

Acetogenesis from CO₂ in an anoxic marine sediment

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Abstract

A combination of radiotracer and pore-water concentration measurements provide evidence for the occurrence of acetogenesis from CO₂ in anoxic marine sediments that are ordinarily dominated by sulfate reduction and methanogenesis. In a month-long incubation experiment using sediments from Cape Lookout Bight, North Carolina, we measured H₂ and acetate concentrations and monitored the incorporation of ¹⁴CO₂ into ¹⁴CH₄ and ¹⁴C-acetate. Depletion of pore-water sulfate resulted in a period of elevated H₂ concentrations that made acetogenic CO₂ reduction thermodynamically favorable. During this period, ¹⁴C-acetate was produced from ¹⁴CO₂ at rates comparable to those of methanogenesis or sulfate reduction during their respective periods of dominance in the incubation. Maintenance of elevated but constant H₂ concentrations immediately following sulfate depletion likely reflects control by acetogenic bacteria, suggesting they were the dominant consumers of H₂ during this period.

Acetogenic bacteria are virtually ubiquitous in anaerobic ecosystems as a result of their metabolic versatility. Schink (1994) notes that “there is hardly any transformation process in an anoxic environment in which homoacetogens do not participate or with which they do not compete.” These organisms produce acetate through both fermentation of organic compounds and reduction of CO₂ (Drake 1994). Pure cultures of acetogens generally catalyze these two reactions simultaneously (Wood 1952), but it has been shown that growth can be based exclusively on either reaction (Daniel et al. 1990; Drake 1994; Schink 1994).

Because molecular hydrogen can serve as the reductant for acetogenic CO₂ reduction (2CO₂ + 4H₂ → CH₃COOH + 2H₂O), the favorability and occurrence of the reaction are highly sensitive to ambient H₂ concentrations. Low H₂ concentrations, as might be maintained by proximal H₂-consuming organisms, have been shown to inhibit (Cord-Ruwisch and Ollivier 1986; Heijthuijsen and Hansen 1986) and even reverse (Lee and Zinder 1988) acetogenic CO₂ reduction. The basis for this inhibition (or reversal) is almost certainly thermodynamic: decreased H₂ concentrations translate to a smaller thermodynamic driving force (ΔG less negative) for the reaction. Progressively lower H₂ concentrations (more positive ΔG values) would at first inhibit acetogenic CO₂ reduction and then cause the reverse process to become energetically favorable.

Such thermodynamic inhibition of acetogenic CO₂ reduction is expected to prevail in most sediment ecosystems, where the activity of terminal metabolic bacteria (e.g., methanogens and sulfate reducers) maintains H₂ concentrations at very low levels (Lovley and Goodwin 1988). For example, at 10°C, Hoehler et al. (1994) measured H₂ concentrations of 0.16 and 1.82 nM for sulfate-reducing and methan-

ogenic sediments, respectively. Acetogenic CO₂ reduction is unfavorable at these concentrations, with $\Delta G = +24.5$ and $+1.63$ kJ per mol acetate formed (given: P_{CO₂} = 0.07 atm (Σ CO₂ = 50 mM); {CH₃COOH} = 4.1×10^{-8} M (Σ CH₃COOH = 10 μ M); $\Delta G^{\circ}_{(10^{\circ}\text{C})} = -91.5$ kJ mol acetate⁻¹). In essence, sulfate-reducing and methanogenic bacteria outcompete acetogens for the common substrate H₂. Hence, while acetogenic CO₂ reduction is well documented in pure culture studies, its role and importance in the environment are poorly understood (Drake et al. 1994).

The few observations of acetogenic CO₂ reduction in sediment ecosystems have all come from freshwater environments, where acetogens must compete with methanogens for H₂ (Lovley and Klug 1983; Phelps and Zeikus 1984; Jones and Simon 1985; Conrad et al. 1989; Nozhevnikova et al. 1993). This study was designed to look for acetogenic CO₂ reduction in marine sediments, where sulfate reducers also compete for H₂.

Seasonal acetate accumulation in Cape Lookout Bight sediments—In the anoxic sediments of Cape Lookout Bight (CLB), North Carolina, an annual temperature variation from 8 to 28°C causes the depth of sulfate penetration into the sediment column to oscillate between 25 cm in the winter and 10 cm in the summer (Fig. 1A) (Crill and Martens 1987; Klump and Martens 1989). As a result of this oscillation, sulfate in the 10–25-cm depth interval is rapidly depleted during the spring (Fig. 1B), and the system shifts from sulfate reduction to methanogenesis as the dominant mode of terminal metabolism. (This sequence of events is hereafter referred to as the “sulfate–methane transition period.”) Sansone and Martens (1982) observed that acetate concentrations in the 10–25-cm interval increase two orders of magnitude to millimolar levels during this transition period (Fig. 1B). It is postulated that this accumulation reflects a temporary decoupling of acetate production (by fermentative bacteria) and consumption (by terminal bacteria) (Alperin et al. 1994). When the supply of sulfate is exhausted, sulfate reduction can no longer provide a sink for acetate. At the same time, the methanogen population is low and unable to keep pace with production of acetate. Acetate accumulates

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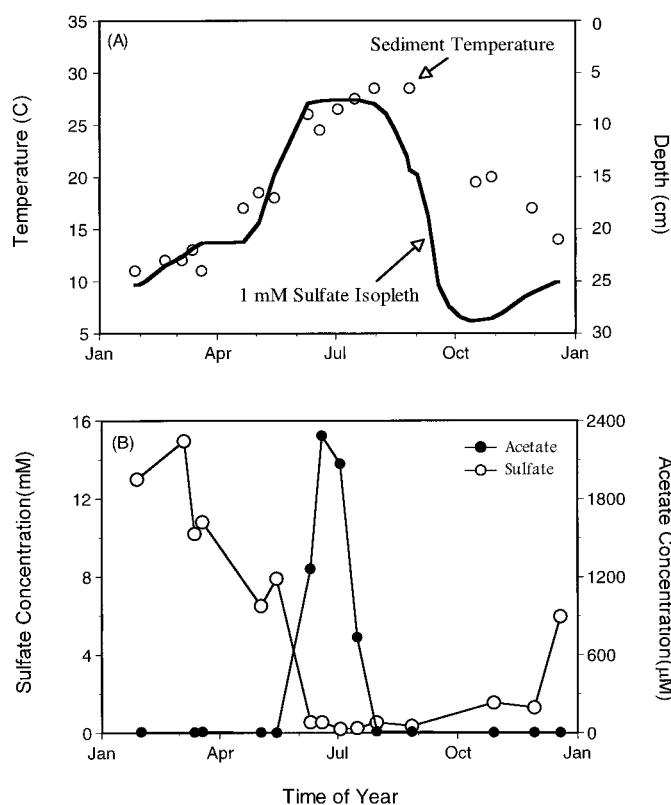


Fig. 1. The seasonal accumulation of acetate in CLB sediments. (A) Seasonal variation in temperature (open circles) and depth of sulfate penetration, defined as the 1-mM sulfate isopleth (solid line). (B) Pore-water concentrations of sulfate (open circles) and acetate (filled circles) at 13.5-cm depth. Data represent a compilation of measurements from 1989 to 1991.

as a result of the production-consumption imbalance. We hypothesize that this mechanism might also create a surplus of H₂ during the sulfate-methane transition, creating a window of opportunity for acetogenic CO₂ reduction.

Alperin et al. (1992) reproduced the acetate accumulation event in a laboratory sediment incubation experiment. This study yielded stable isotopic evidence that suggested that acetogenic CO₂ reduction may occur briefly during the sulfate-methane transition. We conducted a sediment incubation of shorter duration that was identical to that of Alperin et al. (1992) with respect to the incubation vessel, sampling/sediment processing methodology, and techniques for measuring methanogenic CO₂ reduction rates and concentrations of sulfate, CO₂, and organic acids. Our experiment used sediments from a broader depth interval (0–15 cm, from cores taken in early May) with an incubation temperature of 22°C. Sulfate reduction rates reported herein are calculated from the decrease in sulfate concentrations observed with time. These and all other rate and concentration data are reported relative to volume of pore water (e.g., moles per liter of pore water).

Measurement of acetogenesis rates and H₂ concentrations—To better assess the role of acetogenic CO₂ reduction

in this system, we also measured concentrations of H₂ and rates of ¹⁴C-acetate production from ¹⁴CO₂.

Rates of acetogenesis from CO₂ were determined by inoculating 2.5-ml aliquots of sediment with 25 μl of aqueous NaH¹⁴CO₃ (5 × 10⁷ cpm injection⁻¹). These samples were incubated at 22°C in the dark for 24 h; incubations were terminated by freezing the samples. Thawed samples were immediately centrifuged, and the supernatant fluid was filtered through 0.45-μm filters. The bulk radioactivity of the supernatant fluid (equivalent to the added ¹⁴CO₂ activity) was determined by liquid scintillation counting a 100-μl aliquot. In a second aliquot, acetate was isolated by high-pressure liquid chromatography (HPLC) (Albert and Martens 1997) with fraction collection; the activity of ¹⁴C-acetate was determined by liquid scintillation counting. Acetogenesis rates were calculated using, rate = [ΣCO₂]αα/2At, where *a* is the recovered activity of ¹⁴C-acetate per unit volume of pore water, *A* is the added ¹⁴CO₂ activity per unit volume of pore water, *t* is the incubation time, and α is the ¹⁴C:¹²C isotope discrimination factor associated with acetogenesis. We used a value of 1.12 for α (Gelwicks et al. 1989). The factor of 2 in the calculation is used to obtain the rate in terms of acetate production, which equals one-half of CO₂ consumption. Precision on replicate sediment samples averaged about ±10%. Tracer recoveries could not be determined due to the inability to extract all pore water from the bulk sediment.

It is important to consider a potential artifact of determining acetogenesis rates in this fashion. It has been shown that the acetate carboxyl group can exchange with the bulk CO₂ pool (Eikmanns and Thauer 1984; Schauder et al. 1986; De Graff et al. 1996). In our system, this could result in incorporation of ¹⁴CO₂ into the acetate pool without acetogenesis taking place—causing an overestimate of rates. However, the significant ¹³C depletion of the acetate carboxyl carbon that would result from an active exchange phenomenon is not evident in acetate from CLB pore water (Blair and Carter 1992)—suggesting that if the exchange occurs at all in this system, it is probably at a rate too small to bias our results.

Hydrogen concentrations were determined by two methods. The first two and last two samples of the experiment (when hydrogen concentrations were at steady state) were analyzed by the headspace equilibration technique of Lovley and Goodwin (1988) as modified by Hoehler et al. (1994). During the intermediate portion of the experiment (days 16–20), potentially rapid changes in pore-water H₂ concentrations necessitated the use of an alternative methodology based on that of Novelli et al. (1987). An aliquot of whole sediment (10 ml) was rapidly dispensed into a nitrogen-flushed vial containing 5 ml of 2 M NaOH. The vial was quickly sealed and shaken, retarding biological activity and partitioning the pore-water pool of hydrogen between the sediment and headspace. The partial pressure of H₂ in the headspace gas was determined in the same fashion as for the headspace equilibration methodology (Hoehler et al. 1994) and was used to calculate the H₂ concentration in the pore water (using Crozier and Yamamoto 1974). The precision of this method (±30% among replicate samples) is not as good as with the headspace equilibration technique (±5%). Comparison of the two methods on identical samples at steady state (both methanogenic and sulfate-reducing

conditions) indicates the alternative methodology yields H_2 concentrations that are generally higher by 3–6 nM than those measured using the headspace equilibration technique. All concentration and rate data for H_2 and other species are reported relative to volume of pore water (e.g., moles per liter of pore water).

Acetate and acetogenesis—During the initial 2 weeks of the experiment, sulfate concentrations decreased at an approximately constant rate of about $300 \mu\text{M d}^{-1}$ (Fig. 2A). Sulfate reduction became sulfate limited sometime after the concentration dropped below $200 \mu\text{M}$ (on day 16). Sulfate reduction effectively stopped on day 17 when the concentration reached $24 \mu\text{M}$, similar to the threshold limit observed for pure cultures of sulfate reducers (Ingvorsen et al. 1984). As sulfate became limiting, acetate concentrations began a period of increase that lasted at least 5 d, reaching a peak of $536 \pm 10 \mu\text{M}$ (Fig. 2A). Because we have no acetate concentration data for the period between 21 and 30 d, we do not know exactly when the acetate concentration began to decrease or if $536 \mu\text{M}$ is the highest level of acetate that occurred during the experiment. This accumulation of acetate is similar to that previously observed in CLB sediments during the sulfate–methane transition (Sansone and Martens 1982; Alperin et al. 1992) and indicates that, for several days, the competition for terminal metabolic substrates (i.e., fermentation products) was significantly relaxed.

Once sulfate became limiting, we observed $^{14}\text{CO}_2$ reduction to both $^{14}\text{CH}_4$ and ^{14}C -acetate (Fig. 2B). Rates of both processes are reported here relative to product formation, which normalizes the two in terms of hydrogen utilization but not CO_2 consumption. Consistent with the hypothesis that methanogens require a “grow-in period” following sulfate depletion, we observed a steady increase in methanogenic CO_2 reduction rates from day 17 to the end of the experiment. It is not clear whether the peak rate of $79 \pm 3 \mu\text{M d}^{-1}$, observed at the end of the experiment, represents a steady-state methanogenesis rate or if the rate increase continued following the end of the experiment. By contrast, acetogenic CO_2 reduction, was a transient phenomenon associated with the sulfate–methane transition dynamics: rates peaked in the several days following sulfate depletion and had decreased substantially by the late stages of the incubation. The process seems to have been quantitatively important with respect to carbon and electron flow during the intermediate stage of the incubation, as the peak rate of acetogenesis ($108 \pm 7 \mu\text{M d}^{-1}$ on day 20) exceeded that of methanogenesis. It is important to note that in our tracer experiments, some of the ^{14}C -acetate produced during an incubation could have been subsequently consumed—so that our calculated rates should be viewed as minimum estimates. While this is unlikely to have been important during the period in which acetate accumulated, it may represent a significant effect on the day-30 acetogenesis rate.

The role of H_2 —The occurrence of acetogenic CO_2 reduction in this experiment appears to be closely tied to variations in H_2 concentrations (Fig. 2C). While the sediments contained sulfate, H_2 concentrations were maintained below 2 nM, as previously observed for sulfate-reducing CLB sed-

iments (Hoehler et al. 1994, 1998). Acetogenic CO_2 reduction is thermodynamically unfavorable under these conditions (Table 1) and was not detected by our tracer experiments. As sulfate became limiting, H_2 concentrations spiked to $2,600 \pm 240 \text{ nM}$, then decreased and leveled off at about 120 nM. These elevated concentrations presumably represent a relaxation of the competition for H_2 among terminal metabolic bacteria and were sufficient to make acetogenic CO_2 reduction thermodynamically favorable (Table 1). During this period, we observed significant production of ^{14}C -acetate from $^{14}\text{CO}_2$. By the end of the incubation, conditions were approaching a methanogenic steady state and H_2 concentrations were again maintained at low levels (13 nM at the last sampled date) that would make acetogenic CO_2 reduction thermodynamically unfavorable (Table 1). Acetogenesis rates had decreased substantially by this point, though some production of ^{14}C -acetate was observed on day 30, when H_2 concentrations may still have been slightly elevated (the closest measured value, 18 nM on day 25, translates to a thermodynamic driving force for acetogenic CO_2 reduction of $\Delta G = -3.5 \text{ kJ mol acetate}^{-1}$).

Because the increase in H_2 concentrations presumably results from the same production–consumption imbalance that causes acetate to build up, one might expect these two fermentation products to accumulate at comparable rates—provided the flow of electrons through the two pools is similar at steady state. However, the accumulation of H_2 we observed following sulfate depletion, though substantial in the relative sense, was minor in duration and absolute magnitude compared to that of acetate. While acetate concentrations increased for 5 d at approximately $100 \mu\text{M d}^{-1}$ (Fig. 2A), H_2 accumulated for only 1 d at <3% of that rate (Fig. 2C). Following this 1-d buildup, H_2 concentrations actually decreased to about 120 nM and remained roughly constant at that level for several days (Fig. 2C). The relatively rapid return to an apparent steady state with respect to H_2 production and consumption requires that a high-capacity H_2 sink be present at the time of sulfate depletion. This requirement is quite consistent with the ecology of homoacetogens. It is likely that these organisms were already active in their capacity as fermenters (Schink 1994) when the sediments contained sulfate. The elevated H_2 concentrations would make the reductive pathway of their metabolism favorable, allowing them to consume H_2 via acetogenic CO_2 reduction. This would not require a cessation of their fermentative activity, since the processes are known to occur simultaneously in pure culture (Wood 1952).

The activity of acetogens appears, in fact, to be a control on H_2 concentrations during the sulfate–methane transition period. For several days following the initial H_2 spike, H_2 concentrations remained constant at a level one to two orders of magnitude higher than those associated with methanogenesis or sulfate reduction. Lovley and Goodwin (1988) showed that H_2 concentrations in sediment environments are typically controlled by hydrogen-consuming bacteria and that differing modes of terminal metabolism give rise to distinctly different steady-state hydrogen concentrations. We hypothesized that the elevated H_2 concentrations on days 17–20 reflected control by acetogenic bacteria. To test this hypothesis, we assumed that H_2 concentrations during the early

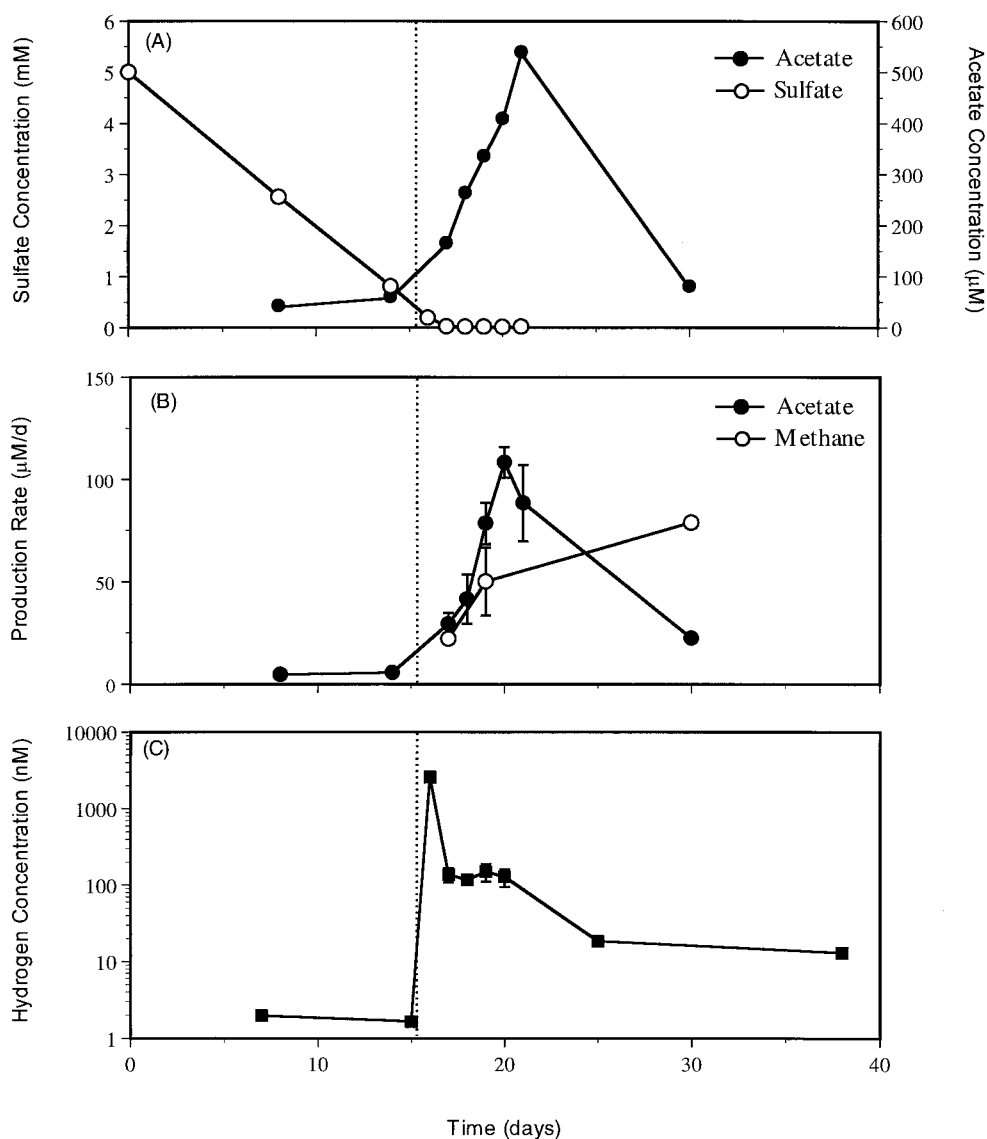


Fig. 2. Dynamics of the sulfate–methane transition in the sediment incubation experiment. (A) Concentrations of sulfate (open circles) and acetate (filled circles) vs. time. (B) ¹⁴C tracer-based rates of methane (open circles) and acetate (filled circles) production from CO₂ vs. time. (C) Pore-water H₂ concentrations (filled squares) vs. time. Note that H₂ concentration is plotted on a log scale. In each plot, the vertical dashed line indicates the time at which sulfate concentrations become limiting to sulfate reduction. For (B) and (C), error bars represent 1 SD about the mean of three replicate sediment samples.

and late stages of the experiment reflected control by sulfate reducers and methanogens, respectively, and used this as a basis for evaluating whether acetogens might have controlled the transition H₂ concentrations.

Hydrogen concentrations strongly influence the bioenergetics of terminal bacteria, and it appears that minimum energy requirements ultimately determine the level of H₂ that is maintained by an organism (Hoehler et al. 1998). Using measured concentrations of H₂ and other relevant species, the free energy yields (ΔG) obtained by methanogens and sulfate reducers can be calculated as -19 and -23 kJ mol⁻¹ of CH₄ or H₂S, during their respective periods of dominance (Table 1). During the period following sulfate depletion, the

elevated H₂ concentrations translate to an energy yield of -18.6 to -21.5 kJ per mol acetate for acetogenic CO₂ reduction (Table 1). The similarity of the acetogenic energy yields to those of methanogenesis and sulfate reduction strongly suggests that hydrogen concentrations during the transition period were controlled by acetogens in exactly the same fashion as they were by methanogens or sulfate reducers during the early and late stages of the experiment.

Overall, these experiments suggest that acetogenic CO₂ reduction may play an important but transient role in marine sediments that are ordinarily dominated by sulfate reduction and methanogenesis. The occurrence and dynamics of the process appear to be intimately tied to variations in H₂ con-

Table 1. Thermodynamics of sulfate reduction, methanogenesis, and acetogenesis.

Day	Active TEAP*	H ₂ (nM)	ΔG [†] for active TEAP (kJ mole ⁻¹)	ΔG for acetogenesis (kJ mole ⁻¹) [‡]
15	Sulfate reduction	1.6 ± 0.1	-23 ± 1.4	+19.5
17-20	Acetogenesis (?)	117-150	-18.6-21.5	-18.6-21.5
38	Methanogenesis	13.0 ± 0.6	-18.8 ± 0.6	+0.1 [§]

* TEAP, terminal electron-accepting process; "active TEAP," the predominant electron-consuming process during a given time period.

[†] ΔG values were calculated using thermodynamic data from Barner and Scheuerman (1977) and are reported in kJ per mole of major product (e.g. H₂S, CH₄, or CH₃COOH); pH and all pertinent concentrations were measured, with the exception of ΣH₂S, which was assumed to have a concentration of 2 ± 1 mM; reported errors result from propagation of individual measurement (and estimated) errors through the ΔG calculation.

[‡] The free energy that would be available from acetogenic CO₂ reduction during the time period indicated. Note that a positive ΔG indicates that acetogenesis would be thermodynamically unfavorable.

[§] Calculated using the last measured acetate concentration, 80 μM (measured on day 30).

concentrations. The relaxation of competition during the sulfate-methane transition period that allows acetogenesis to occur in our system may occur in other environments as well. Any system that undergoes a rapid change from one mode of terminal metabolism to another might be subject to similar effects. Shannon and White (1996) report a seasonal accumulation of acetate in peats that may result from such a mechanism. An analogous situation could also be generated by periodic flooding of rice paddy or forest soils and the resulting oscillation between aerobic and anaerobic respiration processes (Sugimoto and Wada 1993). Thus, acetogenic CO₂ reduction may represent a common periodic occurrence in many environments.

References

- ALBERT, D. B., AND C. S. MARTENS. 1997. Determination of low molecular weight organic acid concentrations in environmental samples via HPLC. *Mar. Chem.* **56**: 27-38.
- ALPERIN, M. J., D. B. ALBERT, AND C. S. MARTENS. 1994. Seasonal variations in production and consumption rates of dissolved organic carbon in an organic-rich sediment. *Geochim. Cosmochim. Acta* **58**: 4909-4930.
- , N. E. BLAIR, D. B. ALBERT, T. M. HOEHLER, AND C. S. MARTENS. 1992. Factors that control the carbon isotopic composition of methane produced in an anoxic marine sediment. *Global Biogeochem. Cycles* **6**: 271-291.
- BARNER, H. E., AND R. V. SCHEUERMAN. 1977. *Handbook of chemical thermodynamic data for compounds and aqueous species*. Wiley.
- BLAIR, N. E., AND W. D. CARTER. 1992. Carbon isotope biogeochemistry of acetate from a methanogenic marine sediment. *Geochim. Cosmochim. Acta* **56**: 1247-1258.
- CONRAD, R., AND OTHERS. 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiol. Ecol.* **62**: 285-294.
- CORD-RUWISCH, R., AND B. OLLIVIER. 1986. Interspecies hydrogen transfer during methanol degradation by *Sporomusa acidovorans* and hydrogenophilic anaerobes. *Arch. Microbiol.* **144**: 163-165.
- CRILL, P. M., AND C. S. MARTENS. 1987. Biogeochemical cycling in an organic-rich coastal marine basin. 6. Temporal and spatial variations in sulfate reduction rates. *Geochim. Cosmochim. Acta* **51**: 1175-1186.
- CROZIER, T. E., AND S. YAMAMOTO. 1974. Solubility of hydrogen in water, seawater, and NaCl solutions. *J. Chem. Eng. Data* **19**: 242-244.
- DANIEL, S. L., T. HSU, S. I. DEAN, AND H. L. DRAKE. 1990. Characterization of the H₂- and CO-dependent chemolithotrophic potentials of the acetogens *Clostridium thermoaceticum* and *Acetogenium kivui*. *J. Bacteriol.* **172**: 4464-4471.
- DE GRAFF, W., P. WELLSBURY, R. J. PARKES, AND T. E. CAPPENBERG. 1996. Comparison of acetate turnover in methanogenic and sulfate-reducing sediments by radiolabeling and stable isotope labeling and by use of specific inhibitors: Evidence for isotopic exchange. *Appl. Environ. Microbiol.* **62**: 772-777.
- DRAKE, H. L. 1994. Introduction to acetogenesis, p. 3-62. *In* H. L. Drake [ed.], *Acetogenesis*. Chapman and Hall.
- , S. L. DANIEL, C. MATTHIES, AND K. KUSEL. 1994. Acetogenesis: Reality in the laboratory, uncertainty elsewhere, p. 273-302. *In* H. L. Drake [ed.], *Acetogenesis*. Chapman and Hall.
- EIKMANN, B., AND R. K. THAUER. 1984. Catalysis of an isotopic exchange between CO₂ and the carboxyl group of acetate by *Methanosarcina barkeri* grown on acetate. *Arch. Microbiol.* **138**: 365-370.
- GELWICKS, J. T., J. B. RISATTI, AND J. M. HAYES. 1989. Carbon isotope effects associated with autotrophic acetogenesis. *Org. Geochem.* **14**: 441-446.
- HEIJTHUISEN, J.H.F.G., AND T. A. HANSEN. 1986. Interspecies hydrogen transfer in co-cultures of methanol-utilizing acidogens and sulfate-reducing or methanogenic bacteria. *FEMS Microbiol. Ecol.* **38**: 57-64.
- HOEHLER, T. M., M. J. ALPERIN, D. B. ALBERT, AND C. S. MARTENS. 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: Evidence for a methanogen-sulfate reducer consortium. *Global Biogeochem. Cycles* **8**: 451-463.
- , ———, AND ———. 1998. Thermodynamic control on H₂ concentrations in an anoxic marine sediment. *Geochim. Cosmochim. Acta* **62**: 1745-1756.
- INGVORSEN, K., A. J. B. ZEHNDER, AND B. B. JØRGENSEN. 1984. Kinetics of sulfate and acetate uptake by *Desulfovibrio postgatei*. *Appl. Environ. Microbiol.* **47**: 403-408.
- JONES, J. G., AND B. M. SIMON. 1985. Interaction of acetogens and methanogens in anaerobic freshwater sediments. *Appl. Environ. Microbiol.* **49**: 944-948.
- KLUMP, J. V., AND C. S. MARTENS. 1989. The seasonality of nutrient regeneration in an organic-rich coastal sediment: Kinetic modeling of changing pore-water nutrient and sulfate distributions. *Limnol. Oceanogr.* **34**: 559-577.
- LEE, M. J., AND S. H. ZINDER. 1988. Isolation and characterization

- of a thermophilic bacterium which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H₂-CO₂. *Appl. Environ. Microbiol.* **54**: 124–129.
- LOVLEY, D. R., AND S. GOODWIN. 1988. Hydrogen concentrations as an indicator of the terminal electron-accepting reactions in aquatic sediments. *Geochim. Cosmochim. Acta* **52**: 2993–3003.
- , AND M. J. KLUG. 1983. Methanogenesis from methanol and methylamines and acetogenesis from hydrogen and carbon dioxide in the sediments of a eutrophic lake. *Appl. Environ. Microbiol.* **45**: 1310–1315.
- NOVELLI, P. C., M. I. SCRANTON, AND R. H. MICHENER. 1987. Hydrogen distributions in marine sediments. *Limnol. Oceanogr.* **32**: 565–576.
- NOZHEVNIKOVA, A. N., O. R. KOTSYURBENKO, AND M. SIMANKOVA. 1993. Acetogenesis at low temperature, p. 416–431. *In* H. L. Drake [ed.], *Acetogenesis*. Chapman and Hall.
- PHELPS, T. J., AND J. G. ZEIKUS. 1984. Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake. *Appl. Environ. Microbiol.* **48**: 1088–1095.
- SANSONE, F. J., AND C. S. MARTENS. 1982. Volatile fatty acid cycling in organic-rich marine sediments. *Geochim. Cosmochim. Acta* **46**: 1575–1589.
- SCHAUDER, R., B. EIKMANN, R. K. THAUER, F. WIDDEL, AND G. FUCHS. 1986. Acetate oxidation to CO₂ in anaerobic bacteria via a novel pathway not involving reactions of the citric acid cycle. *Arch. Microbiol.* **145**: 162–172.
- SCHINK, B. 1994. Diversity, ecology, and isolation of acetogenic bacteria, p. 197–235. *In* H. L. Drake [ed.], *Acetogenesis*. Chapman and Hall.
- SHANNON, R. D., AND J. P. WHITE. 1996. The effect of spatial and temporal variations in acetate and sulfate on methane cycling in two Michigan peatlands. *Limnol. Oceanogr.* **41**: 435–443.
- SUGIMOTO, A., AND E. WADA. 1993. Carbon isotopic composition of bacterial methane in a soil incubation experiment: Contributions of acetate and CO₂/H₂. *Geochim. Cosmochim. Acta* **57**: 4015–4027.
- WOOD, H. G. 1952. A study of carbon dioxide fixation by mass determination on the types of C¹³-acetate. *J. Biol. Chem.* **194**: 905–931.

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