UC Irvine

UC Irvine Previously Published Works

Title

Biogeochemistry of acetate in anoxic sediments of Skan Bay, Alaska

Permalink

<https://escholarship.org/uc/item/6j96c6vq>

Journal

Geochimica et Cosmochimica Acta, 48(9)

ISSN

0016-7037

Authors

Shaw, DG
Alperin, MJ
Reeburgh, WS
et al.

Publication Date

1984

DOI

10.1016/0016-7037(84)90035-8

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Biogeochemistry of acetate in anoxic sediments of Skan Bay, Alaska

D. G. SHAW, M. J. ALPERIN, W. S. REEBURGH and D. J. MCINTOSH
Institute of Marine Science, University of Alaska, Fairbanks, Alaska 99701, USA

(Received January 30, 1984; accepted in revised form June 8, 1984)

Abstract—The role of acetate in the biogeochemical cycling of organic matter in contemporary marine anoxic sediments of Skan Bay, Alaska was investigated with inhibition and *quasi in situ* turnover experiments. The turnover time for acetate oxidation in the upper 30 cm of the sediment column is ca. 1 hr. A molybdate inhibition experiment indicated that sulfate reducing bacteria were responsible for more than 95% of acetate oxidation. However, measured acetate oxidation rates exceeded sulfate reduction rates indicating that acetate oxidation rates are overestimated. Values for acetate concentration calculated from sulfate reduction rates ($0.3\text{--}3.4\ \mu\text{M}$) were considerably lower than directly measured acetate concentrations ($3.1\text{--}10.8\ \mu\text{M}$). Much of the chemically measured acetate may be microbially unavailable, perhaps in the form of a soluble or colloidal complex. A sorption experiment indicates that 10% to 40% of added acetate associates with Skan Bay sediment particles. Production of methane from acetate was detected only at 2 m depth.

INTRODUCTION

ORGANIC MATTER is recycled in marine sediments to carbon dioxide and other simple molecules by a complex and incompletely understood set of biogeochemical processes. In anoxic sediments especially, these processes are selective, indirect and incomplete. Developing a more detailed understanding of the molecular aspects of carbon remineralization in anoxic marine sediments is the object of research in several laboratories (REEBURGH, 1983) and the subject of this paper.

The complexity of carbon remineralization in anoxic marine sediments is reflected in the diverse microbial communities that reside there. While the anaerobic microbes as a group are quite versatile, a given strain may be rather limited in the substrates it can use and the products it excretes as waste. It is only through the use of the partially remineralized wastes of one group of microbes as substrate by another that full remineralization is accomplished. This gives rise to extracellular pools of intermediates in the sediment porewater which are both wastes and substrates. It appears that several key intermediates play important roles in the overall remineralization process.

A rapidly growing body of experimental results (BARCELONA, 1980; CHRISTENSEN and BLACKBURN, 1982; SANSONE and MARTENS, 1982; KING *et al.*, 1983) indicates that acetate is one such key intermediate. Acetate is an end product for fermenters of carbohydrates and other substrates (ANDRESEN *et al.*, 1973; SØRENSEN *et al.*, 1981) as well as a substrate for both sulfate reducers (WIDDEL and PFENNIG, 1977; MOUNTFORT and ASHER, 1981) and methanogens (MAH *et al.*, 1981; GUNNARSSON and RÖNNOW, 1982). It appears that acetate occupies an important branch point in the catabolism of organic matter: one branch leading to methanogenesis and the other to carbon dioxide formation *via* sulfate reduction.

Although sulfate reduction generally appears to be preferred when sulfate is available, the factors which control the relative importance of these pathways in anoxic marine sediments are not yet fully understood.

We have sought to determine the rates at which acetate is consumed by sulfate reducing bacteria and by methanogens in order to establish the relative importance of these two catabolic pathways for acetate and the importance of acetate in overall carbon remineralization. We have also carried out an inhibition experiment to investigate the extent to which acetate oxidation is dependent on sulfate reduction. In the course of this work we found a discrepancy in the measured sedimentary redox budget which suggests that a substantial portion of the acetate is not microbially available.

The sediments examined in this study were taken in September 1982 from Skan Bay ($53^{\circ}37'\text{N}$, $167^{\circ}03'\text{W}$), a basin of 65 m maximum depth and 10 m sill depth on the north side of Unalaska Island in the Aleutian Chain. The sediments of the Skan Bay basin are permanently anoxic. The water column becomes oxygen depleted each summer; in some years it becomes seasonally anoxic. Kelp (*Alaria pistulosa*) appears to be a major carbon source to the basin sediments. Skan Bay has been the site of previous biogeochemical studies (HATTORI *et al.*, 1978; REEBURGH, 1980).

METHODS

General

Sediments were obtained by box corer or piston corer from the deepest part of Skan Bay. Immediately upon recovery each box core was subsampled by inserting nine 50 cm lengths of 6.5 cm diameter transparent plastic coreliner. Care was taken to ensure that at least 10 cm of bottom water remained above the sediment in the subcore, which was tightly capped and placed in a water bath at *in situ* temperature (4°C). The sediment was processed within 12 hours of collection. Each rate determination used subcores from a single box core.

Acetate oxidation rate constants

The method used in our rate constant measurements is a modification of the technique described by JØRGENSEN (1978a). Experimental core liners, made by taping together ten 3 cm segments, were equipped with silicone septa to allow syringe injection of ^{14}C acetate solutions. Sediment was positioned within the segmented core liner by extruding a core upward, retaining the overlying water. Solutions of radiolabeled acetate were prepared from $\text{U-}^{14}\text{C}$ sodium acetate (60 mCi/mmol, Amersham) and sterile, oxygen-free saline solution of density approximately equal to that of the sediment porewater. Two separate experiments were carried out with solutions having acetate concentrations of $0.8\ \mu\text{M}$ ($10^2\ \text{dpm}\ \mu\text{L}^{-1}$) and $8\ \mu\text{M}$ ($10^3\ \text{dpm}\ \mu\text{L}^{-1}$). These solutions were stored in sealed glass ampoules.

The labeled substrate ($100\ \mu\text{L}$) was injected into the middle of core segments at four depths (3–6 cm, 9–12 cm, 18–21 cm, and 27–30 cm) to ensure that the reaction took place in a minimally disturbed environment. As long as the labeled substrate was added at concentrations below *in situ* levels and all products were recovered from a given segment, it was not necessary that the substrate be mixed uniformly with the sediment. Parallel cores were incubated for 5 min, 30 min, and 1 hr. Calculations and measurements show that oxygen diffusion to the center of the core is not significant over these short incubation times (JØRGENSEN, 1978a; REEBURGH, 1980). Millimolar sulfide concentrations in the sediment poison the system against redox changes caused by air oxidation. At the end of incubation, each segment was transferred to a nitrogen-flushed jar containing 50 mL of 1 M potassium hydroxide to halt microbial reactions and retain any carbon dioxide produced. The jar was quickly sealed with a rubber-gasketed metal lid fitted with a gas inlet, a gas outlet and a septum constructed from Swagelok fittings. The sediment was well mixed with the base and frozen until stripping (usually within 24 hours).

Methane produced from acetate oxidation was analysed by thawing the alkaline, frozen samples and attaching the jar to a stripping line. Helium was used to purge the stirred sediment slurry and the methane was passed through a cupric oxide filled quartz combustion tube at 600°C . Combustion efficiency was greater than 85%. The resulting carbon dioxide was trapped in a Harvey Biological Oxidizer trap (Harvey Instrument Co., Patterson, NJ) containing Woeller's solution, a phenethylamine based, carbon dioxide absorbing scintillation fluid (WOELLER, 1961). After the removal of the methane, the samples were acidified with 20 mL of 10 M sulfuric acid. The resulting carbon dioxide was trapped separately with Woeller's solution. To avoid quenching, hydrogen sulfide was trapped with cupric sulfate coated on calcined diatomite (Chromosorb P).

A "killed control" experiment showed that acidification and purging did not cause stripping of acetic acid. Sediment was placed in a stripper jar, slurried with base to halt acetate oxidation, injected with labeled acetate, acidified and finally stripped with helium. No radioactivity above background was detected. An aliquot of the slurry was centrifuged and a portion of the supernatant was counted in Aquasol. This showed that the added radioactivity remained in the slurry.

Samples in Woeller's solution or Aquasol were analysed using a Beckman LSC100 liquid scintillation counter. Quench corrections were applied using the external standard method. Acetate oxidation rate constants, k , were calculated from the equation of a first order reaction, $kt = \ln[A/(A - a)]$ where A is the amount of labeled acetate added (in dpm) and a is the amount of carbon dioxide (in dpm) formed at time t . For each depth, the values of $\ln[A/(A - a)]$ were plotted as a function of time and the rate constant k was taken as the slope of the best fit line.

Sulfate reduction rate constants

This incubation procedure was similar to the acetate rate constant measurements except that only one incubation

time, 20 hr, was employed. The ^{35}S sulfate solution was prepared by diluting a carrier-free sodium sulfate solution ($10^6\ \text{mCi}\ \text{mmol}^{-1}$) with degassed saline solution to give a final sulfate concentration of $3\ \text{nM}$ ($10^4\ \text{dpm}\ \mu\text{L}^{-1}$). The final solutions were stored in sealed glass ampoules.

Following incubation, sulfide was released from the sediment with sulfuric acid and stripped into a Harvey Biological Oxidizer trap containing 20 mL of 10% (w/v) zinc acetate solution. The resulting dispersion of zinc sulfide was diluted to volume and a subsample was mixed with Aquasol for liquid scintillation counting. Sulfate reduction rate constants were calculated by the fractional turnover rate constant method (REEBURGH, 1983) according to the equation $k = a/At$, where a is the recovered sulfide (in dpm), A is the added sulfate (in dpm) and t is time. Since the amount of sulfide formed never exceeded 2% of the added sulfate, the fractional rate constant method gives results equivalent to the semi-log treatment used for acetate.

Acetate concentration

Sediment porewater samples for acetate concentration measurements were obtained by low speed centrifugation for 20 min, made basic ($\text{pH} > 8$) and frozen until analysis. P-bromophenacyl esters of volatile fatty acids were prepared using the procedure of BARCELONA *et al.* (1980), except that the derivatization reaction was carried out at 100°C for one hr using toluene as solvent. This derivative of acetic acid was quantified by gas chromatography using a fused silica column (0.32 mm id by 25 m coated with SP-2100) and flame ionization detection. This procedure had a blank value $< 0.02\ \mu\text{mole}$ of acetate and gave a linear response down to $0.1\ \mu\text{mole}$. For the sample size and concentration steps used in this work, this gave a detection limit of $1\ \mu\text{M}$. Determinations were run in triplicate; means and standard deviations are reported. The method used quantifies acetate, propionate, butyrate, and iso-butyrate with similar precision and sensitivity, although only acetate was detected in Skan Bay pore waters.

Sulfate concentration

Porewater for sulfate determination was collected by centrifugation for 20 min at low speed. Hydrogen sulfide was removed by adding 3 mL of 10% hydrochloric acid to 15 mL of porewater and purging with a stream of helium for 30 min. Samples were sealed and stored frozen until analysis. Sulfate concentrations were determined on a Dionex 2010i ion chromatograph using a HPIC Anion S4 separator column, a continuous flow suppressor column and a conductivity detector. A $50\ \mu\text{L}$ sample of thawed porewater which had been diluted 25 to 100 fold with deionized water was eluted with a solution prepared from 2.9 mmole sodium bicarbonate and 2.3 mmole sodium carbonate per L deionized water. We have analysed porewater sulfate samples collected in Skan Bay in 1981 by both this ion chromatographic method and by the titrimetric method of REEBURGH and SPRINGER-YOUNG (1983) and obtained comparable results.

Inhibition of sulfate reduction

Molybdate, a known inhibitor of sulfate reduction (SØRENSEN *et al.* 1981), was used to examine the relationship between sulfate reduction and acetate oxidation. A slurry of sediment from 15–20 cm was prepared by mixing sediment with an equal volume of degassed seawater. This was anaerobically dispensed in 10 mL portions into helium filled 30 mL serum vials, which were capped and placed in an incubator at 4°C . After 2 hours, 8 vials were treated with $0.2\ \text{mL}$ of $1.0\ \text{M}$ sodium molybdate (prepared in 3.5% saline and degassed) to give a final molybdate concentration of $20\ \text{mM}$. Following an additional 24 hours in the incubator $100\ \mu\text{L}$ of $0.8\ \mu\text{M}$ $\text{U-}^{14}\text{C}$ sodium acetate ($10^4\ \text{dpm}$) were added to each of the molybdate amended vials and also to 8 vials of unamended slurry. Pairs of vials were treated with $3\ \text{mL}$

of 1 M potassium hydroxide after incubation times ranging from 0 to 96 hrs and frozen for later analysis of radiolabeled carbon dioxide and methane as described above.

Sediment sorption of acetate

The extent to which acetate associates with Skan Bay sediment was investigated through a displacement experiment similar to that reported by CHRISTENSEN and BLACKBURN (1982). Solutions of either 1.0 mM or 1.0 M sodium acetate were added to glass, screw cap centrifuge tubes. To each tube was also added 100 μ L of 8 μ M $U-^{14}C$ sodium acetate and 10.0 mL of sediment from either 5–10 cm or 20–25 cm. The resulting mixture was vortex mixed and then centrifuged to yield a slightly yellow supernatant, 1.0 mL of which was taken for liquid scintillation counting in Aquasol. The total elapsed time between addition of labeled acetate to the slurry and the transfer of the supernatant to Aquasol was approximately 20 minutes. Wet and dry weights of the sediment samples were determined so that results could be expressed on a weight basis. A second experiment was performed to investigate the role of bacterial activity in the sorption process. Sediment (4.0 mL) was treated with 0.1 mL chloroform and then carried through the above procedure.

RESULTS

Acetate oxidation rates

Measured acetate consumption rates (*i.e.*, the product of rate constant and acetate concentration at a given depth) as a function of depth in Skan Bay sediments are shown in Fig. 1, which also shows the sulfate reduction rate profile for these sediments. The rate of acetate oxidation ranges from 2.4 μ M hr^{-1} at 3–6 cm to 17 μ M hr^{-1} at 18–21 cm and exceeds sulfate reduction at depths below 6 cm. Measured acetate concentration also increases with depth as shown in Fig. 2 which also shows acetate concentrations calculated by a procedure described in the Discussion. Two *quasi in situ* rate constant determinations were performed. The first used 100 μ L of 0.8

FIG. 1. Depth profiles of measured acetate oxidation rate (O) and sulfate reduction rate (*) in Skan Bay sediments.

FIG. 2. Porewater acetate concentration profiles in Skan Bay as measured directly (O) and calculated (*) from sulfate data (see text). Error bars show one standard deviation.

μ M labeled sodium acetate (10^4 dpm) in each experimental core section. This concentration probably gave a true tracer experiment (*cf.* porewater concentrations, but also see Discussion). The second determination used the same volume of 8 μ M labeled acetate (10^5 dpm) and thus may be approaching saturation. This is indicated by the fact that the apparent rate constants found in the second determination for 3–6 and 18–21 cm are lower than the values obtained in the first (Fig. 3). We have therefore used the constants from the first determination in our calculations of acetate consumption rate (Fig. 1). The same conclusions about the relative magnitudes of acetate oxidation rate and sulfate reduction rate are reached if we use rate constant data from the second determination.

Our rate calculations are based on the assumption that tracer consumption is first order with respect to acetate concentration. However, if the entire consumption process were a single first order reaction, the semi-log plots of Fig. 3 would extrapolate through 1.0 at zero time. The *quasi in situ* method does not allow this point to be determined experimentally, so we have performed "killed controls" in which labeled acetate is added to sediment previously slurried with sodium hydroxide. These controls showed no acetate consumption. For the determination of the rate constant using 0.8 μ M acetate, some rapid process consumed roughly 30% of the acetate in 0.05 hour. This is not due to the oxidation of a labile impurity since the same fraction is not rapidly consumed when 8 μ M acetate (prepared from the same reagents at the same time) was used.

Inhibition of sulfate reduction

Molybdate dramatically inhibited carbon dioxide production from acetate (Fig. 4). More than 90% of

FIG. 3. Semi-log plots from which acetate oxidation rate constants are obtained as the slopes of the lines. In both panels "O" indicates 3–6 cm; "X", 9–12 cm; "+", 18–21 cm; and "*", 27–30 cm. The upper panel shows data from experiments with 0.8 μM labeled acetate; the lower panel with 8 μM . The corresponding rate constants (hr^{-1}) are: upper panel, 3–6 cm, 0.78; 9–12 cm, 0.34; 18–21 cm, 1.57; 27–30 cm, 0.97; lower panel, 3–6 cm, 0.55; 9–12 cm, 0.85; 18–21, 0.56.

the control acetate was oxidized in 96 hours while less than 5% of the acetate in samples to which molybdate had been added was oxidized. This work used slurried sediment rather than the *quasi in situ* approach recently demonstrated by CHRISTENSEN

FIG. 4. The effect of inhibition of sulfate reducing bacteria by the addition of molybdate (O) on acetate oxidation in Skan bay sediment. Controls (*) lack molybdate.

(1984). Although this clearly affected the rate of acetate oxidation, there is no evidence that it changed the dominant pathway. The first order rate constant for acetate oxidation in the control is 0.026 hr^{-1} , about 60 times lower than the value found by the *quasi in situ* technique (Fig. 3). In a comparison of sulfate reduction rate measurements by these two techniques, JØRGENSEN (1978a) also observed smaller rate constants for slurried sediments and was able to conclude that physical disturbance (mixing) of the sediment accounted for the decrease.

Sediment sorption of acetate

The results of the acetate sorption experiment (Table 1) indicate that 10% to 40% of the added acetate sorbs to sediment particles within 20 min. The decline in percentage activity in the supernatant with added unlabeled acetate at 20–25 cm was unexpected since we had anticipated that the addition of unlabeled acetate would compete for sorption sites. A second experiment in which sediment was treated with chloroform to halt microbial activity prior to addition of acetate, showed similar percentages of acetate-sediment association.

Methane production

No detectable methane was formed in the acetate turnover experiments just described. Even when sulfate reduction of acetate was inhibited with molybdate, methane formation was not detected. The production of ^{14}C -methane was observed in an experiment using sediment collected by piston corer from a depth of 2 m. In this experiment a single sediment sample was inoculated with 100 μL of 8 μM ^{14}C -sodium acetate and incubated for 48 hrs. Analysis showed that 1.3% of the added ^{14}C was converted to methane and 13.2% to carbon dioxide. Experiments performed at Skan Bay in September 1981 with $1\text{-}^{14}\text{C}$ -lactate or methyl- ^{14}C -methionine also showed no detectable labeled methane production from those substrates in the upper 30 cm of the sediment column.

DISCUSSION

Results from studies of two other marine anoxic sediment environments suggest that the acetate OXI-

Table 1. Association of Skan Bay sediment with radiolabeled acetate at conditions noted as "treatment". The experimental conditions are described in the text.

Depth cm	Treatment	% Activity In Supernatant
5–10	Water	60
5–10	1mM NaOAc	66
5–10	1M NaOAc	67
20–25	Water	41
20–25	1mM NaOAc	74
20–25	1M NaOAc	68

duction rates in excess of sulfate reduction rates may be a widespread phenomenon. Blackburn and co-workers studied acetate metabolism in Danish marine sediments (ANSBAEK and BLACKBURN, 1980; CHRISTENSEN and BLACKBURN, 1982). Of the sediments investigated, probably the most similar to Skan Bay are those of the Limfjorden, where values for acetate concentration ($0.1\text{--}6\ \mu\text{M}$) and rate constant ($2\ \text{hr}^{-1}$) (ANSBAEK and BLACKBURN, 1980) are similar to the Skan Bay values reported here. Blackburn and co-workers found acetate oxidation rates in excess of both sulfate reduction rates and carbon mineralization rates calculated from ammonium turnover data. CHRISTENSEN and BLACKBURN (1982) showed for sediment from the Danish Belt Sea that a major fraction of added acetate was associated with particulate material and that radiolabeled acetate behaved somewhat differently than total acetate in a gel filtration separation. These results were taken as indication that a portion of the chemically measured acetate pool was unavailable for microbial consumption.

Acetate metabolism has also been investigated in the sediments of Cape Lookout Bight by SANSONE and MARTENS (1981a,b, 1982). These sediments are more active metabolically than Skan Bay because of higher water temperature and ten-fold greater organic input. Our acetate consumption rate constants (*ca.* $1\ \text{hr}^{-1}$, Fig. 3) are similar to those measured in Cape Lookout Bight (SANSONE and MARTENS, 1981a, 1982). However, as pointed out by those workers (SANSONE and MARTENS, 1982), acetate concentration was determined on whole sediment, probably including cellular and sorbed as well as porewater acetate. Their acetate concentration values are at least an order of magnitude greater than ours. This leads to acetate oxidation rates of about $200\ \mu\text{M}\ \text{hr}^{-1}$ in the sulfate reducing zone where, in a separate study, sulfate reduction rates in the range $20\text{--}50\ \mu\text{M}\ \text{hr}^{-1}$ were measured (KLUMP, 1980).

Our results for Skan Bay show that the rate of acetate oxidation exceeds that of sulfate reduction at depths greater than 6 cm. Since the stoichiometry of this reaction is 1:1 and since sulfate reduction is thought to utilize other substrates as well, we expected the acetate oxidation rate to be less than that of sulfate reduction. JØRGENSEN (1978b,c) has shown that the ^{35}S sulfate technique gives rates that are consistent with microbiological data and numerical models. This suggests that the discrepancy in our results can be attributed to the acetate oxidation rates rather than the sulfate reduction rates. We will consider three hypotheses to account for the apparent overestimation of acetate oxidation rates.

1. *Acetate may be oxidized at the expense of substrates other than sulfate.*
2. *The acetate oxidation rate constants may be overestimated.*
3. *Acetate concentrations may be overestimated.*

Our inhibition experiment sheds considerable light on the first hypothesis. The strong inhibition of

acetate oxidation by molybdate (Fig. 4) indicates that sulfate is the predominant electron acceptor. This conclusion is based on molybdate's ability to inhibit sulfate reduction in the laboratory (PECK, 1959) and at 20 mM in marine sediments (OREMLAND and TAYLOR, 1978; SØRENSEN *et al.*, 1981). In our work the slurry necessary for the inhibition experiment was prepared using degassed seawater and therefore was enriched in sulfate, so our result should be accepted with caution. Inhibition of acetate oxidation by molybdate in marine sediments has been observed by others (BANAT *et al.*, 1981; WINFREY and WARD, 1983). Both of these studies report no methane production from acetate either with or without molybdate. OREMLAND and POLCIN (1982) and SENIOR *et al.* (1982) have presented data showing that methane production from acetate is retarded by sulfate. Although we cannot exclude the possibility that the added sulfate or molybdate may have interfered with methanogenesis from acetate, the most likely explanation of our results is that at least in the upper 20 cm of Skan Bay sediment more than 95% of acetate oxidation is accomplished by sulfate reducing bacteria. Based on these results we reject the hypothesis that acetate is oxidized at the expense of substrates other than sulfate as an explanation of the observed rate discrepancy.

The second and third hypotheses need to be considered together since formation of an acetate complex could influence the values measured for either acetate oxidation rate constant or concentration and since sorption of acetate to sediment particles could also affect the rate constant. The tracer solution was made by dissolving labeled acetate in organic free saline solution. It is reasonable to assume that the tracer acetate is completely uncomplexed and therefore microbially available. Although the concentration of the tracer solution ($0.8\ \mu\text{M}$) was below the measured acetate concentrations, the solution may have exceeded free acetate concentrations and led to a stimulation of acetate oxidizing bacteria. Another potential artifact stems from the fact that a portion of the added acetate is rapidly scavenged from solution by sediment particles. Once sorbed, this acetate may be less available to microbes. However, this effect would tend to underestimate acetate oxidation rate constants.

Our results (Table 1) suggest that there is some association of acetate with Skan Bay sediments. Our findings are comparable to those of HORDIJK and CAPPENBERG (1983) who found that a single centrifugation recovered 60% of added acetate from Lake Vechten sediments. SANSONE (1982) has reported that acetate association with sterilized carbonate sediments is minor. CHRISTENSEN and BLACKBURN (1982) showed that the extent of sorption is time dependent for the Danish sediments which they studied. Our results correspond to an acetate-sediment contact time of approximately 20 minutes. We do not know how our results would vary with contact time. Neither can we rule out the possibility that Skan Bay sediments have a greater capacity to irre-

versibly sorb acetate, but were already nearly saturated. These various experiments are difficult to compare with one another because differences in experimental technique and between the sediments studied cannot be resolved. These results suggest that sediment sorption occurs to some extent. Since this would tend to underestimate acetate oxidation rate constants, it would have to be offset by some larger effect.

Sorption of acetate to sediment cannot account for an overestimation of acetate concentration, since sediment bound acetate would be neither measurable in porewater nor microbially available. An explanation consistent with our data is that the bulk of the porewater acetate forms a complex with dissolved or colloidal material which has sufficient stability to render the acetate unavailable to immediate microbial attack but which is decomposed in the course of our acetate determination. If this speculation is correct, it appears (Fig. 1) that the degree of complexation increases with depth. While this possibility is consistent with our results and those of other workers, there is as yet little direct data to support it. Until there is sufficient data to balance the redox budget for organic matter remineralization, all data as well as their interpretation must be regarded as tentative. We can estimate microbially available acetate concentrations using our acetate oxidation rate constants and assuming that the acetate oxidation rate is equal to the sulfate reduction rate. This approach gives acetate concentration values which are close to the measured one near the surface but then decrease with depth (Fig. 2). We cannot exclude the possibility that our measured acetate concentrations are high because of an artifact introduced during the isolation of porewater by centrifugation.

Either sorption or complexation of acetate taking place over a period of minutes could explain the large initial acetate oxidation rate constant observed in our experiment using $0.8 \mu\text{M}$ tracer. If the initial concentration of free acetate was high enough to be stimulatory, but was soon reduced by sorption or complexation, the experimental result would be to initially overestimate the rate constant. At present our understanding of the effects of sorption and complexation of acetate on our measured acetate oxidation rate constants and acetate concentrations is unclear. We cannot now reach a firm conclusion about the source of our overestimation of acetate oxidation rates.

We observed the production of methane from acetate only at a depth of 2 m in our tracer experiments. Even at this depth, where sulfate is presumably exhausted, the ratio of carbon dioxide to methane was 10:1, much greater than the value of 1:1 expected for the simple fermentation of acetate. One explanation, put forward by SANSONE and MARTENS (1982) to explain a similar observation is that methanogenesis takes place *via* interspecific hydrogen transfer which allows a dilution of the carbon label into the sediment carbonate pool. The interspecific hydrogen transfer mechanism (WOLIN, 1982) involves conversion of

acetate to carbon dioxide plus hydrogen by one organism. These products are excreted and the hydrogen scavenged by another bacterial species for recombination with carbon dioxide to form methane. However, the carbon dioxide used in this second step contains stable carbonate species from the porewater pool (mM concentrations) as well as carbon dioxide from acetate oxidation (sub μM concentrations). Thus methanogenesis could be occurring, without being detectable from a carbon tracer. Another possible explanation is that methanogenesis proceeds by co-metabolism of acetate and other initially less oxidized substrates. MAH *et al.* (1981) have demonstrated in culture that such a co-metabolism can lead to the preferential oxidation of acetate and reduction of methanol. This would also result in the formation of methane at the expense of acetate but using another carbon source.

CONCLUSIONS

From this work we draw five conclusions about the role of acetate in the biogeochemical cycling of organic matter at Skan Bay.

1. The measured rate of acetate oxidation is too great to be balanced by the measured rate of sulfate reduction. One explanation for this fact is that only a fraction of the measured porewater concentration of acetate is microbially available, leading to errors in the rate determinations.
2. Acetate is oxidized to carbon dioxide in Skan Bay sediments with rate constants comparable to those observed in other anoxic marine sediments.
3. Acetate is not converted to methane in the upper 30 cm of sediment. Methane production from acetate occurs only at 2 m.
4. Most of the acetate oxidation is inhibited by 20 mM sodium molybdate and is therefore probably mediated by sulfate reducing bacteria.
5. A portion, perhaps 10% to 40%, of added acetate is rapidly sorbed to sediment particles.

Acknowledgments—This research was supported by the Marine Chemistry Program of the National Science Foundation through Grant 81-17882. We thank S. M. Henrichs and K. H. Nealson for reviewing a draft of this paper and the crew of R/V Alpha Helix for making work at Skan Bay possible and pleasant. This is contribution number 550 of the Institute of Marine Science, University of Alaska.

REFERENCES

- ANDRESEN J. R., SCHAUPP A., NEURAUER C. J., BROWN A. and LJUNGDAHL L. J. (1973) Fermentation of glucose, fructose, and xylose by *Clostridium thermoaceticum*: effect of metals on growth yield, enzymes, and the synthesis of acetate from CO_2 . *J. Bact.* **114**, 743–751.
- ANSBAEK J. and BLACKBURN T. H. (1980) A method for the analysis of acetate turnover in coastal marine sediments. *Microb. Ecol.* **5**, 253–264.
- BANAT I. M., LINDSTROM E. B., NEDWELL D. R. and BALBA M. T. (1981) Evidence for coexistence of two distinct functional groups of sulfate-reducing bacteria in salt marsh sediment. *Appl. Environ. Microbiol.* **42**, 985–992.

- BARCELONA M. J. (1980) Dissolved organic carbon and volatile fatty acids in marine sediment pore waters. *Geochim. Cosmochim. Acta* **44**, 1977–1984.
- BARCELONA M. J., LILJESTRAND and MORGAN J. J. (1980) Determination of low molecular weight volatile fatty acids in aqueous samples. *Anal. Chem.* **52**, 321–325.
- CHRISTENSEN D. (1984) Determination of substrates oxidized by sulfate reduction in intact cores of marine sediments. *Limnol. Oceanogr.* **29**, 189–192.
- CHRISTENSEN D. and BLACKBURN T. H. (1982) Turnover of ^{14}C -labelled acetate in marine sediments. *Mar. Biol.* **71**, 113–119.
- GUNNARSSON L. A. H. and RÖNNOW P. H. (1982) Interrelationships between sulfate reducing and methane producing bacteria in coastal sediments with intense sulfide production. *Mar. Biol.* **69**, 121–128.
- HATTORI A., GOERING J. J. and BOISSEAU D. W. (1978) Ammonium oxidation and its significance in the summer cycling of nitrogen in oxygen depleted Skan Bay, Unalaska Island, Alaska. *Mar. Sci. Commun.* **4**, 139–151.
- HORDIJK K. A. and CAPPENBERG T. E. (1983) Quantitative high-pressure liquid chromatography-fluorescence determination of some important lower fatty acids in lake sediments. *Appl. Environ. Microbiol.* **46**, 361–369.
- JØRGENSEN B. B. (1978a) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. *Geomicrobiol. J.* **1**, 11–27.
- JØRGENSEN B. B. (1978b) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. II. Calculation from mathematical models. *Geomicrobiol. J.* **1**, 29–47.
- JØRGENSEN B. B. (1978c) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. III. Estimation from chemical and bacteriological field data. *Geomicrobiol. J.* **1**, 49–64.
- KING G. M., KLUG M. J. and LOVLEY D. R. (1983) Metabolism of acetate, methanol, and methylated amines in intertidal sediments of Lowes Cove, Maine. *Appl. Environ. Microbiol.* **45**, 1848–1853.
- KLUMP J. V. (1980) Benthic nutrient regeneration and the mechanisms of chemical sediment-water exchange in an organic-rich coastal marine environment. Ph.D. dissertation Univ. of N. Carolina at Chapel Hill.
- MAH R. A., SMITH M. R., FERGUSON T. and ZINDER S. (1981) Methanogenesis from $\text{H}_2\text{-CO}_2$, methanol, and acetate by *Methanosarcina*. In *Microbial Growth on C-1 Compounds*, (ed. H. DALTON), pp 131–141. Heyden, London.
- MOUNTFORT D. O. and ASHER R. A. (1981) Role of sulfate reduction versus methanogenesis in terminal carbon flow on polluted intertidal sediment of Waimea Inlet, Nelson, New Zealand. *Appl. Environ. Microbiol.* **42**, 252–258.
- OREMLAND R. S. and POLCIN S. (1982) Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. *Appl. Environ. Microbiol.* **44**, 1270–1276.
- OREMLAND R. S. and TAYLOR B. F. (1978) Sulfate reduction and methanogenesis in marine sediments. *Geochim. Cosmochim. Acta* **42**, 209–214.
- PECK H. D. JR. (1959) The ATP-dependent reduction of sulfate with hydrogen in extracts of *Desulfovibrio desulfuricans*. *Proc. Nat. Acad. Sci. U.S.A.* **45**, 701–708.
- REEBURGH W. S. (1980) Anaerobic methane oxidation: rate depth distributions in Skan Bay sediments. *Earth Planet. Sci. Lett.* **47**, 345–352.
- REEBURGH W. S. (1983) Rates of biogeochemical processes in anoxic sediments. *Ann. Rev. Earth Planet. Sci.* **11**, 269–298.
- REEBURGH W. S. and SPRINGER-YOUNG M. (1983) New measurements of sulfate and chlorinity in natural sea ice. *J. Geophys. Res.* **88**, 2959–2966.
- SANSONE F. J. (1982) Adsorption of acetate and butyrate onto marine sediment surfaces (abstract). *EOS* **63**, 1015.
- SANSONE F. J. and MARTENS C. S. (1981a) Determination of volatile fatty acid turnover rates in organic-rich marine sediments. *Mar. Chem.* **10**, 233–247.
- SANSONE F. J. and MARTENS C. S. (1981b) Methane production from acetate and associated methane fluxes from anoxic coastal sediments. *Science* **211**, 707–709.
- SANSONE F. J. and MARTENS C. S. (1982) Volatile fatty acid cycling in organic-rich marine sediments. *Geochim. Cosmochim. Acta* **46**, 1575–1589.
- SENIOR E., LINDSTROM E. B., BANAT I. M. and NEDWELL D. B. (1982) Sulfate reduction and methanogenesis in the sediment of a saltmarsh on the east coast of the United Kingdom. *Appl. Environ. Microbiol.* **43**, 987–996.
- SØRENSEN J., CHRISTENSEN D. and JØRGENSEN B. B. (1981) Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. *Appl. Environ. Microbiol.* **42**, 5–11.
- WIDDEL F. and PFENNIG N. (1977) A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. *Arch. Microbiol.* **112**, 119–122.
- WINFREY M. R. and WARD D. M. (1983) Substrates for sulfate reduction and methane production in intertidal sediments. *Appl. Environ. Microbiol.* **45**, 193–199.
- WOELLER F. H. (1961) Liquid scintillation counting of C^{14}O_2 with phenethylamine. *Anal. Biochem.* **2**, 508–511.
- WOLIN M. J. (1982) Hydrogen transfer in microbial communities. In *Advances in Microbial Ecology*, Vol. 3 (eds. A. T. BULL and J. H. SLATER), pp. 323–356. Academic Press.