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Evidence for Coexistence of Two Distinct Functional Groups of Sulfate-Reducing Bacteria in Salt Marsh Sediment

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Oxidation of acetate in salt marsh sediment was inhibited by the addition of fluoroacetate, and also by the addition of molybdate, an inhibitor of sulfate-reducing bacteria. Molybdate had no effect upon the metabolism of acetate in a freshwater sediment in the absence of sulfate. The inhibitory effect of molybdate on acetate turnover in the marine sediment seemed to be because of its inhibiting sulfate-reducing bacteria which oxidized acetate to carbon dioxide. Sulfide was not recovered from sediment in the presence of molybdate added as an inhibitor of sulfate-reducing bacteria, but sulfide was recovered quantitatively even in the presence of molybdate by the addition of the strong reducing agent titanium chloride before acidification of the sediment. Reduction of sulfate to sulfide by the sulfate-reducing bacteria in the sediment was only partially inhibited by fluoroacetate, but completely inhibited by molybdate addition. This was interpreted as showing the presence of two functional groups of sulfate-reducing bacteria—one group oxidizing acetate, and another group probably oxidizing hydrogen.

Sulfate-reducing bacteria (SRB) have until recently been regarded as metabolizing only a narrow range of organic electron donors, principally lactate (17), although some at least are known to utilize hydrogen (2, 4, 20). Abram and Nedwell (1, 2) have shown that these hydrogen-utilizing SRB are capable of scavenging hydrogen, causing the "hydrogen transfer" reaction initially described (6) when methanogenic bacteria were the principal hydrogen scavengers. The SRB outcompeted methanogenic bacteria for the available hydrogen. In addition, it has been demonstrated (5) that the SRB are the predominant hydrogen scavengers in the sediment of the Colne Point salt marsh (the site has been described by Nedwell and Abram [12]). A similar observation has been reported by Mountfort et al. (11) in New Zealand intertidal sediments. The reported inhibition of methanogenesis in the presence of active sulfate reduction in field situations (7, 15, 23) has therefore been at least partially attributed to the SRB outcompeting methanogens for available hydrogen (2, 15).

It has previously been suggested (9, 11) that the SRB might be the major acetate oxidizers in at least some anaerobic sediments, and Widdel and Pfennig (22) have already described an acetate-oxidizing SRB named *Desulfotomaculum acetoxidans*. To investigate whether the SRB were significant acetate oxidizers in the Colne Point salt marsh sediment, the following experiments were carried out.

MATERIALS AND METHODS

Products of acetate metabolism in salt marsh sediments. A large vertical core was taken with a plastic coring tube (7-cm diameter) from intertidal sediment in Ray Creek, Colne Point salt marsh. Small cores were removed from the large core at depths of 0 to 1, 4 to 5, and 9 to 10 cm by using 5-ml plastic hypodermic syringes with the needle end removed. The syringes were capped with rubber teats to exclude air and returned to the laboratory. Each core was injected via the rubber seal with 50 μl of [*U*-^{14}C]acetate solution (10 μCi·ml^{-1}, 57.8 mCi·mmol^{-1}), withdrawing the syringe while injecting to distribute the tracer solution as evenly as possible. The samples were incubated for 2 h at the temperature of the sediment which had been measured in the field.

After incubation each sediment core was extruded into a flask containing 35 ml of potassium hydroxide solution (1 M). The flask was immediately stoppered, and the sediment was dispersed in the liquid by shaking and then gassed with a stream of air from which carbon dioxide had been removed by a soda lime trap. The alkali present in the flask prevented carbon dioxide being carried over during this first stage of distillation. This was checked by adding $H^{14}CO_3$ solution to a control sediment sample containing no labeled acetate. No radioactivity was detected when the air stream was passed through a carbon dioxide-absorbing scintillant (^{14}C Absorber P; Fisons, United Kingdom),

and then scintillation counted. Methane in sediment samples injected with [U-^{14}C]acetate was carried by the air stream through a copper oxide column heated to 850°C, which oxidized methane to carbon dioxide. This was then absorbed by bubbling through 5 ml of carbon dioxide-absorbing scintillant. The efficiency of methane oxidation was checked by passing known volumes of methane through the copper oxide column, collecting the effluent gas over water, and analyzing for residual methane by gas-liquid chromatography (Perkin Elmer F33 gas chromatograph with flame ionization detector; Poropak N column at 70°C; nitrogen carrier gas at 20 ml·min^{-1}). The oxidation efficiency of methane was higher than 99%.

In the second stage of the distillation the residual suspension was acidified to lower than pH 1.0 by the addition of 50% (vol/vol) hydrochloric acid and again gassed with air. The effluent gas was passed through duplicate scintillation vials in series, each containing 5 ml of carbon dioxide-absorbing scintillant, to trap $^{14}CO_2$ formed from labeled acetate.

To measure radioactivity in the residual acetate the sediment suspension in the flask was finally centrifuged at 2,000 × g for 15 min, and 0.5 ml of the clear supernatant was added to 4.5 ml of Triton X scintillant [Triton X-100, 0.33 liters; toluene, 0.66 liters; 2,5-diphenyloxazole, 2.64 g·liter^{-1}; 1,4-bis-(5-phenyloxazolyl)benzene, 0.033 g·liter^{-1}]. This scintillant was routinely used because it was miscible with up to 10% (vol/vol) water, whereas the ^{14}C Absorber P was miscible with less than 1% aqueous volume.

The counting efficiency for [U-^{14}C]acetate differed in the two scintillants, so the radioactivity measured in the Triton X scintillant was corrected to that in ^{14}C Absorber P by using a correction factor previously determined by counting a 10-μl sample of [U-^{14}C]acetate solution in each scintillant.

The radioactivity in all samples was counted in a scintillation counter (Packard Tri-Carb 460C), using an external standard to correct for quenching. The radioactivity of residual acetate was corrected for subsample volume. It was assumed in these experiments that residual dissolved radioactivity was due to labeled acetate. This had been previously checked by extracting a sediment slurry sample with ether and analyzing the extract for radioactivity in organic acids by gas-liquid chromatography linked to a gas proportional counter. The only radioactivity detectable was in the acetate peak.

The radioactivity of residual dissolved acetate could be affected by low extraction if some labeled acetate adsorbed to, or was entrained by, the centrifuged sediment particles. This was corrected for by using a reference sediment core which was injected with the same volume of [U-^{14}C]acetate solution and then immediately extracted by the method described above. As the radioactivity injected into the sample was known, the soluble acetate radioactivity measured in water from this control sample could be used to correct other extracted samples for low extraction efficiency. Moreover, although the gas stream was bubbled through ^{14}C Absorber P, no radioactivity was detectable. This confirmed that there was no significant volatilization and carry-over of labeled acetate from the acidified sediment suspension during the gassing period used here to strip carbon dioxide.

Experiments with sediment slurries. Material was collected from the top 5 cm of sediment in Ray Creek, Colne Point salt marsh in completely filled and capped glass jars. Sediment slurries were prepared in the laboratory by mixing equal volumes of anaerobic sediment and aged seawater deoxygenated by flushing with oxygen-free nitrogen. Hungate anaerobic technique was used to maintain anaerobiosis during preparation. The sediment contained abundant sulfide, which is an effective redox buffer, and the slurry remained black and reduced.

(i) Experiment with [^{14}C]acetate in salt marsh sediment. Samples (160 ml) of homogenized slurry were added to each of 10 250-ml conical flasks. Duplicate flasks were prepared with the following treatments: (i) no addition; (ii) sodium monofluoroacetate (5 mM final concentration); (iii) sodium molybdate (20 mM final concentration); (iv) no addition; and (v) monofluoroacetate as for (ii).

All chemicals used were of analytical reagent grade. The flasks were closed with Suba-seals and gassed for 15 min. Flasks i through iii were gassed with 80% (vol/vol) N_2–20% (vol/vol) CO_2, and flasks iv and v were gassed with 80% (vol/vol) H_2–20% (vol/vol) CO_2.

The flasks were incubated at 25°C overnight on an orbital shaker at a speed just sufficient to prevent settlement of sediment particles. This preincubation was necessary as experience had shown that sometimes a lag occurred before molybdate or fluoroacetate became totally inhibitory. As acetate turnover was so rapid, it was necessary to ensure that inhibition had occurred before labeled acetate was added to the slurry. Each flask was then injected with 500 μl of sodium [U-^{14}C]acetate solution (10 μCi·ml^{-1}; 57.8 mCi·mmol^{-1}, Radiochemical Centre, Amersham).

Samples (3 ml) were withdrawn with a 5-ml plastic hypodermic syringe and introduced in a universal container closed with a Suba-seal and containing 0.5 ml of concentrated hydrochloric acid. The universals were then gassed with oxygen-free nitrogen for 15 min, and the effluent gas was passed through 4 ml of carbon dioxide-absorbing scintillant in a plastic scintillation tube to collect labeled carbon dioxide.

The acidified slurry was then centrifuged at 2,000 × g for 10 min, and 0.5 ml of the clear supernatant was added to 4 ml of Triton X scintillant to count the remaining radioactive acetate.

During the experiment a sample was taken immediately after the addition of reagents to the slurry to provide a zero time measurement of radioactivity, and samples were thereafter taken at regular intervals up to 48 h. The flasks were regassed only once after 2 h during the course of the experiment.

(ii) Experiment with [U-^{14}C]acetate in freshwater sediment. To elucidate the effect of the inhibitors molybdate and fluoroacetate upon acetate turnover an identical experiment was carried out with [U-^{14}C]acetate in slurries of an anoxic freshwater sediment devoid of sulfate. This sediment was obtained from the lake in the grounds of Wivenhoe Park, University of Essex, Colchester, United Kingdom. A sample of sediment was removed from the top 5 cm of the lake sediment and used, with the precautions to exclude oxygen described for the salt marsh sediment, to make a 50% (vol/vol) slurry of sediment with distilled water. Distilled water was used rather than lake water

as the latter contained low levels of sulfate. The sulfate content of the slurry was below the limits of detection (<0.1 mM) when measured by a barium sulfate turbidimetric technique (3). The slurry was dispensed into flasks and gassed out with N_2–CO_2. The details of the experiment were identical to those described for that with the salt marsh sediment, except that the lake slurry was preincubated in the presence of the added inhibitors for only 3 h, rather than overnight, before labeled acetate was added, and the experiments were only carried out under N_2–CO_2. Residual dissolved radioactivity was measured as described for the experiment with salt marsh sediment and subsamples of slurry were periodically withdrawn over a 96-h period.

(iii) Recovery of sulfide from sediment in the presence of molybdate. We used [^{35}S]sulfate as a tracer to measure the rates of sulfate reduction in slurries of salt marsh sediment in the presence of different inhibitors. The method used was that described by Sorokin (19) and depends upon the acidification of a sample of sediment to volatilize any [^{35}S]-sulfide which is then gassed over with oxygen-free nitrogen into traps containing zinc acetate solution. We frequently observed that when 20 mM molybdate was added to a sediment sample to inhibit sulfate reduction little or no sulfide was subsequently evolved or trapped after acidification of the sample, even though some sulfide was initially present. This has also been reported by Oremland and Silverman (14). At the same time, in the presence of molybdate a dark blue color developed in the sediment interstitial water after acidification of the sample. A centrifuged sample of this interstitial water showed an absorption peak at 870 nm. The addition of further sulfide to the sample intensified the blue color, indicating that its optical density was dependent upon the amount of sulfide present. Thus, the apparent lack of measured [^{35}S]-sulfide activity recovered in the zinc acetate traps may be a reflection of sulfide chemically reacting in the sediment rather than inhibition of sulfate reduction to sulfide.

Strickland and Parsons (21) described a method for analysis of phosphate in seawater where a phosphomolybdate complex is formed under acidic conditions in the presence of added molybdate solution, and this is then reduced by ascorbic acid to a blue-colored complex. Trivalent antimony is also required to form this blue complex, which has a peak absorption at 885 nm.

To check whether this blue complex could be formed by sulfide we added excess phosphate to seawater followed by the standard reagents described by Strickland and Parsons (21), but omitted the ascorbic acid. When the solution was acidified and then hydrogen sulfide was passed through, the characteristic blue color developed. Thus, it appears that the lack of any evolved sulfide from acidified sediment in the presence of molybdate may be the result of the sulfide acting as a reducing agent in forming the blue phospho-molybdate complex. Many anoxic sediments contain abundant phosphate, and the availability of sulfide as a reducing agent appears to be the factor limiting the complex formation. The slight difference in wavelength of the absorbtion peak of the complex formed in the sediment (870 nm) compared with that produced in the standard phosphate analysis (885 nm) may be because trivalent ions other than antimony were involved in the formation of the colored complex in the sediment.

However, the reoxidation of sulfide upon acidification in the presence of molybdate interfered with measurements of the [^{35}S]sulfide evolved. To overcome this we added an excess of strong reducing agent which would be preferentially used to form the blue phospho-molybdate complex, leaving the sulfide unaffected. If excess titanium chloride solution was added immediately before acidification, sulfide was evolved when the sample was acidified and gassed over through zinc acetate traps. In the absence of titanium chloride, no sulfide was evolved. In the present work 65 mM titanium chloride proved to be sufficient for this purpose, but the amount required may differ in other sediments with higher concentrations of phosphate. Quantitative recovery was checked by using a slurry of 50% (vol/vol) sediment in deoxygenated seawater. Samples of sediment slurry (50 ml) were placed in 150-ml conical flasks sealed with Suba seals and gassed out with 80% (vol/vol) H_2–20% (vol/vol) CO_2. To each flask 0.1 ml sodium [^{35}S]sulfate solution (10 μCi·ml^{-1}) was added, and three treatments were used (four replicates for each): (i) no addition as controls, (ii) 20 mM molybdate added at the start of the incubation, and (iii) 20 mM molybdate added at the end of the incubation. The flasks were incubated at 23°C for 1 week, after which titanium chloride solution was added to the treatments ii and iii. The flasks were then acidified and gassed out with oxygen-free nitrogen, and evolved sulfide was trapped in zinc acetate solution (1%, wt/vol). Half of the zinc acetate trap was titrated iodometrically to measure total evolved sulfide; the other half was filtered, and radioactivity was measured by scintillation counting (see below).

(iv) Experiments with [^{35}S]sulfate in salt marsh sediment. Samples (160 ml) of salt marsh sediment slurry were added to 250-ml conical flasks closed with Suba-seals. Three flasks (i through iii) were gassed with 80% (vol/vol) N_2–20% (vol/vol) CO_2, and two flasks (iv and v) were gassed with 80% (vol/vol) H_2–20% (vol/vol) CO_2. To each flask was added 400 μl of sodium [^{35}S]sulfate solution (20 μCi·ml^{-1}, 2,500 Ci·mmol^{-1}; Radiochemical Centre), and the flasks were incubated at 25°C on an orbital shaker. After 2.5 h the following additions were made: (i) no addition (as control); (ii) sodium monofluoroacetate solution (5.0 mM final concentration); (iii) sodium molybdate solution (20 mM final concentration); (iv) no addition; and (v) sodium monofluoroacetate as in ii. The incubation was continued at 25°C on an orbital shaker. Samples of slurry (3 ml) were withdrawn with sterile plastic hypodermic syringes and introduced into a universal tube closed with a Suba-seal and containing 0.3 ml of concentrated hydrochloric acid. The universals were previously gassed out with oxygen-free nitrogen to avoid oxidation of sulfide by air. Gassing with oxygen-free nitrogen was then continued, and evolved sulfide was trapped in 10 ml of 4% (wt/vol) zinc acetate solution. In sediment samples where molybdate was present 0.2 ml of titanium chloride solution (25%, wt/vol) was added to the subsamples of

sediment to liberate sulfide (65 mM final concentration). The zinc sulfide precipitated in the trap was filtered through a Sartorius membrane filter (0.45-μm pore size, 2.4-cm diameter) and dissolved in 2 ml of Bray scintillant. Sufficient fumed silicate (Fisons, United Kingdom) was then added to form a gel in which the sulfide particles were evenly distributed. Radioactivity was measured in a scintillation counter by using the external standard method to correct for quenching. Initial samples were taken from all flasks immediately after the addition of radiotracers to provide a zero time reading; samples were then taken at regular intervals to determine the reduction of sulfate to sulfide with time.

RESULTS

Products of acetate metabolism in salt marsh sediment. The results of the experiment using injected [*U*-^{14}C]acetate in sediment samples are shown in Table 1. Approximately 80% of the added label was recovered as methane, carbon dioxide, and residual soluble radioactivity. The proportion of acetate metabolized to methane was negligible, carbon dioxide being the major end product of acetate turnover.

Experiments with sediment slurries. (i) Experiment with acetate in salt marsh sediment. For the first experiment with salt marsh sediment slurry using labeled acetate the relative rates of removal of acetate are shown in Fig. 1 as the radioactivity due to [*U*-^{14}C]acetate in a 0.5-ml subsample of the water from the slurry after centrifugation. Radioactivity appearing in dissolved carbon dioxide is shown in Fig. 2 as activity in a 3-ml subsample of slurry. The dissolved carbon dioxide is assumed to be in equilibrium with that in the overlying gas atmosphere, and the graphs therefore show relative rates of conversion of acetate to carbon dioxide. The apparent decrease in radioactivity after 2 h (Fig. 2) is due to the flasks being regassed after this time to maintain an abundant supply of hydrogen in these slurries held under a hydrogen atmosphere.

The experiment with labeled acetate revealed that its turnover was rapid under both a hydrogen and a nitrogen atmosphere, the majority (90%) of the label having disappeared from the slurry within the first 2 h of incubation. The

TABLE 1. *Percentage conversion after 2 h of incubation of [U-^{14}C]acetate to $^{14}CO_2$ and $^{14}CH_4$ in cores of salt marsh sediment from Ray Creek, Colne Point salt marsh*

Depth of cores (cm)	% Acetate remaining	% $^{14}CO_2$ formed	% $^{14}CH_4$ formed	% Recovery
0 to 1	18.0	62.6	0	80.6
4 to 5	16.0	74.7	0	90.7
9 to 10	44.7	39.9	0	84.6

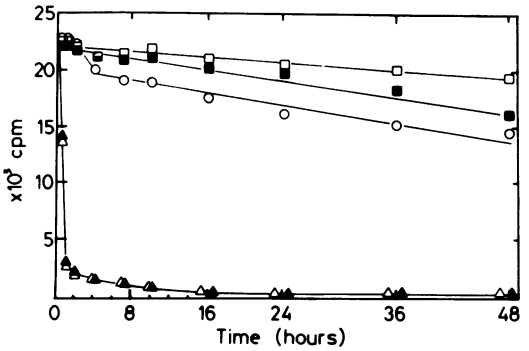

FIG. 1. *Radioactivity remaining in [U-^{14}C]acetate in 0.5-ml subsamples of slurries of salt marsh sediment. Symbols: △, slurry under N_2-CO_2; ▲, slurry under H_2-CO_2; ○, slurry plus 20 mM molybdate under N_2-CO_2; □, slurry plus 5 mM fluoroacetate under N_2-CO_2; ■, slurry plus 5 mM fluoroacetate under H_2-CO_2.*

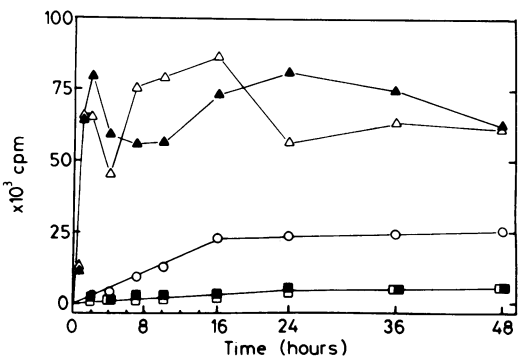

FIG. 2. *Radioactivity from [U-^{14}C]acetate appearing as dissolved carbon dioxide in 3-ml subsamples of slurries of salt marsh sediment. Symbols as for Fig. 1.*

turnover constant (k) for acetate in the controls was 2.07 h^{-1}. The presence of fluoroacetate, an inhibitory analog of acetate, virtually stopped acetate turnover ($k = 2.84 \times 10^{-3}$ h^{-1}), with 85% of the label still remaining in the slurry under nitrogen after 48 h. The presence of 20 mM molybdate, which is an inhibitor of sulfate reduction (15, 16) also inhibited acetate turnover ($k = 1.82 \times 10^{-3}$ h^{-1}), although a very slow but continued rate of disappearance of label seemed to proceed throughout the experiment, with 65% of the initial acetate label remaining after 48 h.

When the accumulation of ^{14}C label in the dissolved carbon dioxide was examined there was rapid accumulation in the controls under both hydrogen and nitrogen during the first 2 h of the experiment when label rapidly disappeared from the acetate. Thereafter, there was little further accumulation of label in the carbon

dioxide. The presence of fluoroacetate almost entirely eliminated the accumulation of label in carbon dioxide throughout the experiment; the presence of molybdate resulted in a small initial amount of accumulation during the first 16 h, but thereafter no further accumulation of label occurred.

(ii) Experiment with [*U*-^{14}C]acetate in freshwater sediments. In the slurries of lake sediment acetate turnover ($k = 1.4 \times 10^{-2}$ h^{-1}) was slower than in the salt marsh sediment, the residual dissolved radioactivity disappearing after approximately 96 h (Fig. 3). Fluoroacetate again almost totally inhibited acetate metabolism ($k = 1.09 \times 10^{-3}$ h^{-1}), but the addition of molybdate had no effect upon acetate turnover. The rate of disappearance of dissolved residual radioactivity due to acetate in the presence of molybdate was identical to that in the controls.

(iii) Recovery of sulfide from sediment in the presence of molybdate. Table 2 shows the effect of the addition of titanium chloride upon recovery of sulfide from sediment in the presence of molybdate. Comparison of treatments i and iii shows that recovery of sulfide was >99% when titanium chloride solution was added to the sediment slurry before acidification. In contrast, in treatment ii, where molybdate (20 mM) was present throughout the incubation period, a smaller amount of sulfide was present at the end of the experiment and in addition there was no reduction of [^{35}S]sulfate to [^{35}S]sulfide. This confirmed that molybdate was an effective inhibitor of sulfate reduction, and that the lack of radioactivity in sulfide was not an artifact resulting from a low recovery of sulfide in the presence of molybdate.

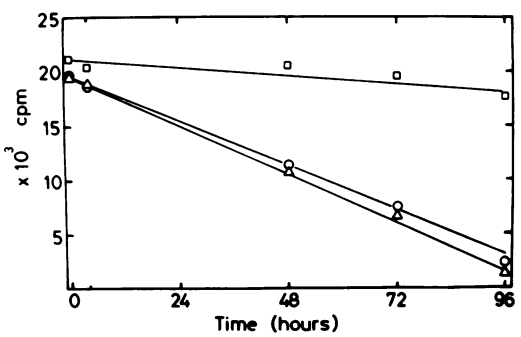

FIG. 3. *Radioactivity remaining in [U-^{14}C]acetate in 0.5-ml subsamples of slurries of freshwater sediment from Wivenhoe Park Lake. Symbols as for Fig. 1.*

(iv) Experiments with [^{35}S]sulfate in salt marsh sediment. The relative rates of sulfate reduction are shown in Fig. 4 as the radioactivity due to [^{35}S]sulfide in a 3-ml subsample of slurry. Under a nitrogen atmosphere the presence of fluoroacetate substantially decreased the rate of sulfate reduction, as measured by the reduction of $^{35}SO_4^{2-}$ to $^{35}S^{2-}$. Over the first 48 h the rate of sulfide formation was reduced by approximately 60% in the presence of fluoroacetate. Molybdate entirely eliminated labeled sulfide formation. No label was present in the sulfide even when titanium chloride was added to ensure successful distillation of sulfide, and inhibition of sulfate reduction was complete.

DISCUSSION

The data from salt marsh sediment cores injected with labeled acetate showed that virtually no methane was derived from acetate metabolism, acetate oxidation to carbon dioxide being the predominant process. This is similar to other workers' data (9, 11, 23) for sediments rich in sulfate, although in low-sulfate sediments methanogenesis predominates (23).

The experiments with slurries of salt marsh sediment showed that labeled acetate was rapidly metabolized under both an N_2–CO_2 and an H_2–CO_2 atmosphere. Label accumulated in carbon dioxide during the initial 2 h when acetate metabolism was rapid (Fig. 2). Acetate metabolism was almost entirely inhibited by the presence of fluoroacetate, whereas the presence of molybdate, an inhibitor of SRB (15, 16), also greatly diminished acetate metabolism to an extent almost equal to that in the presence of fluoroacetate.

An overnight preincubation of slurry was used

TABLE 2. *Sulfide concentrations and radioactivity present as [^{35}S]sulfide in sediment slurry after 1 week of incubation at 23°C*

Treatment	Final sulfide concn (mmol·ml^{-1} of sediment)	Radioactivity as [^{35}S]sulfide (cpm·ml^{-1} of sediment)
i Control	10.2×10^{-3} ($\pm 1.77 \times 10^{-4}$)[a]	80,797 (± 671)
ii 20 mM molybdate added at start of incubation[b]	0.38×10^{-3} ($\pm 7.22 \times 10^{-5}$)	18 (± 4)
iii 20 mM molybdate added at end of incubation[b]	9.94×10^{-3} ($\pm 1.88 \times 10^{-4}$)	81,063 (± 2159)

[a] Numbers within parentheses indicate standard errors.
[b] Titanium chloride (65 mM) added before acidification and distillation of sulfide.

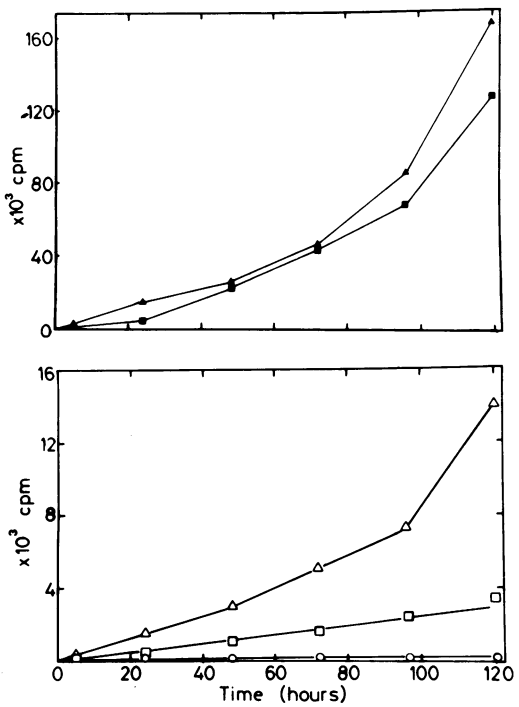

FIG. 4. *Appearance of radioactivity from $^{35}SO_4^-$ as $^{35}S^{2-}$ in 3-ml subsamples of slurries of salt marsh sediment. Symbols as for Fig. 1.*

to ensure complete effectiveness of the added inhibitors before labeled acetate was injected into the slurry, as its turnover was so rapid. This preincubation period did not result in depletion of acetate metabolism which was active in the unsupplemented controls when labeled acetate was later injected. The lack of activity in the presence of both molybdate and fluoroacetate must have been the result of effective inhibition of acetate-metabolizing bacteria during the preincubation period.

Our data strongly suggested that SRB were involved in acetate turnover in the salt marsh sediment, as suggested by other workers (9, 23). Peck (16) and Oremland and Taylor (15) have shown that SRB are inhibited by molybdate, and our data indicated that acetate oxidation was largely inhibited by molybdate addition. However, caution is required when interpreting data using supposedly specific inhibitors in natural mixed microbial communities where there may possibly be nonspecific side effects by the inhibitor on other groups of microorganisms. For example, Wolin and Miller (24) have shown that a molybdate-sulfide complex affects synthesis of hydrogenase, but not activity of preformed hydrogenase, in *Ruminococcus albus*, although the complex had no effect at all in five other bacteria examined. The other major group of acetate-metabolizing bacteria, the methanogens, do not appear to be adversely affected by molybdate, as addition of 20 mM molybdate almost invariably stimulates methanogenic rates in slurries of salt marsh sediment (15; Nedwell and Banat, Microb. Ecol., in press). Therefore, inhibitory side effects of molybdate upon acetate-dissimilating methanogens seem to be discounted. Indeed, the slow residual rate of dilution of labeled acetate in the presence of molybdate (Fig. 1) indicates the presence of acetate-dissimilating bacteria which are not molybdate sensitive.

However, the experiment with labeled acetate in slurries of lake sediment suggested that molybdate had no significant side effects upon other groups of bacteria involved in acetate turnover. In this experiment fluoroacetate again totally inhibited acetate turnover, but molybdate had no inhibitory influence. In the absence of sulfate in this freshwater sediment the SRB would not be actively respiring sulfate and hence would not be susceptible to inhibition by molybdate. The identical results for acetate turnover in both the controls and the molybdate-treated slurry (Fig. 3) argue that molybdate had no effect upon other groups of bacteria, other than the SRB, which influenced acetate turnover. While not entirely conclusive, this evidence from a freshwater sediment suggests that molybdate has little significant influence other than upon the SRB, at least as far as acetate turnover is concerned, and that the effect of molybdate upon acetate turnover in the salt marsh sediment must be because of its effect upon acetate-oxidizing SRB.

This conclusion was in turn supported by the slurry experiment investigating reduction of [^{35}S]sulfate. Fluoroacetate, which has been shown to totally inhibit acetate metabolism, depressed the measured rate of sulfate reduction under an N_2–CO_2 atmosphere (Fig. 4) during the first 2 days by about 60% of the rate in the absence of fluoroacetate. However, fluoroacetate did not entirely inhibit sulfate reduction. A slower but continued rate of sulfate reduction proceeded throughout the experiment. We interpret this as showing the presence of at least two distinct functional groups of SRB both of which are inhibited by the addition of molybdate, which entirely inhibits sulfate reduction (Fig. 4). Molybdate apparently acts by inhibiting adenosine triphosphate sulfurylase (18), which is presumably common to both groups. One of the groups of SRB comprises acetate oxidizers which are inhibited by the addition of fluoroacetate, thus depressing the rate of sulfate reduction. A second group of SRB comprises organisms that metabolize electron donors other than acetate

and are not inhibited by the presence of fluoroacetate. Thus, this second group continues to reduce sulfate in the presence of fluoroacetate. As the SRB seem to be the predominant hydrogen scavengers in this sediment the implication is that hydrogen is the major electron donor for this second group.

This interpretation is supported by the observation (Fig. 4) that under a hydrogen atmosphere the rate of sulfate reduction was greatly stimulated, and although fluoroacetate diminished the amount of sulfate reduction the inhibitory effect was proportionately less than under a nitrogen atmosphere. It has been shown elsewhere (16) that sulfate reduction is limited by electron donor availability in these sediments, and the presence of abundant hydrogen would stimulate the activity of the hydrogen-metabolizing SRB, but not that of the acetate-oxidizing SRB. We propose, therefore, that the SRB are not only the predominant hydrogen-scavenging bacteria in this sediment (5), but that they are also the predominant acetate oxidizing bacteria.

Most of the SRB known, predominantly of the genus *Desulfovibrio* (17), are able to utilize a restricted range of reduced organic acids such as pyruvate and lactate or ethanol. Many of these bacteria are also known to utilize hydrogen, and indeed some are able to use hydrogen as the sole electron donor (4). Acetate is the predominant end product when organic electron donors are being metabolized by these SRB, and it is not further oxidized to carbon dioxide. A few acetate-oxidizing sulfate reducers have been reported, including *Desulfotomaculum acetoxidans* (22) and more recently *Desulfobacter* (9a). Both of these reported genera have been isolated from marine anoxic sediments, and both oxidize acetate to carbon dioxide. Neither is able to metabolize hydrogen. It seems, therefore, that these acetate-oxidizing sulfate reducers represent a distinct functional group occupying a metabolic niche separate from that of the majority of sulfate reducers so far known. Their distribution may be wider than so far appreciated.

The observation that active sulfate reduction inhibits methanogenesis has already at least partially been attributed to competition for available hydrogen (2, 15), but our present data suggest that competition for acetate between the acetate-oxidizing SRB and the acetate-dissimilating methanogenic bacteria may also be significant. Winfrey and Zeikus (23) have proposed a similar competition in Lake Mendota sediment, where sulfate inhibition of methanogenesis was reversed by the addition of either hydrogen or acetate.

Figure 5 illustrates the suggested relationship between SRB and the organotrophic bacteria preceding them in the pathway of carbon flow in the salt marsh sediment. The hydrogen-scavenging SRB cause the release of hydrogen by hydrogen transfer from the preceding bacteria with the concomitant formation of acetate. It has been demonstrated with rumen microorganisms that hydrogen transfer stoichiometrically increases the proportion of acetate in the metabolic products of the fermentative-hydrogen-transferring bacteria preceding the hydrogen-scavenging organism (8, 10, 18), whereas in the absence of hydrogen-scavenging bacteria there is a stoichiometric increase in the proportion of reduced fermentative products such as lactate, propionate, succinate, etc., at the expense of acetate, because hydrogen transfer is inhibited by the accumulation of hydrogen. The maintenance of low sedimentary hydrogen concentrations by H_2-scavenging bacteria presumably has the same effect in anoxic sediments.

Such a scheme suggests two possible mechanisms for competition among the SRB and methanogenic bacteria, namely, between the hydrogen-scavenging SRB and the methanogens using the H_2 plus CO_2 pathway to methane and among the acetate-oxidizing SRB and the acetate-dissimilating methanogens (Fig. 5). The situation apparently existing in the surface salt marsh sediment at these two sites of potential competition is indicated in Fig. 5, the SRB apparently outcompeting the methanogens for both substrates. In comparison, a number of authors (9, 11, 23) have shown that in sulfate-depleted sediment a much greater proportion of the acetate is metabolized to methane than to carbon dioxide, presumably because lack of sulfate inhibits the SRB and prevents them from competing either for acetate or for hydrogen.

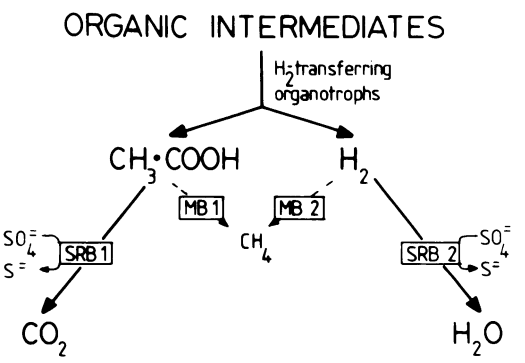

FIG. 5. *Illustration of the proposed role of SRB in electron flow in salt marsh sediment. Abbreviations: SRB_1, acetate-oxidizing SRB; SRB_2, H_2-oxidizing SRB; MB_1, acetate-dissimilating methanogenic bacteria; MB_2, H_2-oxidizing methanogenic bacteria.*

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