Geophysical Research Letters

RESEARCH LETTER
10.1029/2018GL081284

Key Points:
- Volatile fatty acids (VFAs) and alcohols were abundant in the hydrothermal sediment of the Guaymas Basin
- Isotopic signatures, thermodynamics, and sediment incubations suggest that the source of VFAs is biological fermentation and acetogenesis
- High oxidation rates of acetate and methanol document utilization predominantly by nonmethanogenic chemoheterotrophs as an energy source

Supporting Information:
- Supporting Information S1

Correspondence to:
S. B. Joye, mjoye@uga.edu

Citation:

1Department of Marine Sciences, University of Georgia, Athens, Georgia, USA, 2MARUM Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany, 3State Key Laboratory of Biogeology and Environment Geology, College of Marine Science and Technology, China University of Geosciences, Wuhan, China, 4Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA, 5Deceased

Abstract
Volatile fatty acids (VFAs) and alcohols are key intermediates of anaerobic carbon metabolism, yet their biogeochemical cycling remains poorly constrained in hydrothermal systems. We investigated the abundance, stable carbon isotopic composition, and metabolic cycling of VFAs and alcohols to elucidate their generation and utilization pathways in hydrothermally influenced sediments (4 °C to 90 °C) from the Guaymas Basin. Acetate (up to 229 μM) and methanol (up to 37 μM) were abundant in porewaters. The δ13C values of acetate varied between −35.6‰ and −18.1‰. Carbon isotopic signatures, thermodynamic predictions, and experimental incubations suggested biological sources such as fermentation and acetogenesis for acetate. Acetate and methanol were predominantly consumed by nonmethanogenic processes (e.g., sulfate reduction), as reflected in high oxidation rates versus low methanogenesis rates, and further evidenced through inhibition experiments with molybdate. These results reveal an important role for VFAs and alcohols as energy sources for diverse chemoheterotrophs in organic-rich hydrothermally influenced sediments.

Plain Language Summary
Hydrothermal systems are unique seafloor habitats that host abundant and diverse microbial communities, but questions remain regarding their energy strategy and metabolic activity. We found that low molecular weight organic compounds such as acetate and methanol were abundant in the hydrothermal sediments of Guaymas Basin. Multiple lines of evidence suggested that these substrates were produced largely via biological pathways. We further investigated the microbial metabolism of acetate and methanol and found that both compounds could be used as an energy source to support various microbial processes in the hydrothermal systems.

1. Introduction
Seafloor hydrothermal systems provide unique habitats for a diverse array of microorganisms that live and thrive under extreme conditions. The Guaymas Basin in the Gulf of California is a deep-sea hydrothermal system characterized by active seafloor spreading coincident with an area of massive sediment accumulation (Teske et al., 2016). The hydrothermally heated sediments in this basin host abundant and diverse microbial communities that mediate active biogeochemical cycles (Dhillon et al., 2003; Edgcomb et al., 2002; Teske et al., 2002). Advection of hydrothermally altered fluids through the organic-rich sediments generates end products such as methane, hydrogen, short-chain alkanes, and even oil that accumulate to considerable abundance (Bazylinski et al., 1988; Kawka & Simonet, 1987; Welhan & Lupton, 1987; Welhan et al., 1988). Energy-laden fluids are transported subsequently through the sediment column, providing organic substrates to fuel microbial activity and growth and promote microbial diversity. Near-surface heating of sediments also facilitates production and discharge of reactive dissolved organic carbon (Liu et al., 2017). Guaymas Basin surficial sediments provide a rare and accessible window for investigating the impact of microbially mediated processes on carbon and other elemental (e.g., sulfur and nitrogen) cycles. Several important microbial processes, such as methane oxidation, sulfate reduction, and sulfide oxidation, have received considerable attention in Guaymas hydrothermally altered sediments (Biddle et al., 2012; Dowell et al., 2016; Jørgensen et al., 1990; Kallmeyer & Boetius, 2004; Nelson et al., 1989). In addition to
methane and other hydrocarbons, low molecular weight (LMW) organic compounds are produced in hydrothermally altered sediments. Martens (1990) found that acetate and propionate concentrations reached up to >1100 and 200 μM, respectively, in the Guaymas Basin. The volatile fatty acids (VFAs) could constitute important substrates for various terminal metabolic processes. A previous study demonstrated that elevated acetate production from organic matter during burial and heating provided a bacterial energy source that fueled the deep subsurface biosphere (Wellsbury et al., 1997). Similar to VFAs, alcohols are another class of key metabolites, but information on their cycling is quite limited in marine sediments. Recently, Tremblath-Reichert et al. (2017) reported that methylated compounds including methanol were used by nonmethanogenic heterotrophic microorganisms in 2 km-deep subseaflor coal and shale beds.

Multiple lines of evidence suggested that VFAs and alcohols serve as important energy sources to support microbial growth in the Guaymas Basin. However, the metabolic pathways and cycling rates of LMW carbon substrates remain unconstrained in these sediments. We sought to track the generation and fate of VFAs and alcohols and to decipher their role as energy sources for microbial processes at hydrothermal sites in the Guaymas Basin. We characterized the pool sizes of VFAs and alcohols, determined the stable carbon isotopic composition of VFAs, and quantified the rates of acetate and methanol oxidation and their conversion to methane. The results provide new insight into the carbon metabolism and cycling in seafloor hydrothermal systems.

2. Materials and Methods

2.1. Sample Collection and Geochemical Analysis

Sediment samples were collected from hydrothermal areas of Guaymas Basin in the Gulf of California during cruise AT37-06 of R/V Atlantis in December 2016. Push cores were obtained using the human-occupied deep diving vehicle Alvin during dives 4867 to 4872. A general description of sampling sites is available in Table S1. At each site, parallel cores were collected, sectioned, and sampled for geochemical characterization and microbial rate measurements.

Porewater was obtained by centrifugation (15 min at 4000 rpm) and then filtered through a precleaned 0.2-μm syringe filter (Target®). Depending on the porosity (e.g., depth), ~15 to 25 mL of pore water was extracted from 45 mL of wet sediment. Samples for quantification of concentrations of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), VFAs, and alcohols were immediately frozen and stored at ~20 °C or ~80 °C (i.e., alcohols) before analysis. For rate assays, approximately 3 mL of live sediment was transferred into a modified Hungate tube as described previously (Bowles, Samarkin, et al., 2011). At each depth, triplicate live samples and one killed control were collected for the quantification of acetate and methanol turnover rates.

The in situ temperature in the sediment was measured at 10-cm intervals over a 0.6-m profile with Alvin’s external temperature probe (McKay et al., 2012). DIC was quantified using a GC-FID outfitted with a methanizer (Shimadzu GC14); the instrument was standardized according to Segarra et al. (2015). The concentration of DOC was quantified using a Shimadzu TOC-V equipped with a nondispersive infrared detector; reagent-grade potassium phthalate was used as the standard (Joye et al., 2004). The concentrations and stable carbon isotopic composition of VFAs were determined using liquid chromatography coupled to an isotope ratio mass spectrometer (DELTA Plus XP IRMS) via a LC Isolink interface (ThermoFinnigan; Heuer et al., 2006). Dissolved methanol and ethanol were quantified using a purge and trap system connected to a GC-FID (SRI) using a method modified from Zhuang et al. (2014).

2.2. Rate Measurements for the Metabolism of Acetate and Methanol

We used a radiotracer approach to quantify the rates of acetate and methanol oxidation to CO₂ and substrate conversion to methane (Zhuang, Montgomery, & Joye, 2019). Quadruplicate samples (one killed control and three live samples) were injected with 100 μL of 13C-labeled 2-13C-acetate (200 kBq) or methanol (42 kBq; American Radiolabeled Chemicals, Inc., specific activity ~1.85–2.2 GBq/mmol) in modified Hungate tubes. Control samples were killed before tracer addition by injecting 3 mL of 2 M NaOH and mixing the samples via vortexing. All samples were incubated at in situ temperature for three days, after which the activity in live samples was terminated in the same manner as the control (i.e., killed with base). The addition of 13C label resulted in a tracer concentration of 42 μM acetate and 8.7 μM methanol, respectively. These rates are
considered environmentally relevant since the added tracer concentrations were similar in magnitude to in situ substrate concentrations.

Methane production rates from acetate and methanol were determined by quantitatively converting produced $^{14}$CH$_4$ to $^{14}$CO$_2$ with copper oxide at 850 °C; the details of the method have been described previously (Zhuang, Heuer, et al., 2018). The pool turnover time in days (d) was calculated based on the time required to metabolize the total amount of added $^{14}$C-labeled acetate and methanol to methane. The turnover rate to methane ($d^{-1}$) was expressed as the reciprocal of the turnover time. Methanogenesis rates were calculated by multiplying the in situ substrate concentration by the turnover rate and isotopic fractionation factor (Zhuang, Heuer, et al., 2018).

The oxidation rates of acetate and methanol were determined by tracking $^{14}$CO$_2$ production from labeled tracers through acidifying the material remaining after the produced $^{14}$CH$_4$ was stripped from the Hungate tubes under basic conditions (Zhuang, Montgomery, & Joye, 2019). Similarly, the turnover time to CO$_2$ (d) was calculated as the time required to oxidize the added $^{14}$C-labeled tracer to CO$_2$. The rate of substrate turnover to CO$_2$ was expressed as the reciprocal of the turnover time ($d^{-1}$). Oxidation rates were calculated by multiplying the in situ substrate concentrations by the turnover rate. All the rates and turnover time calculations were corrected by killed sample counts. After removing the produced $^{14}$CH$_4$ and unused residual $^{14}$CO$_2$ by acidifying and purging the sediment, we used a Soxhlet extraction to quantify $^{14}$C organic intermediates in the liquid phase; this provided an estimate of the production rate of volatile organic carbon (VOC), such as acetate, from bicarbonate (see supporting information).

2.3. Effect of Inhibitors on the Utilization of Acetate and Methanol

Inhibition experiments were conducted to estimate the role of sulfate-reducing bacteria and methanogens in acetate and methanol metabolism. Slurries were prepared by mixing sediment with anoxic artificial seawater medium (1:1 ratio; slightly modified from Parkes et al. (2010)). A final concentration of 25 mM molybdate and 30 mM 2-bromoethanesulfonate were employed to inhibit the activity of sulfate reduction and methane production, respectively (Zhuang, Montgomery, Sibert, et al., 2018). Rates were determined as described above.

3. Results and Discussion

3.1. Distribution and Stable Carbon Isotopic Composition of LMW Dissolved Organic Carbon in Guaymas Basin Sediments

In the hydrothermal sediments of Guaymas Basin, the concentrations of DOC ranged from 0.3 to 8.8 mM (Figures 1a–1f). High DOC concentration was documented in the cold Northern Towers mat (>5.7 mM for ~10 °C sediments; Dive 4871; Figure 1e), while DOC concentration was less than 1 mM at the higher-temperature sites, Ultra Mound and Cathedral Hill (>60 °C; Figures 1b and 1f). DOC concentrations generally increased with depth (Figures 1a, 1c, and 1e). In contrast, a decrease in DOC with increasing depth and temperature was observed at some hot sites (>60 °C; Figures 1b and 1f), and this could be indicative of inhibition of biological DOC production under elevated temperature or reduced DOC production at these sites due to previous prolonged exposure of the sediment to elevated temperature, which had resulted in increased abiotic DOC production and subsequent loss by advection (Lin et al., 2017).

Formate, acetate, and lactate were the VFAs detected in the porewaters. Acetate was the most abundant VFA (Figures 1g–1l), and its concentration reached up to 229 μM (Dive 4871-1, hot Northern Towers mat; Figure 1j). These concentrations were similar to previous reports for Guaymas Basin sediment (McKay et al., 2012; Teske et al., 2016), and comparable to values observed in subseafloor sediments (e.g., northern Cascadia Margin; Heuer et al., 2009). While acetate increased with depth at the Aceto Balsamico site, a subsurface maximum of >100 μM acetate was observed at the Northern Towers mats that were dominated by white Beggiatoa. Lactate was detected at most of the sites (Figures 1g–1l), with a maximum of 53 μM observed at the Guaymas 9A site (Dive 4867). In contrast, formate was only detected in the surface sediments of Aceto Balsamico mat (upper 13.5 cm, concentration: 6 to 9.3 μM) and in deep sediments of the hot Northern Towers mat (Dive 4871: below 11.5 cm, concentration: 24 to 72 μM). Generally, acetate and lactate were minor constituents of bulk DOC, contributing 2.9% ± 2.6% and 3.6% ± 6.6% of the bulk DOC pool,
respectively. However, acetate constituted up to 11% of bulk DOC in Northern Towers-1 sediment and lactate accounted for >12% of DOC in the low organic carbon mat at Ultra Mound. Methanol and ethanol were also detected in the porewaters, although the concentrations were lower than those of VFAs (methanol: 0.2 to 36.7 μM, ethanol: 0.6 to 15.3 μM; Figures 1p–1r). Interestingly, methanol increased significantly with increasing depth and temperature in the hot Northern Towers mat (Figure 1p), where concentrations reached ~37 μM near the bottom of the core; this value is among the highest values reported for marine sediments (Yanagawa et al., 2016; Zhuang et al., 2014). Ethanol concentrations were elevated at this site (~3 μM), compared to other sites which exhibited concentrations of ~1 μM.
As a metabolic intermediate of organic matter degradation, methanol can be produced from lignin, pectin, and carbohydrates (Donnelly & Dagley, 1980; Schink & Zeikus, 1980). The increase in methanol concentration over depth could indicate the elevated production due to the thermal alteration of organic matter.

The stable carbon isotopic composition of formate varied between $-19.6\%$ and $-15.2\%$ in the Northern Towers-1 mat (Figure 1m), and formate was progressively enriched in $^{13}\text{C}$ with depth. Acetate isotopes spanned a wide range from $-35.6\%$ to $-18.1\%$ at Guaymas Basin sites and averaged $-24\% \pm 5\%$ (Figure 1n). In the Aceto Balsamico and Northern Towers-12 mats, $^{13}\text{C}$ values for acetate became increasingly heavy with depth, but the opposite pattern was observed for the Northern Towers-1 mat, where the $^{13}\text{C}$ content of acetate decreased slightly in deeper sediments. In contrast to formate and acetate, lactate was more depleted in $^{13}\text{C}$, with an average isotope value of $-30.6\%$. Its isotopic composition was also less variable with depth (Figure 1o).

Collectively, the concentration of VFAs and alcohols reflects the balance of production and consumption processes. Differences in the stable carbon isotope values of VFAs suggested variability in carbon precursors used to generate these intermediates as well as differences in the relative rates of processes that control pool sizes within and between sites. This information helps to constrain the pathways of formation and utilization of LMW compounds, and to identify biogeochemical processes involved in their cycling in the hydrothermal sediments.

3.2. Generation and Fate of VFAs and Alcohols in the Guaymas Basin Sediments

Theoretically, both abiotic and biological sources may contribute to production of VFAs in hydrothermal systems. Lang et al. (2010) observed elevated concentrations of formate and acetate in sediment-starved samples from the Lost City hydrothermal field and suggested an abiotic formation mechanism for formate. Abiotic formation of organic acids from inorganic carbon can be thermodynamically favorable under hydrothermal conditions (high $\text{H}_2$, $\text{pH}$, and temperature; Shock & Schulte, 1998). Further, abiotic reduction of carbon dioxide to formate has been demonstrated experimentally in the laboratory (McCollom & Seewald, 2003).

Evidence for abiotic production of VFAs in these sediment-rich samples is lacking. In the case of abiotic formation, formate concentrations should exceed those of acetate, since formate is the simplest VFA and because it is a primary product of the abiotic synthesis from $\text{CO}_2$/H$_2$ (Lang et al., 2010). In these samples, formate concentrations were much lower than those of acetate; multiple VFAs including formate, acetate, and lactate were detected concurrently and $\sum$VFAs accounted for a minor fraction of DOC. VFAs could be also produced from thermocatalytic degradation of kerogen in formation waters (Carothers & Kharaka, 1978; Fisher, 1987), but no oil was observed in these samples. Instead, VFAs were likely produced from the biologically mediated degradation of organic matter at relatively low temperatures. Below we discuss the biological production and consumption of VFAs using stable carbon isotopic compositions as an indicator.

In marine sediment, VFAs are often considered to be common products of organic matter fermentation. The $^{13}\text{C}$ values of fermentation products are determined by the isotopic composition of precursors and isotopic fractionation resulting from fermentative processes. Previous studies showed that the $^{13}\text{C}$ value of total organic carbon in Guaymas Basin mat sediments varied between $-21.6\%$ and $-25.7\%$ (Pearson et al., 2005; Schouten et al., 2003). There is no significant isotopic fractionation ($<3\%$) associated with acetate formation via fermentation (Penning & Conrad, 2006), so fermentation should produce acetate with $^{13}\text{C}$ values from $-18.6\%$ to $-22.7\%$. Likewise, lactate production from fermentation should yield $^{13}\text{C}$ values between $-22.6\%$ and $-31.7\%$, based on the reported fractionation factor for lactate ($-1\%$ to $-6\%$; Penning & Conrad, 2006). The $^{13}\text{C}$ values of lactate in these samples ($-30.6\% \pm 4.1\%$) did not always reflect fermentation of total organic carbon, as lactate was relatively depleted in $^{13}\text{C}$ relative to bulk organic matter. The relatively negative $^{13}\text{C}$ values of lactate indicated a carbon source with lighter carbon isotope signatures contributed to lactate generation. Isotopically lighter substrates are possible because Guaymas Basin sediments contain microbially derived organic matter that is strongly depleted in $^{13}\text{C}$ due to the incorporation of methane-derived carbon into biomass (Schouten et al., 2003; Teske et al., 2002).
At some depths, acetate was relatively depleted in $^{13}$C (<$-30^{\circ}$), raising the possibility of formation from H$_2$/CO$_2$ via biological acetogenesis (Chapelle & Bradley, 1996). Acetogenesis exerts a large isotopic fractionation that results in acetate with negative $\delta^{13}$C values. Although hydrogen concentration data were not available, the likelihood of acetogenesis can be assessed from thermodynamic calculations. Assuming a minimum biological energy quantum for sustaining microbial life of $-10$ kJ/mol (Hoehler et al., 2001), acetogenesis via CO$_2$ reduction with H$_2$ could meet this threshold if hydrogen concentrations were between 2.9 and 9.8 nM in the Aceto Balsamico mat and from 2.9 to 5.1 nM in the Northern Towers-12 mat (Table S2); these are both sites with a temperature <20 °C. At hot mats such as Ultra Mound and Northern Towers-12, a minimum H$_2$ of 25 to 41 nM and 230 to 482 nM, respectively, would be required to facilitate acetogenesis (Table S2).

Dowell et al. (2016) measured in situ hydrogen concentration in Mat Mound sediments of Guaymas Basin at different temperatures. They found that hydrogen concentrations peaked between 2 and 10 nM when samples were incubated at 20 °C, and that incubation at elevated temperature (>50 °C) led to localized H$_2$ accumulation with concentrations exceeding 300 nM. The thermodynamic predictions for acetogenesis fall well within the range of hydrogen concentrations documented for Guaymas Basin sediments and suggest that acetogenesis contributed to the light isotopic values of acetate.

The potential importance of acetogenesis is also supported by the formation of volatile organic carbon (VOC) from $^{14}$C-labelled bicarbonate (Figure S1). Intriguingly, the VOC formation rate reached up to 3.67 pmol cm$^{-3}$ d$^{-1}$ in Cathedral Hill sediments (Figure S1). As VFAs (particularly acetate) are probably the dominant components of VOC compounds produced from bicarbonate, these results provide further evidence for the occurrence of acetogenesis in Guaymas Basin sediments.

A previous study reported an isotopic fractionation of $-58.6^{\circ}$ ± $0.7^{\circ}$ during the conversion of carbonate to acetate via acetogenesis (Gelwicks et al., 1989). Since the stable carbon isotopic composition of DIC ranged widely from $-1^{\circ}$ to $-22^{\circ}$ in Guaymas Basin mats (McKay et al., 2012), acetogenesis could potentially produce acetate with $5^{13}$C values between $-59.6^{\circ}$ and $-81.6^{\circ}$. These values are much more negative than the values observed in our samples, suggesting that both fermentation and acetogenesis contributed to acetate production in tandem and that the isotopic composition of the acetate pool is dependent on the relative contribution of each process.

Isotopic fractionation during biological consumption also leads to enrichment of $^{13}$C in VFAs. For example, the heavier isotopic compositions (>−20%) in addition to the downcore enrichment of $^{13}$C in formate (Dive 4871-1) and acetate (Dive 4871-12) could be attributed to isotopic fractionation resulting from consumption. This is supported by the high oxidation rates of acetate via sulfate reduction, or to a lesser extent, methanogenesis by methanogens (see below).

Stable carbon isotope fractionation during acetoclastic methanogenesis and acetotrophic sulfate reduction has been studied with cultures of methanogenic archaea and sulfate-reducing bacteria, respectively. Depending on the species and metabolic pathway, both processes can generate a wide range of fractionation effects (Goevert & Conrad, 2008; Krzycki et al., 1987; Londry & Des Marais, 2003; Penning et al., 2006; Valentine et al., 2004). Together, isotopic signatures, thermodynamic predictions, and data from experimental incubations suggest that fermentation, acetogenesis, and microbial consumption act in concert to control the pool size and stable carbon isotopic compositions of VFAs.

### 3.3. Metabolism of VFAs and Alcohols and Related Biogeochemical Processes

To explore the biological consumption of VFAs and alcohols, we studied the utilization of acetate and methanol by methanogenic (i.e., CH$_4$ production) and nonmethanogenic heterotrophic (i.e., oxidation leading to CO$_2$ production) processes. Generally, methane production rates from acetate were relatively low, ranging from 0.003 to 510 pmol cm$^{-3}$ d$^{-1}$, in Guaymas Basin sediments (Table S3). The highest rates of methanogenic activity were observed in the Aceto Balsamico mat, where both the turnover rates and acetate concentrations were high. Rates at this site were 2 to 5 orders of magnitude higher than the rates observed at other sites. The turnover of methanol to methane was 1 to 3 orders of magnitude faster than the turnover of acetate to methane (Table S3), but the methanogenesis rates from methanol were similar (0.04 to 69.5 pmol cm$^{-3}$ d$^{-1}$) because the methanol pool was smaller than the acetate pool.
The low rates of methanogenic activity are not surprising. Surface sediments in the Guaymas Basin are typically characterized by methane-oxidizing and sulfate-reducing conditions with microbial communities dominated by methanotrophic archaea and sulfate-reducing bacteria (Dowell et al., 2016; Teske et al., 2002). The documented high activity for anaerobic methane oxidation and sulfate reduction suggests that these processes are thermodynamically favored and may outcompete methanogens, limiting methanogenic activity. This idea is supported by the results of inhibitor experiments, as methanogenesis rates from acetate and methanol increased by 3 to 5 times following addition of molybdate (Figure S2). Furthermore, the stable carbon isotopic compositions of methane (generally ~$-50\%$) suggested that the majority of methane in Guaymas Basin sediments was of thermogenic origin (Biddle et al., 2012; McKay et al., 2012).

In contrast to methane production, the turnover of acetate to CO$_2$ was much more rapid ($4-1417$ versus $53-2.4 \times 10^6$ days for CO$_2$ versus CH$_4$ production from acetate; Table S4), and the oxidation rates were about 2 to 5 orders of magnitude higher. The maximum acetate oxidation rate, $2.1 \times 10^5$ pmol cm$^{-3}$ d$^{-1}$, was documented at the cold Northern Towers mat (Dive 4871-12; Figure 2). This site also had the highest DOC concentrations. Abundant DOC provides sufficient energy-rich substrates to fuel diverse microbial communities, and these communities use acetate as an energy source. Acetate oxidation was not detectable at 50-cm depth in the hot Northern Towers mat core (Dive 4871-1, >80 °C), suggesting temperature limitation of the process. Likewise, the turnover of methanol to CO$_2$ was also fast and the turnover time ranged from 7 to 186 days (Table S4). The methanol oxidation rates ($577$ pmol cm$^{-3}$ d$^{-1}$) were much higher than methanogenesis rates from methanol ($12.4$ pmol cm$^{-3}$ d$^{-1}$). High methanol oxidation rates occurred at the hot Northern Towers mat (Dive 4871-1; Figure 2), where maximum concentrations of methanol were observed.

Higher acetate oxidation rates than methanogenesis rates suggest that acetate was predominantly used as an energy source through nonmethanogenic processes, that is, sulfate reduction. This is supported by the addition of molybdate, which resulted in a significant decrease in the CO$_2$ production from acetate (Figure S2), indicating that acetate was largely utilized by sulfate-reducing bacteria. Acetate could be an energetic substrate for methanogens at depths in the Aceto Balsamico mat, as the methanogenesis rates were higher or comparable to the acetate oxidation rates. This pattern could be attributed to the relief of competition with sulfate reducers, since sulfate is often depleted in the upper 8 cm at this site (Teske et al., 2016).

Methanol is often considered a noncompetitive substrate for methanogens in different sedimentary settings (Maltby et al., 2016; Oremland et al., 1982; Zhuang, Montgomery, & Joye, 2019; Zhuang, Montgomery, Sibert, et al., 2018). In these samples, >95% of methanol was channeled into the oxidation pathway, suggesting that methanol was primarily used as an energy source by nonmethanogenic heterotrophs. Methanol oxidation was likely coupled to terminal processes other than sulfate reduction, since addition of molybdate did not significantly change the oxidation rate of methanol at the Ultra Mound mat (Figure S2). A recent study demonstrated that methanol could be coupled to denitrification (Zhuang, Montgomery, & Joye, 2019), and
high denitrification rates have been reported in Guaymas Basin sediments (Bowles, Nigro, et al., 2011). Given their abundance and rapid turnover, VFAs and alcohols are important energy substrates that support diverse chemoheterotrophs and various microbial processes in the hydrothermally altered sediments of the Guaymas Basin.

4. Conclusions

We investigated the abundance and stable carbon isotopic compositions of VFAs and alcohols in surficial sediments of Guaymas Basin across a number of hydrothermal sites. Abundant acetate (up to 229 μM) and methanol (up to 37 μM) were detected in the porewaters, indicating the high potential as energetic substrates for microbes in hydrothermal systems. Stable carbon isotopic signatures, thermodynamics, and experimental incubations suggested that biological source including fermentation and acetogenesis contributed to the generation of formate and acetate, and microbial consumption could be a dominant sink that controlled their pool sizes. We further investigated the processes that turned over acetate and methanol. High oxidation rates of acetate and methanol suggested that they were predominantly used as an energy source for nonmethanogenic processes, although methanogenic activity was also detected with low rates. Acetate was used largely by sulfate-reducing bacteria, but the oxidation of methanol might be also coupled to other terminal processes. Taken together, our findings suggested that VFAs and alcohols served as an energy source that support various microbial processes in the hydrothermally altered sediments, the role of which could be more important than previously thought.

Acknowledgments

We thank the captain, crew, and the Alvin team of R/V Atlantis for their kind assistance during expeditions AT15-56 (2009) and AT37-06 (2016). We thank K. Hunter for the help with cruise preparation and for the generation of geochemical data. This research was funded by the National Science Foundation (NSF) Biological Oceanography Program (grants OCE-0959337 and OCE-1157380 to S. Jooy and OCE-0647633 and OCE-1357238 to A. Teske). This research was also made possible, in part, by a grant from the Gulf of Mexico Research Initiative to support the “Ecosystem Impacts of Oil and Gas in the Gulf” (ECOGIG) research consortium. Data are publicly available through the Gulf of Mexico Research Initiative to support the Gulf of Mexico Research Initiative to the China Scholarship Council. F. Zhuang was sponsored by the Gulf of Mexico Research consortium. Data are publicly available through the Gulf of Mexico Research Initiative to support the Gulf of Mexico Research Initiative to support the China Scholarship Council.

ZHUANG ET AL.

References


