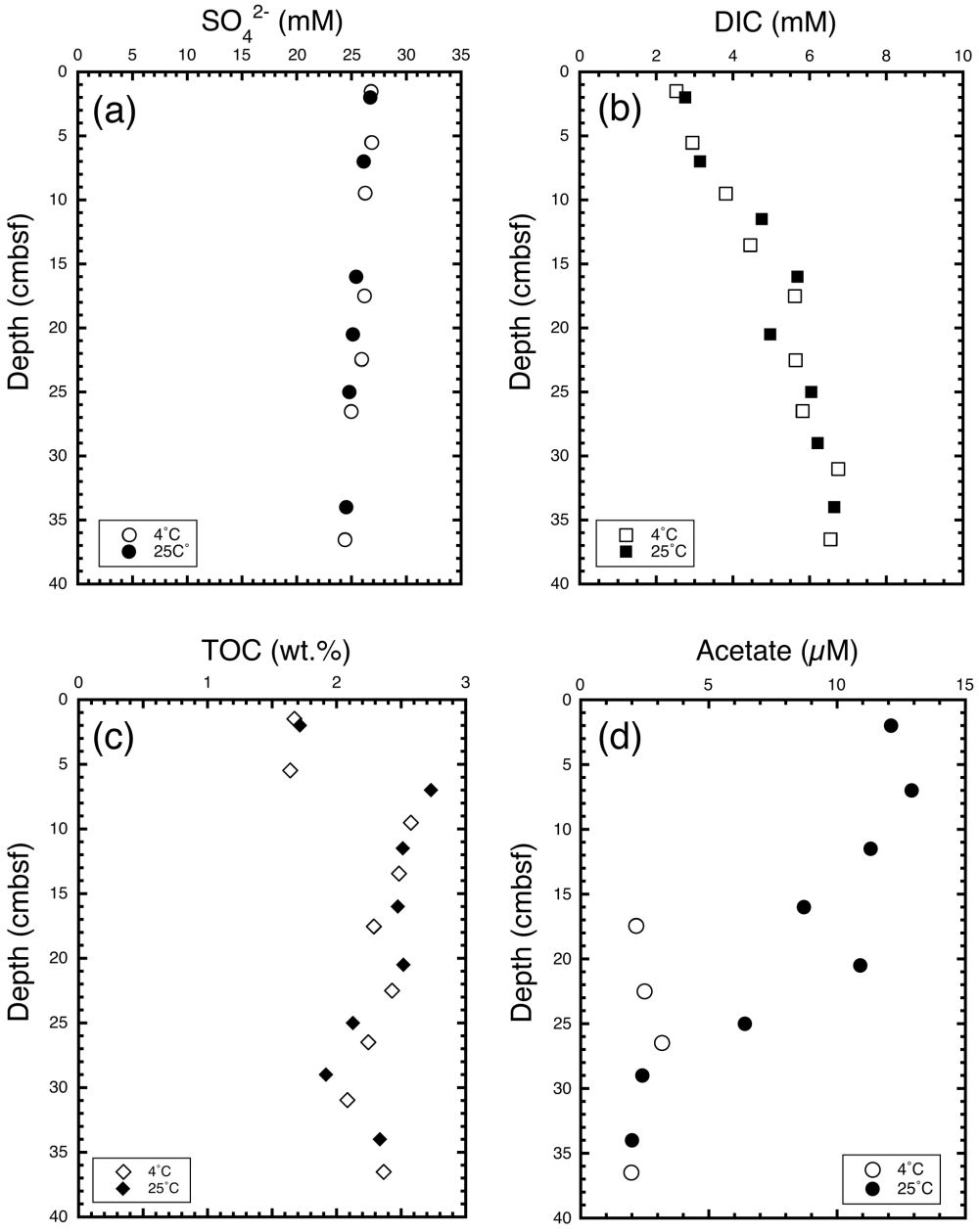

Fig. 1. Depth profiles of (a) SO_4^{2-}, (b) DIC, (c) TOC, and (d) acetate concentrations in pore-water of push-core sediment samples stored at 4°C and 25°C. The analytical precisions for SO_4^{2-}, DIC, and acetate were 0.4%, 3%, and 4%, respectively.

sure the concentration and $\delta^{13}C$ of acetate, total organic carbon (TOC) and dissolved inorganic carbon (DIC) as well as sulfate concentrations, and discuss the requirements of shipboard sample processing and storage for geochemical analyses.

Materials and Methods

We used two near-surface sediment push cores (about 40 cm long) collected from the Integrated Ocean Drilling Program (IODP) Site C0020A off Shimokita Peninsula in northeast Japan (41°10.60′ N, 142°12.03′ E; water depth, 1182 m; bottom water temperature, 2.5°C: see Inagaki *et al*., 2012) by the remotely operated vehicle (ROV) *Hyper-Dolphin* (Dive #1339) on 18 November 2011 during the KY11-E06 site survey cruise of R/V *Kaiyo*. The two push-cored sediment samples were collected within 1 m of horizontal distance to each other.

After the push-core samples were recovered onboard, one corer was put into a refrigerator kept at 4 ± 0.1°C

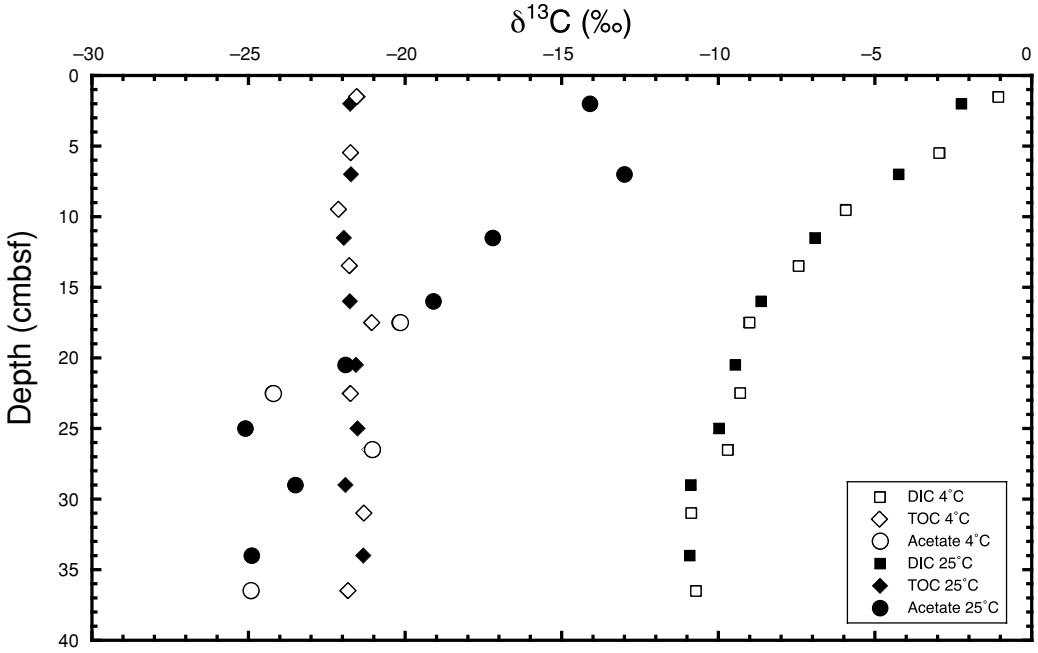

Fig. 2. Carbon isotopic compositions of DIC, TOC, and acetate in pore-water of push-core sediment samples stored at 4°C and 25°C. The analytical precisions for the isotopic compositions of DIC, and acetate were <0.2‰ and <1‰, respectively.

within 3 hours to minimize temperature change from *in-situ* condition, and the other was stored at room temperature (25 ± 1°C) for 19 hours, and then pore-water samples were extracted in a laboratory on the *Kaiyo*. The rubber stoppers at the top and bottom of the corer were kept in place during storage to avoid evaporation, degassing, and air contamination.

Procedures for sub-sampling, pore-water extraction, and storage of pore water are described in Tsunogai *et al.* (2002), Manheim (1968), and Ijiri *et al.* (2012), respectively. After the extraction of pore water, the squeezed sediment was kept at –20°C for analysis of TOC.

Analytical methods for acetate, DIC, and SO_4^{2-} in pore-water samples are previously described (Ijiri *et al.*, 2012). Sediment TOC content and δ^{13}C-TOC were determined by using an EAirMS (Flash EA 1112 coupled to Finnigan DeltaPlus Advantage IRMS). The analytical error for determination of organic carbon with the method used was less than 0.01 wt%. The precision of the δ^{13}C-TOC determination was better than 0.1‰.

Results and Discussion

Depth profiles of SO_4^{2-}, TOC and DIC concentrations, as well as δ^{13}C values of TOC and DIC, in the push-core samples stored at 4°C within 3 hours and 25°C for 19 hours (hereafter, 4°C-core and 25°C-core) were almost the same within the range of analytical error (Figs. 1 and 2). The depth profiles of SO_4^{2-} concentration show a slight depletion with increasing depth. The DIC concentrations and δ^{13}C-DIC values increased and decreased with increasing depth, respectively. Those characteristics have been commonly observed in marine sediment and can be explained by consumption of SO_4^{2-} by sulfate-reducing bacteria and mixing with DIC from the overlying seawater as well as the oxidation of buried organic matter, which possibly includes the end product of sulfate reduction. The almost identical trend with depth of DIC and SO_4^{2-} between the 4°C- and 25°C-cores suggests that organic matter oxidation, including sulfate reduction, was not affected by the storage temperature for 19 hours, or that the effect was too small to detect in DIC and SO_4^{2-}.

In marked contrast, the depth profiles of acetate were substantially different between the two cores under different storage temperatures (Fig. 2d). The acetate concentrations in the 4°C-core were less than 3 μM and the concentrations shallower than 17.5 cm below the seafloor (cmbsf) were below the detection limit (i.e., 2 μM). In the 25°C-core, however, the concentrations shallower than 25 cmbsf were as high as 13 μM, which are notably higher than those in the 4°C-core. These concentrations are comparable to those previously reported for the sulfate reduction zone of marine sediments (<15 μM), in which the acetate concentration is kept very low by the well-balanced acetate production and sulfate reduction (e.g., Wellsbury *et al.*, 1997). We calculated the maximum pro-

duction rate for acetate in pore water as 16 nmol/mL/day assuming that the maximum acetate concentration (13 μM) at 5.5 cm in the 25°C-core increased from zero during the 19 hours of storage. However, the concentrations deeper than 25 cmbsf are comparable to those in the 4°C-core. The higher acetate concentrations in the shallow 25°C-core sediment suggest that the microbial community in shallow sediment is metabolically highly active, and its metabolic activity producing acetate can be rapidly stimulated by the warmer conditions. Another reason for the high acetate concentrations in the shallow sediment could be that the freshly buried shallow organic matter, which had been less degraded than those in deeper sediments, was more bioavailable and sensitive to the temperature change. Additionally, air contamination through the rubber stopper over 19 hours may stimulate the oxidation of organic matter to acetate or prevent anaerobic sulfate reduction from consuming acetate in shallow sediment; this could also result in increase of acetate concentration. For the 4°C-core, acetate concentration would not be affected by the small temperature change from 2.5°C to 4°C for 3 hours, or the effect was too small to detect. Consequently, our result means that even sample storage within a day on the ship may result in substantially increased acetate concentrations in the sediment pore water.

The co-relation between δ^{13}C-acetate and δ^{13}C-TOC is a useful indicator of acetate sources and sinks (Heuer *et al*., 2010; Ijiri *et al*., 2012). In this study, the δ^{13}C-acetate profiles deeper than 16 cmbsf in both cores were similar, although the δ^{13}C-acetate values shallower than 17.5 cmbsf in the 4°C-core are unknown because they were below the detection limit. The δ^{13}C-acetate values shallower than 25 cmbsf were enriched in ^{13}C compared to δ^{13}C-TOC. The δ^{13}C-acetate values increased with increasing acetate concentrations, up to –13.0‰ and 13 μM at 7 cmbsf in the 25°C-core, respectively. The δ^{13}C-acetate was 8.7‰ enriched in ^{13}C relative to TOC at this depth.

A conceivable explanation for the ^{13}C-enrichment in acetate relative to TOC with increasing acetate concentrations is the fermentative production of acetate: e.g., laboratory experiments with pure cultures of *Clostridium papyrosolvens* showed that mixed-acid fermentation of saccharides resulted in the production of acetate slightly enriched in ^{13}C (<3‰) as compared with TOC (Penning and Conrad, 2006). This is because δ^{13}C-acetate is enriched in ^{13}C compared to the alternative product (e.g., ethanol) at a branching point of the acetyl-CoA pathway. Moreover, Penning and Conrad (2006) reported that a high carbon conversion to ethanol would result in the production of acetate, which was relatively strongly enriched in ^{13}C compared to TOC (<17‰). Another laboratory incubation of lake sediments, in which fermentation is the dominant microbiological process, also showed that δ^{13}C values of acetate were close to or slightly higher (4.5 ± 1.4‰) than those of TOC in the sediment solid phase (Heuer *et al*., 2010).

In this study, we observed the rapid increase of acetate concentrations in surface marine sediment by warming and possible air contamination during a short period of the sample processing and storage after recovery. The observation provides a warning about the analytical requirements for pore-water geochemistry in marine sediment samples, especially for seafloor to shallow subsurface sediments that contain freshly buried organic matter and metabolically highly active microbial communities. In addition, our study suggests that microbial activity in seafloor sediment has a wide range of metabolic potential in response to temperature change.

Acknowledgments—We acknowledge the officers, crew, and shipboard scientific party of the KY11-E06 site survey cruise of R/V *Kaiyo* for the sample retrieval and technical support. This work was supported in part by the Grant-in-Aid for Young Scientists (A) No. 23681007 (to A.I.) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT), and the Japan Society for the Promotion of Science (JSPS) Funding Program for Next Generation World-Leading Researchers (NEXT Program; to F.I.).

References

De Lange, G. J., Cranston, R. E., Hydes, D. H. and Boust, D. (1992) Extraction of porewater from marine sediments: A review of possible artifacts with pertinent examples from the North Atlantic. *Mar. Geol.* **109**, 53–76.

Heuer, V., Elvert, M., Tille, S., Krummen, M., Prieto Mollar, X., Hmelo, L. R. and Hinrichs, K.-U. (2006) Online δ^{13}C analysis of volatile fatty acids in sediment/pore water systems by liquid chromatography-isotope ratio-mass spectrometry. *Limnol. Oceanogr. Methods* **4**, 346–357.

Heuer, V. B., Krüger, M., Elvert, M. and Hinrichs, K.-U. (2010) Experimental studies on the stable carbon isotope biogeochemistry of acetate in lake sediments. *Org. Geochem.* **41**, 22–30.

Ijiri, A., Harada, N., Hirota, A., Tsunogai, U., Ogawa, N. O., Itaki, T., Khim, B.-K. and Uchida, M. (2012) Biogeochemical processes involving acetate in sub-seafloor sediments from the Bering Sea shelf break. *Org. Geochem.* **48**, 47–55.

Inagaki, F., Hinrichs, K.-U., Kubo, Y. and Expedition 337 Scientists (2012) Deep coalbed biosphere off Shimokita: microbial processes and hydrocarbon system associated with deeply buried coalbed in the ocean. *IODP Prel. Rept.* **337**, 1–62; doi:10.2204/iodp.pr.337.2012.

Manheim, F. T. (1968) Disposable syringe techniques for obtaining small quantities of pore water from unconsolidated sediments. *J. Sediment. Petrol.* **38**, 666–668.

Masuzawa, T., Kanamori, S. and Kitano, Y. (1980) The reversible effect of temperature on the chemical composition of

interstitial water of marine sediment. *J. Oceanogr. Soc. Japan* **36**, 68–72.
Penning, H. and Conrad, R. (2006) Carbon isotope effects associated with mixed-acid fermentation of saccharides by *Clostridium papyrosolvens*. *Geochim. Cosmochim. Acta* **70**, 2283–2297.
Tsunogai, U., Yoshida, N. and Gamo, T. (2002) Carbon isotopic evidence of methane oxidation through sulfate reduction in sediment beneath cold seep vents on the seafloor at Nankai Trough. *Mar. Geol*. **187**, 145–160.
Wellsbury, P., Goodman, K., Barth, T., Cragg, B. A., Barnes, S. P. and Parkes, R. J. (1997) Deep marine biosphere fuelled by increasing organic matter availability during burial and heating. *Nature* **388**, 573–576.