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Measurement of Acetate Concentrations in Marine Pore Waters by Using an Enzymatic Approach†

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Acetate concentrations in marine and freshwater matrices were measured by an enzymatic technique which coupled the synthesis of acetyl coenzyme A to AMP production. The resulting AMP was assayed by a sensitive and relatively rapid high-pressure liquid chromatography method, using an aqueous, isocratic mobile phase for elution. The method was insensitive to the presence of seawater salts and required no sample prepurification or distillation. Propionate caused a minor, but statistically insignificant, interference when equimolar with acetate; butyrate caused no interference, even at relatively high concentrations. Detection limits for acetate were approximately 100 nM with a precision of about 5%. Pore waters from two intertidal sediments contained approximately 1 to 12 μM acetate; the concentrations were linearly but inversely correlated with porewater sulfate.

Acetate is a key intermediate during anaerobic degradation of organic matter (34). In mixed cultures as well as natural systems, acetate is produced during the fermentation of saccharides, amino acids, nucleic acids, and lipids, particularly when interspecies hydrogen transfer to either methanogenic or sulfidogenic bacteria results in low hydrogen partial pressures (e.g., see references 17, 20, and 34). The significance of acetate as a fermentation end product and as a carbon source for sulfate reduction and methanogenesis has been documented for sludge digestors and various freshwater and marine sediments among other systems (e.g., see references 18–20, 29, 30, and 35).

However, while the significance of acetate is undisputed, the analysis of its concentrations remains problematic. The low micromolar concentrations in surface waters and sediments are particularly troublesome. Gas chromatographic (GC) methods have been applied successfully to samples with relatively high concentrations, even in difficult matrices such as sludge (e.g., see references 10 and 12). For saline samples with low acetate concentrations, GC procedures are more difficult, often requiring time-consuming purification and concentration steps (1, 8, 27). Even then, analytical precision can be less than desirable. High-pressure liquid chromatographic (HPLC) methods provide alternative approaches, particularly when coupled with conductivity detection. Relatively high sensitivity and improved precision facilitate the analysis of low concentrations, although prepurification steps are still recommended (24, 29).

I report here an alternative, enzymatic method for determining acetate concentrations in both marine pore waters and freshwater samples. The method is based on the reaction of acetate with acetyl coenzyme A (CoA) synthase according to the following stoichiometry:

$$\text{acetate} + \text{ATP} + \text{CoA} \xrightarrow{\text{acetyl CoA synthase}} \text{acetyl CoA} + \text{AMP} + \text{PP}_i$$

The reaction produces AMP, which can be determined by HPLC with a high degree of precision and low detection limits. Since enzymes that form AMP instead of ADP from ATP are relatively uncommon, problems with nonspecific interferences are minimized. The method is simple, requiring no sample purification or concentration, and works equally well in ionic and nonionic matrices, thus eliminating the distillations used for other methods and the necessity for modifying sample matrix composition (8, 24). Precision is very good, and detection limits are submicromolar. In addition, the enzyme reaction goes to completion rapidly (≤1 h), while the subsequent HPLC procedure is rapid, using only an aqueous mobile phase, isocratic elution, and detection of A_{260}.

MATERIALS AND METHODS

Sample collection. Pore waters for acetate analysis were collected from two sites, Lowes Cove and Cod Cove, Maine. The organic-poor sediments of the former have been described in detail elsewhere (e.g., see references 15, 18, and 22). The latter site, characterized by a high sedimentation rate, an extensive salt marsh, and organic-rich, sulfidic sediments (unpublished observations), was formerly an open mudflat that has become a mixed marsh-mudflat system (see Belknap et al. [4] for a description of the recent sedimentological and geological history). Sediments from both sites were collected with 6.2-cm-inner-diameter acrylic core tubes. At Cod Cove, sediments were collected from an open area within the marsh; these sediments and those from Lowes Cove were devoid of plant roots. Cores were returned to the laboratory and sectioned at approximately 2-cm intervals. The sections were placed into centrifuge tubes that were capped afterward. Pore water was obtained after centrifugation at a maximum of about 12,000 × *g* for 10 min at 4°C (see Shaw and McIntosh [29] for a discussion of possible uncertainties due to methods of porewater collection). No precautions were taken to limit exposure to the atmosphere. The pore waters were subsequently filtered through glass fiber filters (Whatman GF/C) that had been incubated in a muffle oven for >3 h at 550°C. Triplicate samples for each depth interval were stored frozen in disposable polypropylene screw-cap vials until further processing.

Acetate analysis. The following stock solutions were prepared in distilled deionized water: acetyl CoA synthase, 20 U ml^{-1} (approximately 6 U mg of $protein^{-1}$); CoA, 10 mM

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(sodium salt, from yeast); disodium ATP, 10 mM; bovine serum albumin (BSA), 200 μg ml^{-1}. Acetyl CoA synthase was used as received without further processing. All glassware was washed extensively and then heated at 500°C for 3 h prior to use. Stock solutions of ATP and BSA were stored frozen after initial preparation and were stable for months. Acetyl CoA and acetyl CoA synthase were prepared in volumes necessary for anticipated sample processing during a period of about 4 weeks; the solutions were stored frozen in 1.5-ml polypropylene centrifuge tubes and repeatedly thawed and refrozen with no apparent loss of activity.

For routine analysis, 10 μl of each of the above solutions was added to 1.0 ml of the desired sample matrix contained in a disposable 7-ml screw-cap polypropylene vial, resulting in final concentrations of 100 μM for ATP and CoA. The vial contents were mixed by shaking and then incubated for 1 h at 37°C. Acetyl CoA synthase activity was terminated by immersing samples in a boiling water bath for 2 min. The efficacy of this procedure was determined by examining the following parameters with acetate standards (2 to 10 μM) prepared in filtered (0.2-μm pore size) artificial seawater (FASW): (i) stability of ATP in the presence of acetyl CoA synthase but no added CoA; (ii) the stability to boiling (5 min) of AMP and ATP in FASW; (iii) the stability to boiling (1 to 5 min) of acetyl CoA synthase; (iv) the effect of incubation time at 37°C; (v) the effect of incubation temperature (23.5, 37, and 50°C); (vi) the effect of matrix pH (6.0 to 9.0) at 37°C; (vi) the effect of ionic strength (10 μM acetate standards in distilled deionized water versus FASW); (vii) interference from propionate and butyrate (5 and about 100 μM, respectively, versus 5 μM acetate). Sample blanks were evaluated by adding heat-inactivated acetyl CoA synthase or by deleting CoA. In addition, the volumes of each of the added reagents were increased from 10 μl (in 10-μl increments) to 40 μl in a factorial experiment designed to evaluate reagent contamination. Each of these optimization assays was run in triplicate. The precision of the method was estimated by using the routine conditions for quadruplicate acetate standards in FASW at 0.2 and 2.0 μM. The limit of quantitation was calculated as three times the standard deviation of the blank value.

Pore waters were assayed under the routine conditions noted above and by direct injection into the HPLC system described below. The latter analysis was necessary to determine whether AMP occurred at observable concentrations in the sample matrix prior to addition of acetyl CoA synthase. The relationship between AMP production and acetate concentration was determined by two methods: (i) pore waters were assayed before and after addition of a known concentration of acetate; (ii) a standard curve (0.1 to 8 μM) was generated by adding acetate to a batch of pore water prepared by mixing equal volumes from all available depths. Triplicate samples were typically analyzed for standardization and for each interval of the sediment depth profile.

Chromatography. AMP concentrations were determined by injecting samples, using a Rheodyne 7125 injection valve fitted with a 20-μl sample loop. The loop was filled completely by inserting a syringe and needle into the needle port of the injector and pulling a volume of 100 to 200 μl through a short length of Teflon tubing attached to port six of the valve. After injection, a small volume of mobile phase was used to rinse the injector according to the manufacturer's instructions. This procedure eliminated cross-contamination or ghosting due to the propensity of AMP to adsorb to stainless steel (metal-free injectors offer another solution). AMP was eluted from an analytical column (25 cm by 4.6 mm; LC-18-T; Supelco, Inc.) with a mobile phase of 0.1 M potassium phosphate (pH 6.0) under isocratic conditions at 35°C with a flow rate of 1.0 ml min^{-1} delivered by a solvent pump (Rabbit HP; Rainin Instruments, Inc.). Lower temperatures were acceptable but resulted in longer retention times and poorer peak geometries. The analytical column was protected with a Brownlee guard column cartridge (RP-18, 2 cm by 4.6 mm). AMP was detected by monitoring either A_{254} or A_{260} by using a variable-wavelength detector (Spectroflow 757; Kratos Analytical Instruments, Inc.) operated at maximum sensitivity (0.005 absorbance unit full scale). The detector response was analyzed with a recording integrator (HP 3390A or 3396A; Hewlett-Packard, Inc.) and calibrated with AMP or by the addition of known concentrations of acetate to the enzyme reaction mixture.

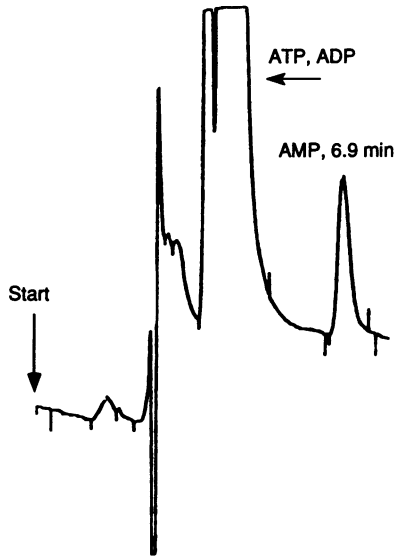

FIG. 1. Typical chromatogram of a marine pore water after the acetyl CoA synthase reaction. Operating and sample reaction conditions are given in the text. ATP and ADP peaks are not well resolved because of the high concentration of added ATP (100 μM). The AMP response is for a 1 μM concentration.

Other analyses. The HPLC detector output was standardized by using AMP at concentrations from 0.05 to 16 μM; precision was evaluated at 0.2, 2, and 16 μM. Sulfate, salinity, and pH in the porewater samples were assayed by using a turbidimetric method (31), a refractometer, and an ion analyzer (Beckman Instruments, Inc.), respectively. Statistical analyses were based on a microcomputer software package, Statview SE.

All reagents used for the enzymatic reaction were purchased from Sigma Chemical Co. Other chemicals and solvents were of reagent grade quality or better and purchased from various commercial sources.

RESULTS AND DISCUSSION

Preliminary observations indicated that AMP was amenable to a simple and sensitive analysis based on an isocratic elution with a buffered aqueous mobile phase (Fig. 1). AMP was well separated from other adenylates, and no interferences were observed for natural freshwater or marine matrices relative to standards. Likewise, ionic composition had no significant effect on adenylate separation (data not

shown). Using the operating conditions described earlier, precision ($n = 4$) was 2.9, 1.0, and 0.1% at 0.2, 2, and 16 μM concentrations, respectively. The limit of quantitation was <0.05 μM. This limit was determined primarily by the volume of the sample loop (20 μl) and the maximum sensitivity of the detector (0.005 absorbance unit full scale), both of which can be increased if lower detection limits are required. Additions of organic modifiers to the mobile phase appeared to offer little increased chromatographic resolution or detection.

Preliminary observations also indicated that ATP was stable in a porewater or FASW matrix in the presence of acetyl CoA synthase but not acetyl CoA synthase plus CoA. In addition, ATP and AMP were heat stable, with either no or insignificant degradation during boiling for up to 5 min. In contrast, acetyl CoA synthase activity was lost rapidly after 1-ml solutions contained in 7-ml vials were immersed in boiling water. These results suggested that the acetyl CoA synthase reaction provided a feasible enzymatic approach for measuring bioavailable acetate concentrations and that the key reactant and product, ATP and AMP, were stable under conditions that facilitated analysis.

With an initial acetate concentration of 10 μM and an incubation temperature of 37°C, the synthase reaction reached completion in approximately 45 min. Thus, for analysis of concentrations of <10 μM, 60 min was selected as a routine incubation time. The time required for completion was, of course, a function of reactant and enzyme concentrations. Increasing or decreasing the amount of added enzyme provided a means for altering predictably the desired incubation time (data not shown).

Incubation temperature was an important reaction variable, with lower AMP production for a given acetate concentration at both 25 and 50°C. While the decrease at 25°C was small (although statistically significant), a more substantial decrease was observed at 50°C. As a consequence, all enzymatic incubations were performed at 37°C; lower temperatures were deemed suitable with increased incubation times or if incubation at elevated temperatures was not feasible. The pH of the reaction mixture was somewhat less critical than incubation temperature over a range from about 7.0 to 8.5; at higher or lower values, decreased AMP production was observed, especially at pH 6.0.

Since neither 0.01 M bicarbonate, carbonate, 2-(*N*-morpholino)ethanesulfonic acid (MES), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), nor Tris appeared to adversely affect acetyl CoA synthase activity independent of pH (data not shown), a variety of buffers provide options for sample pH adjustment. Fortunately, this may not be necessary for many marine pore waters. For example, the pore waters from both sites examined in this study varied from approximately pH 7.0 to 7.5. This range is typical for sediments not affected significantly by sulfide or metal oxidation (5). Regardless, analysis of sample pH is advised since values of less than 6.5 are not uncommon, particularly in freshwater or salt-marsh sediments, and since significant pH gradients occur as a function of sediment depth (see, for example, references 11 and 16).

In contrast to the effects of pH and temperature, acetyl CoA synthase activity was virtually insensitive to the ionic strength of the reaction mixture. Identical AMP production was observed from 10 μM acetate standards whether prepared in distilled deionized water or FASW. As a result, addition of ionic cofactors was unnecessary for freshwater or marine samples. Moreover, neither deionization nor distillation was necessary to provide a suitable matrix for either the enzymatic reaction or subsequent HPLC. This is a considerable advantage relative to the prepurification steps recommended (or necessary) for GC analysis of low micromolar acetate concentrations in marine samples (e.g., see references 8 and 25).

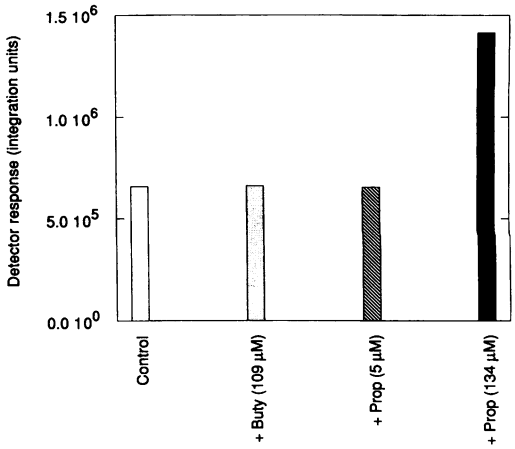

FIG. 2. AMP production in FASW containing 5 μM acetate only (Control, □); 5 μM acetate plus 109 μM butyrate (+Buty, ▨); 5 μM acetate plus 5 μM propionate (+Prop [5 μM], ▧); 5 μM acetate plus 134 μM propionate (+Prop [134 μM], ■). Each value represents the mean of triplicate determinations; error bars are not distinguishable.

Although common inorganic solutes had no apparent effect, the presence of propionate stimulated AMP production slightly (Fig. 2). However, the molar response for propionate was substantially lower than that for acetate: 31 μM propionate was equivalent in response to 1 μM acetate. There was no evidence for AMP production resulting from longer-chain fatty acids, e.g., butyrate, even at 109 μM. When propionate and acetate were incubated at equimolar concentrations (e.g., 5 μM), there was no statistically significant increase in AMP production relative to acetate only.

Since propionate seldom exceeds or even equals acetate concentrations in sediments (Table 1), it is unlikely that it will contribute to >5% uncertainty in the measured acetate concentration. This level of uncertainty is comparable to the standard deviations observed for replicate samples of marine pore waters (see below). Of course, in some freshwater or sludge matrices or circumstances in which interspecies hydrogen transfer is inactive, propionate could accumulate to levels comparable to those of acetate (e.g., see references 12, 17, and 20). However, even if propionate exceeds the acetate concentration by twofold, the resulting error will still amount to ≤10%. Since propionate is less difficult to assay by GC than acetate, the potential for interference is readily checked. Although interference from acrylate was not examined specifically, it is not likely to cause greater interference than propionate. However, since acrylate might occur at high concentrations in some restricted circumstances (e.g., during rapid hydrolysis of locally high levels of dimethylsulfoniopropionate), some caution is advised.

Standard curves for acetate in FASW were linear over about 2 orders of magnitude (Fig. 3), with a detection limit of about 0.1 μM and a precision of 3.0 and 1.5% at 0.2 and 2.0 μM, respectively ($n = 4$). Concentrations of >10 μM were not analyzed, but linear responses can be expected assuming that levels of added ATP and CoA are sufficient and that incubation times or enzyme concentrations are increased.

TABLE 1. Concentrations of acetate and propionate in various sediments and sludges[a]

Site location	Acetate (μM)	Propionate (μM)	Reference
Salt marsh, U.K.	5.3	0.5	Balba and Nedwell (2)
Flax Pond salt marsh	15–70	NR[b]	Michelson et al. (23)
Limfjord, Denmark	0.1–6	0.1–6	Ansbæk and Blackburn (1)
Santa Barbara Basin	33–471	<0.1	Barcelona (3)
Skan Bay, Alaska	1–14	NR	Shaw and McIntosh (29)
Lowes Cove, Maine	1–3	NR	This study
Cod Cove, Maine	5–11	NR	This study
Loch Eil, Scotland	3–22	NR	Parkes and Taylor (24)
Cape Lookout Bight	55–660[c]	1–16	Sansone and Martens (27)
Continental slope	10.7–69.0[c]	1.2–11.4	Sansone and Martens (27
Wintergreen Lake	100	14	Lovley and Klug (20)
Sludge digestor	400	60	Zinder (35)
Sludge digestor	33.9–56.8[d]	15.4–31.6	Henderson and Steedman (12)

[a] All data with the exception of those from this study and references 24 and 29 were based on GC methods. The latter two studies were based on an ion chromatographic technique.
[b] NR, not reported.
[c] Micromoles per liter of sediment; concentrations based on solvent extraction.
[d] Millimolar.

Detection limits were determined primarily by the amount of acetate in the reagents (no blank AMP was observed). When all reagents were added in increasing volumes to FASW with no acetate, the AMP peak increased linearly (Table 2); extrapolation to zero added reagent suggested final blank acetate levels of about 0.03 μM. Based on results from a factorial experiment, acetyl CoA synthase was the major source of the contamination. The amount of contaminant acetate was probably amenable to reduction by dialysis, although this was not specifically examined. Reduction in the blank value would potentially lower the detection limit. For the samples analyzed in this study, lower limits were unnecessary.

Although detection limits and precision were comparable, the relationship between AMP production (detector response) and acetate concentration differed for pore water and FASW (Fig. 3). Specifically, AMP production per mole of acetate in pore water was about 72% of that in FASW or distilled deionized water. This result was consistent for pore waters from the two sampling sites, even though there were significant differences in pore water salinity, sulfide, and dissolved iron (data not shown). Reasons for the lower yield of AMP in pore water were not evident. Differences in the kinetics of acetyl CoA synthase could have decreased the extent of the reaction, or acetate could have formed unreactive complexes in pore water relative to other matrices. These possibilities were not explored since detection limits were still much lower than necessary for porewater analyses and standard curves were linear to at least 8 μM. The absolute production of AMP per mole of acetate was not optimized for a 1:1 yield for similar reasons.

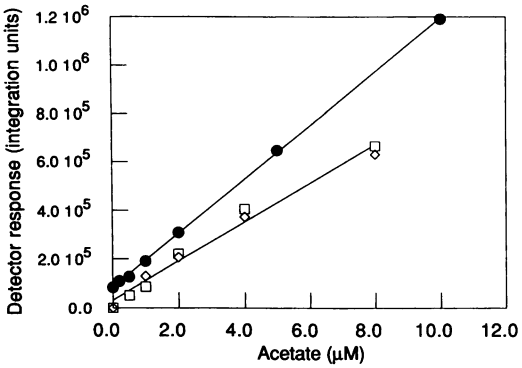

FIG. 3. AMP production versus acetate concentration for an FASW matrix (●) and pore waters from Lowes Cove (□) and Cod Cove (◇). All points are based on triplicate determinations; error bars are coincident with the symbols. For both the FASW matrix and the pooled pore waters, the relationship between AMP production and acetate is highly significant (for FASW, $r^2 = 1.00$; $P = 0.0001$; for pore waters, $r^2 = 0.99$; $P = 0.0007$). Differences among the intercepts are due to different reagent blanks.

Acetate was readily detected in the porewaters from two intertidal sediments (Fig. 4A). In pore water from Lowes Cove, acetate concentrations were approximately uniform at 1 to 2 μM over the upper 10 cm. Over the same interval, there was little significant sulfate depletion. In contrast, sediments from Cod Cove contained approximately 1 to 12 μM acetate with a trend for increasing concentrations with increasing depth (Fig. 4A). The trend for sulfate was opposite, with significant depletion occurring over 18 cm. Concentrations of sulfate and acetate were significantly inversely correlated (Fig. 4B; acetate [μM] = 14.90 − 0.52 (±0.043) sulfate [mM]; $r^2 = -0.938$; $P = 0.0001$). Increasing concentrations of acetate with depth have been reported for Skan Bay, Alaska (28), while decreases were generally observed for Danish coastal sediments (8). Reasons for the different trends are unclear.

TABLE 2. Evaluation of acetate concentrations in the reagents used for the acetyl CoA synthase reaction[a]

Total reagent vol (μl)	Apparent acetate concn (μM ± 1 SE)
40	0.70 ± 0.04
80	1.24 ± 0.08
120	1.76 ± 0.10
160	2.12 ± 0.02

[a] Each of four separate reagents (acetyl CoA synthase, CoA, ATP, and BSA) was added in increasing volumes to replicate samples of FASW (1 ml per sample; triplicates for each treatment). The synthase reaction was allowed to proceed for 1 h at 37°C and was followed by AMP analysis as described in the text.

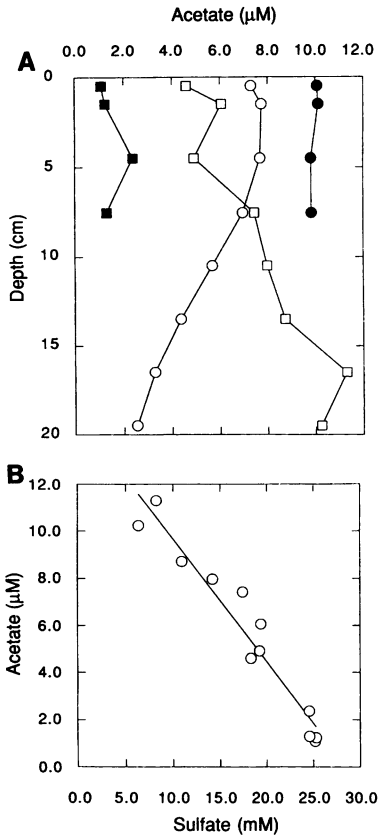

FIG. 4. (A) Concentrations of acetate (■,□) and sulfate (○,●) in pore waters from Lowes Cove (■,●) and Cod Cove (□,○). All points are means of triplicate determinations. Coefficients of variation for sulfate in Lowes Cove and Cod Cove are <1.0 and 5.0%, respectively; error bars for acetate are ±1 standard error. (B) Correlation between acetate and sulfate concentrations for pooled data from Lowes Cove and Cod Cove.

The relationship between acetate and sulfate in this study is also noteworthy because sulfate reduction, the major sink for acetate in marine sediments, is not limited at the sulfate concentrations observed (>5 mM; Boudreau and Westrich [6] report saturation values of about 3 mM for sediments from Long Island Sound). Thus, the increasing acetate levels cannot be attributed to a substrate-dependent decrease in sulfate reduction rates. Other factors, such as decreases in the biomass of sulfate reducers, decreases in the affinity of sulfate-reducing bacteria for acetate, or imbalances in the relative rates of acetate production by fermentors and consumption by sulfate-reducing bacteria, must affect the depth profile. For example, changes in the kinetics of acetate uptake have been previously invoked to explain acetate depth profiles in fresh water (21). The changes with depth have been attributed to transitions from sulfate-reducing to methanogenic systems. In Cod Cove, changes in affinity may be linked to seasonal transitions in the depth of the sulfate-reducing zone. During summer, intense sulfate reduction rates driven by high concentrations of organic matter deplete sulfate to micromolar values within 5 to 10 cm (unpublished data). This may stabilize relatively low-affinity sulfate-reducing bacteria populations at depths below the permanent sulfate-reducing zone.

Regardless of the trends with depth, the acetate concentrations in Lowes Cove and Cod Cove are among the lower values reported for marine sediments (Table 1). The relatively low concentrations and variability among replicates suggest that processing problems as described by Shaw and McIntosh (29) were minimal. Differences among the concentrations in this and other studies probably reflect the methods used, with the emphasis here on bioavailable pools, as well as true variability among sites.

The acetate concentrations reported here probably provide reasonable estimates of the bioavailable pool. Unlike other HPLC or GC methods, the acetyl CoA synthase technique measures only acetate that reacts enzymatically. This would include the free, dissolved pool as well as any adsorbed pools that equilibrate rapidly with the free pool. Acetate fractions that are kinetically slow to equilibrate or that require some harsh chemical conditions to desorb bound pools are not measured. These fractions are not bioavailable as defined by their kinetic characteristics, even though they are detected by other methods. The distinction between the bioavailable pool and total detectable acetate has been made by others (e.g., see references 1, 8, 25, and 28), who indicate that about 10 to 80% of the total detectable pool is bioavailable in marine pore waters. On the other hand, Michelson et al. (23) also provided evidence for a bioavailable fraction but cautioned that artifacts in gel chromatography could lead to underestimates of its concentration.

The significance of the bioavailable fraction is illustrated by the overestimation of mineralization that results from the use of total acetate concentrations determined by typical GC or HPLC methods. Ansbæk and Blackburn (1), Christensen and Blackburn (8), and Shaw et al. (28) have reported that acetate mineralization exceeds rates of sulfate reduction or other indices of carbon metabolism measured in parallel (and expressed on a carbon basis). These results differ markedly from those of Christensen (7), who measured acetate accumulation in intact cores after inhibition of sulfate reduction by molybdate. Although Christensen's GC method measures total acetate concentrations, the short-term accumulation of acetate most likely reflected changes in the bioavailable pool. On the basis of the observed accumulation rate, Christensen (7) calculated that acetate oxidation supported 65% of sulfate reduction in intact cores. This is consistent with results from other anoxic systems (e.g., see references 20, 27, and 29). However, despite the evidence supporting an unavailable acetate pool, Shaw and McIntosh (29) have observed that discrepancies between rates of acetate oxidation and other metabolic processes can be attributed to problems resulting from the method used to collect pore water without the necessity of invoking an unavailable pool of acetate.

In addition to facilitating the analysis of acetate, the HPLC method described here has adequate sensitivity and resolution for determining dissolved or particulate adenylate concentrations. In fact, ATP, ADP, and AMP have all been observed at concentrations up to 100 nM in the pore waters examined in this study (data not shown). Others have previously used the concentrations and dynamics of dissolved ATP as an indication of heterotrophic activity in the water column (e.g., see references 13 and 26); similar applications may be appropriate for sediments (9). Analysis of particulate adenylates for determining biomass is also feasible since common extractants such as Tris and bicarbonate buffers (e.g., see reference 14) do not interfere with the adenylate chromatography. Given the low adenylate detection limits possible by monitoring A_{254} to A_{260}, derivitization and fluorescence detection (e.g., see reference 33)

may be unnecessary (see also Viarengo et al. [32] for a gradient-based absorbance method). Finally, other solutes of ecological interest, including glucose, galactose, gluconate, pyruvate, and succinate, are amenable to analysis by using simple modifications (e.g., enzyme substitution, ADP analysis) of the approach described here.

In summary, porewater acetate concentrations are determined by a simple, sensitive enzymatic method subject to few interferences. Only small volumes and minimal processing are required for either freshwater or marine samples; in particular, prepurification or distillation are unnecessary for marine matricies. The availability of an alternative to a bioassay (e.g., see reference 25) should promote a more detailed understanding of acetate's role in the carbon cycle of marine sediments and a resolution of questions concerning bioavailable versus total acetate pools.

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