Glucose Uptake and End Product Formation in an Intertidal Marine Sediment†

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Glucose uptake was monitored on a seasonal basis, using [6-³H]glucose and undisturbed cores collected from an intertidal mud flat. The fate of glucose carbon, including the formation of CO₂ and biomass, was assayed by using undisturbed cores and [U-¹⁴C]glucose; the production of short-chain fatty acids was monitored with [U-¹⁴C]glucose and sediment slurries. Rate constants for glucose uptake varied temporally, with temperature accounting for much of the variability; turnover times ranged from about 2 to 10 min. Rate constants decreased with increasing sediment depth and in the following order for several common monosaccharides: glucose > galactose > mannose ~ fucose. Time course analyses of ¹⁴CO₂ production provided evidence of significant isotopic dilution; although pore water glucose turnover times were on the order of minutes, ¹⁴CO₂ did not plateau until after approximately 6 h of incubation. At this time a maximum of about 40% of the added radioglucose had been respired. The extent of respiration varied as a function of sediment depth and season, with the highest values below the surface (4 to 7 cm) and in summer and fall. Incorporation of radiolabelled glucose into biomass also varied seasonally, but the greatest extent of incorporation (about 40%) was observed in the fall and for the 0- to 1-cm depth interval. The production of short-chain fatty acid end products was largely limited to acetate, which accounted for only a small percentage of the added radiolabel. Other organic acids, pyruvate in particular, were observed in pore water and were due to artifacts in the heat-kill procedure used to terminate incubations. An accurate assessment of the distribution and importance of short-chain fatty acids as end products required the use of an enzymatic technique coupled with high-pressure liquid chromatography to verify qualitative identities.

Analyses of benthic metabolism in marine sediments have generally focused on integrative parameters (e.g., oxygen uptake, CO₂ production, or sulfate reduction) that provide a systems level perspective (14, 17, 19, 20, 23, 26, 41). Measurements of sulfate reduction are especially common (see Skyring [40] for a review); rates of sulfate reduction are readily expressed in terms of anaerobic carbon flow by making a simple and widely accepted assumption about the stoichiometry between sulfate reduction and carbon mineralization (10). As a consequence, a considerable data base exists on the spatial and temporal patterns of benthic metabolism and relationships among benthic processes, sedimentation, bioturbation, and nutrient exchange (4; see also Capone and Kiene [5] for a review).

Somewhat less information exists on specific aspects of intermediary metabolism or the pathways of carbon flow in marine sediments. Earlier studies often used sediment slurries and tended to focus on the extent of respiration for various substrates, rather than on the details of pathways (e.g., see references 8, 9, 12, 13, and 15). Many of these studies have shown only limited ¹⁴CO₂ production from radiolabelled amino acids and sugars. Such observations have been criticized, however, as they did not account for isotopic dilution (22, 24). More recent studies have established the key role of acetate as an intermediate in organic matter mineralization (6, 7, 37–39), but few studies have documented the relationship between acetate (or other fermentation end products) and the metabolism of more complex organic substrates or potential acetate precursors. In contrast, such relationships are well known for other sys-

tems in which anoxic metabolism is important: sludge digestors, the rumen, and freshwater sediments (27, 45, 46).

We report here results from a seasonal study of glucose metabolism, including patterns of end product formation, rate constants for uptake, and relationships to other parameters. Acetate was the only short-chain fatty acid (SCFA) excreted; both ¹⁴CO₂ production and microbial biomass represented important fates for glucose carbon. Glucose uptake varied seasonally and as a function of sediment depth, with pore water glucose turnover times ranging from about 2 to 10 min.

MATERIALS AND METHODS

Site description. Sediments were obtained by hand coring with 6.7-cm (inner diameter) polycarbonate tubes along the landward side of a mussel bar in Lowes Cove, Maine. General aspects of this site have been described previously (1, 25, 30, 36). Briefly, the sediment contained 3 to 5% organic matter. The water content decreased from about 0.8 ml cm⁻³ in the 0- to 2-cm interval to about 0.7 ml cm⁻³ in the 8- to 10-cm interval. The salinity was about 30‰, and oxygen penetrated only the top several millimeters.

For comparative purposes, sediments collected in Triton Bay, St. Croix, U.S. Virgin Islands, in March 1989 were used for several of the assays described below. These sediments were collected near a sand spit on the eastern side of the bay at a water depth of about 1 m. They consisted of unconsolidated carbonates and amorphous mangrove and seagrass detritus; water contents were 0.9 ml cm⁻³. Partially decomposed plant material was observed to about 30-cm depth.

Glucose uptake. Rate constants for D-[6-³H]glucose (referred to hereafter as ³H-glucose) uptake were determined

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by using techniques modified from King and Klug (24). Subcores were collected from the 0- to 2-, 4- to 6-, and 8- to 10-cm depth intervals of triplicate cores, using cutoff 5-ml syringes which were sealed with butyl rubber septa (Supelco, Inc.) after sample collection. The subcores were equilibrated for 1 h at ambient sediment temperature. Each was then injected with 10 μ l of an aqueous solution containing 3.7 kBq of ^3H -glucose ($1.11 \text{ TBq mol}^{-1}$; New England Nuclear) and incubated in the dark at the ambient sediment temperature for 0, 1, 5, and 15 min. Incubations were terminated by extruding the subcores into 15-ml disposable centrifuge tubes containing 2 ml of glutaraldehyde (10% [vol/vol] glutaraldehyde in deionized water; Kodak) and shaking the tubes vigorously for about 1 min. For the zero-time incubations, the subcores were extruded into the glutaraldehyde just prior to the addition of label.

After overnight storage at 3°C , the sediment-glutaraldehyde mixture was centrifuged for 10 min at 6,000 rpm. Three 0.5-ml subsamples of the supernatant were collected for further processing. One subsample was added to 6.5 ml of deionized water in a scintillation vial to determine total soluble activity. The second subsample was mixed with 200 μ l of sodium acetate (0.35 M sodium acetate in deionized water) and sodium carbonate buffer (0.25 M NaHCO_3 and 0.1 M Na_2CO_3 in deionized water, pH 11) in a scintillation vial and dried under a heat lamp to determine the amount of nonaqueous (non- $^3\text{H}_2\text{O}$) radioactivity. Sodium acetate and buffer addition prevented SCFA loss during drying. After drying, 7 ml of deionized water was added to the vial. The third subsample was passed through an ion-exchange column containing 5 cm^3 of Dowex-1 resin (Sigma Chemical Co.) in the F^- form to fractionate nonvolatile label into acidic (SCFA) and nonacidic components. The fluoride form was necessary for complete SCFA adsorption. After loading, the ion-exchange column was washed with 7 ml of deionized water and the eluant was collected in a scintillation vial. Seven milliliters of water was sufficient to elute >97% of any glucose present. Radioanalyses were conducted by scintillation counting with an LKB 1218 counter (LKB Pharmacia, Inc.) after the addition of 10 ml of Scintiverse II scintillation cocktail (Fisher Scientific Co.) to each scintillation vial. Radioactivity was standardized by using quench correction and external standards. Rate constants for ^3H -glucose uptake were calculated from the amount of unreacted glucose remaining in solution at various intervals, using triplicates for each of four time points. Unreacted glucose was estimated by subtracting the $^3\text{H}_2\text{O}$ radioactivity (subsample 1 - subsample 2) from the radioactivity in the ion-exchange column eluants (subsample 3). The same procedures were also used for determining the uptake rate constants for D-[6- ^3H]galactose ($1.12 \text{ TBq mol}^{-1}$; Amersham) and D-[2- ^3H]mannose ($0.68 \text{ TBq mol}^{-1}$; Amersham) in October 1989 and for D-[6- ^3H]galactose, D-[2- ^3H]mannose, and L-[6- ^3H]fucose ($0.92 \text{ TBq mol}^{-1}$; ICN Biomedicals) in December 1989.

Carbon dioxide production. Sediments used for the determination of D-[U- ^{14}C]glucose (referred to hereafter as ^{14}C -glucose) incorporation into biomass and $^{14}\text{CO}_2$ were collected within 1 week of the glucose uptake analyses. The top 1 cm and the 4- to 7-cm interval were subcored as described above. A total of 45 or 54 subcores were taken from the 0- to 1-cm interval and 30 to 36 subcores were taken from the 4- to 7-cm interval for triplicate analysis of five or six time points in a 24-h time course. The subcores were equilibrated at ambient sediment temperature for 30 min prior to injection of 50 μ l of an aqueous solution containing 0.925 kBq of

^{14}C -glucose ($11.3 \text{ GBq mol}^{-1}$; Sigma Chemical Co.). The subcores were then incubated in the dark at ambient sediment temperature. The final concentration of added glucose in 3 cm^3 of sediment was 34 nM. Incubations were terminated at appropriate intervals by immersing the subcores in boiling water for about 1 min; subcores were then frozen. Zero-time subcores were immersed in boiling water prior to injection of radiotracer and then boiled and frozen immediately after injection.

The frozen subcores were extruded into 60- cm^3 glass tubes which were sealed, fitted with gas-sparging tubes, and purged with nitrogen (60 ml min^{-1}). The outlets from each tube were connected to a series of three $^{14}\text{CO}_2$ traps (scintillation vials containing 6 ml of 2 M sodium hydroxide). Ten milliliters of 1 M HCl was injected into the tubes, and inorganic carbon was stripped for 1.5 h. Ten milliliters of scintillation cocktail was added to the traps for radioassay. The trapping efficiency for carbon dioxide (87%) was determined concurrently with sediments from the 0- to 1-cm interval that were injected with 50 μ l of [^{14}C]sodium carbonate; corrections were also made for small losses (about 10%) which occurred during sample incubation and storage as indicated by controls injected with [^{14}C]sodium carbonate and processed analogously to samples with ^{14}C -glucose.

Several additional control experiments were conducted with subcores from 0 to 1 cm. Unlabelled glucose (100 μM final concentration in 3 cm^3 of sediment) was injected into subcores after incubation for 5 h with ^{14}C -glucose. The subcores were then incubated for up to 19 h, and results were compared with a parallel set of subcores to which only ^{14}C -glucose was added. The effect of varied ^{14}C -glucose concentrations on $^{14}\text{CO}_2$ production after 5 h of incubation was determined by injecting 50 μ l of an aqueous solution containing 0.037 to 1.85 μCi (66 nM to 3.3 μM) of ^{14}C -glucose. The effect of preincubation on $^{14}\text{CO}_2$ production was determined by using subcores stored at ambient field temperature for up to 24 h prior to label injection; subcores preincubated for only 1 h were used for a control. The effect of disturbance on $^{14}\text{CO}_2$ production was analyzed by slurring 1:1 sediment from the 0- to 1-cm interval under oxic conditions with filtered (0.2- μm pore size) seawater collected at the sampling site. The slurries were incubated and processed as described above.

Incorporation of glucose into biomass. Subcores were collected, injected with ^{14}C -glucose, and incubated concurrently with the subcores used for $^{14}\text{CO}_2$ production. Incubations were terminated by boiling the subcores for 1 min and then freezing them. Further processing was initiated by extruding the frozen subcores into 15-ml disposable centrifuge tubes. After centrifugation for 10 min at $2,800 \times g$, the supernatants were decanted, filtered (0.45- μm pore size), and transferred to scintillation vials. The contents of the vials were adjusted to 6 ml with 2 M sodium hydroxide, and 10 ml of scintillation cocktail was added to each. The filters were saved for subsequent oxidation. Each of the sediment pellets was then resuspended, washed with 5 ml of sodium acetate (0.1 M), and centrifuged; the resulting supernatants were treated as described before. The acetate wash was repeated twice, and the resulting sediment pellet was frozen until further analysis. For some experiments, processing was modified by acidifying the initial supernatants and acetate washes to remove inorganic carbon prior to radioassay.

Two to four days after the washing procedure, the frozen pellets and corresponding filters were placed in round-bottomed boiling flasks connected to an N_2 source and a series of three scintillation vials containing 6 ml of 2 M

sodium hydroxide. A flow of nitrogen was initiated (60 ml min^{-1}), and 50 ml of potassium persulfate (30 g of potassium persulfate liter $^{-1}$ in 0.1 M sulfuric acid) was added. The sediment was boiled for 2 h to convert particulate ^{14}C in the pellet and on the filters to $^{14}\text{CO}_2$.

Oxidation and recovery efficiencies were estimated by using cells from a sediment enrichment culture containing ^{14}C -glucose as the sole carbon source. Cells from six 1-ml samples of the culture were harvested by filtration on a $0.2\text{-}\mu\text{m}$ cellulose acetate filter. Triplicate filters were assayed for radioactivity after dissolution in 10 ml of scintillation cocktail; the remaining three filters were oxidized as described above with unlabeled sediment. The combined oxidation and trapping efficiency was determined by comparing radioactivity recovered from both sets of filters.

SCFA analysis. Sediments from the 0- to 1-, 4- to 6-, and 8- to 10-cm intervals of three cores were collected and pooled. Sediments from the 4- to 6- and 8- to 10-cm intervals were immediately placed in a glove bag containing 100% N_2 ; surface sediments were handled in air. Slurries were produced by mixing an equal volume of filtered ($0.2\text{-}\mu\text{m}$ pore size) seawater with the sediment; the seawater added to the 4- to 6- and 8- to 10-cm intervals was bubbled with 100% N_2 for 1 h before addition. Twenty subsamples were collected from each slurry with cutoff 5-ml syringes that were sealed with butyl rubber septa. The subsamples were equilibrated at the ambient sediment temperature for 1 h prior to injection of 100 μl of an aqueous solution containing 18.5 to 37 kBq of ^{14}C -glucose. Slurries were used for SCFA end product analysis to minimize possible artifacts from locally high added substrate concentrations. The final added glucose concentration in the slurries was 700 nM. Metabolism was terminated by extruding the subsamples into 15-ml disposable centrifuge tubes which were immersed in boiling water for 1 min. The subsamples were centrifuged at $2,800 \times g$ for 10 min; pore water from replicate subsamples at a given depth and incubation time was pooled, filtered ($0.45 \mu\text{m}$), and frozen for subsequent analysis.

The distribution of radiolabel in pore water SCFAs was determined by high-pressure liquid chromatography (HPLC), using a Milton Roy minipump, an HPX-87 ion-exchange column (Interaction Chemicals Inc.), and a Kratos model 757 variable-wavelength detector (24). Samples (100 μl) were eluted with a mobile phase of 0.01 N H_2SO_4 (Fisher Scientific Co.) at 0.6 ml min^{-1} . Eluant fractions corresponding to individual SCFAs were collected. Organic acid elution times were determined with authentic standards added to pore water. Each of the fractions collected was adjusted to 3 ml with deionized water and assayed for radioactivity. To verify the identity of the radiolabel in various fractions, an enzymatic technique was used to convert specific SCFAs to products which would not coelute with the parent compound. The amount of radiolabel lost from a given fraction after treatment provided an estimate of the purity of the fraction. For example, acetate was reacted with acetyl coenzyme A (CoA) synthetase ($0.88 \text{ U mg of solid}^{-1}$) by adding 50 μl (about 1 U) of acetyl-CoA synthetase (in 0.1 M Tris HCl, pH 7.2), 100 μl of CoA (10 mM CoA, lithium salt, in 0.1 M Tris HCl), and 100 μl of ATP (10 mM ATP, disodium salt, in 0.1 M Tris HCl) to 0.75 ml of pore water containing radiolabelled SCFAs. A 0.75-ml volume of a parallel pore water sample was mixed with 0.25 ml of deionized water as a control. Both samples were incubated at 37°C for 2 h and then filtered through 1-ml columns containing an octadecylsilyl adsorbent (Supelco, Inc.). The eluant was collected, and 100- μl samples were analyzed for

FIG. 1. Seasonal patterns of temperature (---) and glucose uptake rate constants for 0- to 2 (○)-, 4- to 6 (●)-, and 8- to 10 (■)-cm depth intervals. Standard errors are typically comparable to symbol sizes and are not shown (see text also).

SCFAs as described above. Lactate oxidase (10 to $30 \text{ U mg of solid}^{-1}$), L-lactic dehydrogenase ($820 \text{ U mg of solid}^{-1}$), and succinic thiokinase ($10.4 \text{ U mg of protein}^{-1}$) were used similarly to confirm the presence of lactate, pyruvate, and succinate, respectively. Lactate oxidase required no cofactors. L-lactic dehydrogenase required NADH and succinic thiokinase required GTP and acetyl-CoA. Incubation times were 2 h at the optimum temperatures for each enzyme; necessary cofactors were added in concentrations similar to those for acetyl-CoA synthetase. The efficiency of each of the above enzymes was determined by adding labelled or unlabelled substrate to pore water and then determining the percentage removed from the appropriate HPLC elution fraction. Acetyl-CoA synthetase consistently removed 80% of the $[1,2\text{-}^{14}\text{C}]$ acetate added to pore water, lactate oxidase removed 50% of $[1\text{-}^{14}\text{C}]$ lactate with the production of an equal amount of pyruvate, and lactic dehydrogenase removed 80% of added pyruvate. These measured efficiencies were used to correct losses observed in the samples.

The effect of the termination method (boiling) on the concentration of radiolabelled SCFAs was determined in January 1989, using 0- to 1-cm interval sediment. One set of subsamples was terminated after 0.5 h by heating as usual. A parallel set of subsamples was centrifuged without heating and the pore water was filtered; the total incubation time, including centrifugation, was about 0.5 h. Radiolabel in the SCFA fractions of both sets was analyzed, and differences were attributed to the release of low-molecular-weight intracellular compounds by heating.

RESULTS

Glucose uptake. Glucose uptake rate constants varied throughout the year and between the same months of different years (Fig. 1). Typically, maxima and minima paralleled temperature (Fig. 1); there was also a tendency for rate constants to decrease with depth. For comparison, rate constants measured in carbonate sediments from St. Croix were 0.301 ± 0.036 , 0.381 ± 0.067 , and $0.251 \pm 0.030 \text{ min}^{-1}$ (mean ± 1 standard error; $n = 3$) for the 0- to 2-, 4- to 6-, and 8- to 10-cm intervals, respectively, in March 1989; these were similar to the rate constants in Lowes Cove during the summer. Standard errors for uptake constants at all times were typically 10 to 20%, expressed as a coefficient of

FIG. 2. Glucose uptake rate constants for the 0- to 2 (A)-, 4- to 6 (B), and 8- to 10 (C)-cm depth intervals versus incubation temperatures. Regression parameters are as follows: $y = 0.010x + 0.152$, $r^2 = 0.535$, and $P = 0.0001$ (0 to 2 cm); $y = 0.010x + 0.098$, $r^2 = 0.592$, and $P = 0.0001$ (4 to 6 cm); $y = 0.007x + 0.106$, $r^2 = 0.429$, and $P = 0.0009$ (8 to 10 cm).

variation. When the number of replicates for the 0- to 2-cm interval was increased from three to nine in February and August 1989, the coefficients of variation were 9 and 7%, respectively.

Glucose uptake rate constants were significantly correlated with incubation temperatures at all depths (Fig. 2). Temperature accounted for approximately 40 to 60% of the observed seasonal variability based on the r^2 values of simple linear regressions (0.429 to 0.592); use of an Arrhenius model explained a comparable level of the variability. Activation energies derived from Arrhenius plots were 6 to 8 kcal (ca. 25 to 33 kJ) $K^{-1} mol^{-1}$.

On two separate occasions, glucose uptake was compared with uptake of several other monosaccharides. In both cases, glucose uptake rate constants exceeded those for the other sugars for each depth interval (Table 1). In general, rate constants decreased in the following order: glucose > galactose > mannose ~ fucose.

TABLE 1. Monosaccharide uptake rate constants for various sediment depths

Monosaccharide	Sediment depth (cm)	Rate constant ($min^{-1} \pm 1 SE$)	
		October 1989 ^a	December 1989
Glucose	0-2	0.232 ± 0.057	0.189 ± 0.016
	4-6	0.282 ± 0.037	0.160 ± 0.021
	8-10	0.253 ± 0.019	0.172 ± 0.030
Galactose	0-2	0.167 ± 0.030	0.110 ± 0.037
	4-6	0.129 ± 0.034	0.065 ± 0.026
	8-10	0.095 ± 0.055	0.051 ± 0.021
Mannose	0-2	0.056 ± 0.005	0.065 ± 0.019
	4-6	0.046 ± 0.009	0.037 ± 0.015
	8-10	0.042 ± 0.010	ND ^b
Fucose	0-2		0.058 ± 0.018
	4-6		0.024 ± 0.018
	8-10		ND

^a Fucose uptake was not assayed in October 1989.

^b ND, no uptake detected.

Carbon dioxide production. There were several potential sources of error in the estimates of $^{14}CO_2$ production. Vigorous mixing during the production of slurries from the 0- to 1-cm interval resulted in a 50% decrease in $^{14}CO_2$ formation (Fig. 3A). In contrast, storage of sediment for up to 24 h prior to glucose addition had no effect (Fig. 3B); likewise, $^{14}CO_2$ production was apparently insensitive to a range of added ^{14}C -glucose concentrations (66 nM to 3.3 μM , in a 50- μl injection [Fig. 3C]).

In general, $^{14}CO_2$ accumulated rapidly for the first 3 to 6 h of incubation and more slowly thereafter, typically reaching a plateau (Fig. 3A). The amount of $^{14}CO_2$ accumulated at 6 h varied significantly throughout the year. About 25 to 35% of the added ^{14}C -glucose was oxidized to $^{14}CO_2$ in the 0- to 1-cm interval in summer and early fall; about 30 to 40% was oxidized in the 4- to 7-cm interval during the same periods (Fig. 4). In the late fall, winter, and spring, $^{14}CO_2$ production in the 0- to 1-cm interval accounted for only 15 to 25% of the added glucose; only 20% of the added glucose was oxidized to $^{14}CO_2$ during December 1988 for the 4- to 7-cm interval. In comparison, 41% (0 to 1 cm) to 58% (4 to 7 cm) of added ^{14}C -glucose was oxidized to $^{14}CO_2$ in sediments from St. Croix after 6 h of incubation at 23°C.

The effect on $^{14}CO_2$ production of adding stable glucose after 5 h of incubation was minimal on the basis of results from an experiment conducted in June 1989 (Fig. 5). The amount of $^{14}CO_2$ recovered from 0- to 1-cm interval sediment slurries was similar with or without the addition of 100 μM (final concentration) unlabelled glucose.

Incorporation of glucose into biomass. The percentage of added ^{14}C -glucose incorporated into biomass was defined as the fraction of label in a particulate form recovered from the sediment pellet after 6 h of incubation. In May, July, October, and December 1989, recovery in these fractions amounted to 31 and 32%, 32 and 21%, 40 and 30%, and 34 and 21% for the 0- to 1- and 4- to 7-cm intervals, respectively. The total recovery of label from all fractions (sediment pellets, $^{14}CO_2$, etc.) ranged from 85 to 104% of the added ^{14}C -glucose (Fig. 6). On the basis of results of an experiment with 0- to 1-cm interval sediment slurries in August 1989, the amount of label in the acidified pore water, acetate rinses, and the washed sediment pellet was unaf-

FIG. 3. Recovery of added ^{14}C -glucose as $^{14}\text{CO}_2$: (A) in slurried sediments ($\circ$) and intact subcores ($\bullet$) as a function of incubation time; (B) from intact subcores preincubated for various times prior to injection of radiolabel. (C) Effect of added ^{14}C -glucose concentrations on recovery of $^{14}\text{CO}_2$. Error bars represent ± 1 standard error.

fect by the addition of excess unlabelled glucose ($100\ \mu\text{M}$ final concentration in $3\ \text{cm}^3$ of sediment [Fig. 5]).

SCFA analysis. Two SCFA end products from ^{14}C -glucose (acetate and pyruvate) were detected by using both HPLC and enzymatic approaches. However, only acetate was detected when incubations were terminated by centrifugation rather than heating. Unidentifiable radiolabelled compounds were routinely observed with or without heating (although heating increased the amount of label in these

FIG. 4. Recovery of added ^{14}C -glucose as $^{14}\text{CO}_2$ after 6 h of incubation in intact subcores from 0 to 2 ($\circ$)- and 4- to 7 ($\bullet$)-cm depth intervals; error bars represent ± 1 standard error.

pools). These compounds eluted with all of the SCFA standards except acetate (Table 2), but were not verified as SCFA by enzyme analyses. For the purposes of calculating end product formation, pyruvate was ignored because it appeared to arise from intracellular pools that were artifacts

FIG. 5. Recovery of added ^{14}C -glucose (A) as $^{14}\text{CO}_2$ and (B) as acidified pore water and acetate rinses ($\circ$, $\bullet$) and sediment pellets ($\square$, $\blacksquare$). Closed symbols represent slurries to which $100\ \mu\text{M}$ (final concentrations) stable glucose was added at time points indicated by the arrows; open symbols represent unamended controls. Error bars represent ± 1 standard error. The slurries were prepared with sediments from the 0- to 1-cm depth interval.

FIG. 6. Proportion of added ^{14}C -glucose recovered as $^{14}\text{CO}_2$ (□), nonglucose metabolites (▨), and biomass (■) for sediments from the 0- to 2 (A)- and 4- to 7 (B)-cm intervals. See text for details.

of the heating procedure used to terminate incubations (Table 2).

The percentage of added ^{14}C -glucose recovered as acetate after 0.5, 1, and 3 h of incubation was very small. After 0.5 h, only about 2% of the added radiolabel was attributed to acetate in the 0- to 1-cm interval and 5% was attributed to acetate in the 4- to 6- and 8- to 10-cm intervals; after 3 h, this percentage was typically <1% in the 0- to 1-cm interval and

TABLE 2. Recovery of added ^{14}C -glucose as SCFAs in triplicate slurried sediments from the 0- to 1-cm interval, incubated for 0.5 h and subjected to heat treatment or centrifugation only to terminate microbial activity^a

HPLC fraction	Heat-treated pore water		Centrifuged pore water	
	dpm ml ⁻¹	% Lost ^b	dpm ml ⁻¹	% Lost ^b
Void ^c	38,820	NA ^d	0	NA
Acetate	5,210	102.6	5,570	89.4
Propionate	6,500	NA	0	NA
Lactate	10,180	0.0	3,230	0.0
Pyruvate	17,120	15.9	5,250	0.0

^a Pore water samples were collected after termination and analyzed by HPLC for the distribution of radiolabel; a parallel set of pore water samples was incubated with enzymes that reacted with specific SCFAs (see text for details). The percentage of radioactivity lost is equivalent to the percentage of identifiable SCFAs in each fraction.

^b Corrected for enzyme reaction efficiencies; see text for details.

^c Eluant collected for the 0- to 5-min interval postinjection.

^d NA, not assayed.

<3% in the deeper intervals. Adsorption of acetate was limited, since 95% of $[1,2-^{14}\text{C}]$ acetate (36.5 mCi/mmol; New England Nuclear) added to heat-inactivated sediment was recoverable from filtered (0.45- μm pore size) pore water.

DISCUSSION

Glucose uptake. Glucose uptake rate constants in Lowes Cove sediments vary by a factor of about 5 on a seasonal basis and tend to decrease with increasing depth (Fig. 1). Turnover times generally vary between 2 and 10 min, consistent with the more rapid estimates for other systems (8, 9, 16, 31, 32). However, the variety of different techniques used for estimating turnover precludes broad comparisons among systems. For example, calculations based on the appearance of $^{14}\text{CO}_2$ (e.g., those of references 9, 16, and 32) probably overestimate turnover times because of isotopic dilution (22, 24). Our technique, which is based on a direct analysis of uptake (24), yields estimates that are unaffected by dilution.

While a number of factors affect rate constants, temperature is often particularly significant (Fig. 2). In this study, temperature accounts for 43 to 59% of the observed seasonal variation. Other parameters, such as microbial biomass and total dissolved monosaccharide concentrations, appear less important (18). Temperature may affect rate constants directly by altering the kinetic properties of enzymes or indirectly by altering ecological or geochemical parameters. For example, benthic algal blooms during winter (29) may support significant microbial activity, thereby decreasing the relative importance of temperature as a proximate control of glucose uptake. In general, though, the seasonal patterns in this study agree with reports for other environments and substrates. For example, King and Klug (24) have observed a two-fold difference in glucose turnover times. Ansbæk and Blackburn (2) and Christensen and Blackburn (7) have observed a range of up to fivefold for acetate uptake over temperatures from 2 to 14°C. Mayer (29) has reported fivefold variations in proteolytic activity in Lowes Cove sediment over a range of 0 to 15°C.

Irrespective of seasonality, the rate constants for monosaccharide uptake decrease in the following order: glucose > galactose > mannose ~ fucose. The rate constants for each monosaccharide also generally decrease from fall to winter and decrease with depth, though the relative decrease is much less for glucose than for the other sugars (Table 1). Since glucose typically dominates pore water monosaccharide pools (33), the rapid decrease in rate constants for nonglucose monosaccharides suggests that the relative importance of glucose increases with depth. This may be due to a more rapid attenuation of nonglucose monosaccharide concentrations in the dissolved or particulate polysaccharide pools or in the rate of release of other monosaccharides from polysaccharides. Such changes could result in a decrease in the number of sugar-specific transport systems for the nonglucose sugars.

Estimates of pore water glucose concentrations at our study site range from about 2 to 17 and 3 to 14 μM for the 0- to 2- and 8- to 10-cm intervals, respectively (18). On the basis of the observed rate constants (Fig. 1), glucose uptake rates range from 0.4 to 7.1 and 0.2 to 4.8 $\mu\text{mol cm}^{-3} \text{ day}^{-1}$ for the two respective depths (using porosities of 0.8 and 0.7 ml). Assuming that respiration accounts for 60% of metabolism (see below) and a molar stoichiometry of 1:3 for glucose respiration and sulfate reduction, sulfate reduction rates of 0.7 to 12.8 and 0.3 to 8.6 $\mu\text{mol cm}^{-3} \text{ day}^{-1}$ could be

supported. These values are approximately 10-fold higher than the observed sulfate reduction rates (18). While processes such as nitrate, manganese, and iron reduction undoubtedly account for some additional glucose metabolism, it is unlikely that uptake actually occurs at the rates calculated above. It is more likely that the contribution of glucose carbon is in error as a result of the use of centrifugation to collect the pore water used for the glucose assay; similar problems with centrifugation have been reported for acetate by Shaw and McIntosh (39).

However, various problems might have contributed to errors in the rate constant estimates. Although the subsampling procedure minimized disturbance, sealing sediment from the 0- to 2-cm interval in syringes might have had an impact on oxygen concentrations or distribution, thereby altering glucose uptake. Such impact would likely have been minimal, though, since (i) oxygen penetration at the sample site is only 1 to 2 mm (21); (ii) the radiotracer was injected primarily into the anoxic region of the subcores; and (iii) incubation times were too short to allow significant diffusion to any oxic zone. In addition, Toerien and Cavari (43) have reported that the maximum rate of uptake, the glucose turnover rate, and the amount of CO_2 produced from added glucose were not affected by the presence or absence of oxygen in either oxic or anoxic sediments.

The distribution of added radiotracer between available and unavailable pools (2, 7, 11, 35) also raises concern. In this study, it appears that the added glucose was overwhelmingly associated with the "bioavailable" pool since up to 90% of the added $[\text{H}^3]\text{glucose}$ was utilized after incubation for only 15 min; during longer incubations (4 h), up to 99% was utilized. These results indicate that the effects of adsorption or exchange were insignificant, at least with respect to availability.

Carbon dioxide production. The recovery of $^{14}\text{CO}_2$ typically plateaued after 6 h of incubation for sediments from all depths and collection dates (Fig. 3A). Subsequent increases in $^{14}\text{CO}_2$ were correlated with and perhaps explained by decreases in pools of ^{14}C biomass or nonglucose metabolites (NGM). Thus, the initial 6 h of incubation were defined operationally as a primary catabolic phase, while longer time points represented a secondary phase that included redistribution of radiolabel within intracellular pools as well as metabolism of glucose fermentation end products. A comparable inflection in the time course of $^{14}\text{CO}_2$ production has been previously attributed to changes in substrate specific activity and to the catabolism of fermentation end products (22, 24).

The amount of $^{14}\text{CO}_2$ accumulation at 6 h varied seasonally and with depth, accounting for 15 to 40% of the added radiolabel (Fig. 5). In comparison, $^{14}\text{CO}_2$ accounted for 8, 5 to 15, and 15 to 20% in undisturbed sandy intertidal sediments (31, 32), slurried lake sediments (43), and anoxic riverine and groundwater sediments (28), respectively. In general, the latter data were derived from analyses with relatively short incubation periods (1 h or less) and may not reflect isotopic equilibrium. King and Klug (24) found that volatile end products ($^{14}\text{CO}_2$ and $^{14}\text{CH}_4$) accounted for only 33% of added radioglucose after a 1-h incubation of anoxic lake sediments, but accounted for approximately 90% after 34 h. Cahet et al. (3) reported that $^{14}\text{CO}_2$ accounted for 20 to 30% of glucose uptake in oxic deep-sea sediments, but time course data were not available to assess the extent of isotopic dilution.

Failure to consider the impact of isotopic dilution results in underestimation of turnover times as well as erroneous

FIG. 7. (A) Regression analysis of $^{14}\text{CO}_2$ recovery and incubation temperature ($r^2 = 0.814$; $P = 0.0004$). (B) Correlation between recovery of $^{14}\text{CO}_2$ and glucose uptake rate constants; $r = 0.726$. Data were obtained with sediments from the 0- to 1-cm depth interval.

interpretation of the relative magnitudes of respiration and biosynthesis, especially if biosynthesis is calculated as $100\% - \% \text{CO}_2$ production. The differences in glucose turnover times calculated from $^{14}\text{CO}_2$ accumulation ($T_t > 3$ h) and $[\text{H}^3]\text{glucose}$ uptake ($T_t \sim 2$ to 10 min) in this study are best explained by isotopic dilution and further illustrate the problem.

In this study, the extent of $^{14}\text{CO}_2$ production strongly correlates with temperature (Fig. 7A; $r^2 = 0.814$; $P = 0.0004$). This may be explained in part by a direct proportionality between biosynthesis and temperature. Such a relationship could occur if, for example, cellular maintenance requirements were directly proportional to temperature, resulting in increased growth efficiencies even as absolute growth rates decline. However, the observed correlation can also be attributed to isotopic dilution, since the extent of isotopic equilibration is likely a function of temperature-sensitive metabolic rates. This interpretation is supported by the correlation between glucose uptake rate constants and $^{14}\text{CO}_2$ production (Fig. 7B; $r = 0.726$), which indicates that the extent of catabolism is a function of the turnover time of extracellular glucose as well as intracellular metabolites. While the relationship between glucose turnover time and temperature is consistent with expectations based on general behavior of enzymatic systems, a positive correlation between turnover time and the extent of $^{14}\text{CO}_2$

production is consistent with greater isotopic equilibration as turnover times for glucose and glucose metabolites decrease. Thus, it may be possible to accurately assess the rates and dynamics of glucose uptake but more difficult to specify the distribution of glucose carbon between anabolic and catabolic pathways simply on the basis of patterns of end product formation.

Biosynthesis. Glucose incorporation and $^{14}\text{CO}_2$ production were analyzed in parallel. Radiolabel remaining in the sediment after repeated washing was defined as a particulate fraction, equivalent to biomass. Radiolabel recovered in the various acidified rinses was defined as NGM distinct from biomass. The presence of glucose and SCFAs in the NGM fraction was excluded on the basis of results which consistently showed consumption of 99% of the added radiolabel after 4 h. Acetate, the only extracellular SCFA observed (see below), accounted for just 1 to 3% of the added radiolabel after 3 h of incubation. Since only 5% of added radioacetate was adsorbed, neither acetate nor other SCFAs contributed significantly to NGM.

A comparison of the distribution of radiolabel among particulate, NGM, and $^{14}\text{CO}_2$ fractions in July, October, and December 1989 for sediments from the 0- to 1- and 4- to 7-cm intervals showed greater incorporation into the particulate and NGM fractions in winter than in summer or fall (Fig. 6). In the 0- to 1-cm interval, increased incorporation into the particulate and NGM fractions was paralleled by a pronounced decrease in the recovery of $^{14}\text{CO}_2$. However, of the three fractions, the NGM showed the greatest seasonal variability. Between 30 to 40% (0 to 1 cm) and 20 to 30% (4 to 7 cm) of the added radiolabel was consistently recovered from the particulate fraction (Fig. 6), while 20 to 40% (0 to 1 cm) and 30 to 50% (4 to 7 cm) of the radiolabel was recovered in the NGM fractions. The temporal trends in the NGM fraction were probably related in part to a temperature-dependent isotopic dilution effect as described for $^{14}\text{CO}_2$ production.

Though the composition of the NGM fraction is uncertain, the particulate label probably consists of macromolecules that represent biomass (3). The relative incorporation of glucose into biomass is probably minimal because of isotopic dilution in pools of biosynthetic intermediates. However, the observed values are consistent with expected growth efficiencies for sulfate-reducing microbial communities in marine sediments (10). King and Klug (24) have reported somewhat lower values for biosynthesis from glucose in a lake sediment (calculated from the total added label minus the sum of $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$). It is tempting to speculate that the difference between the two sediments reflects differences in the energetics of catabolism in freshwater versus marine systems. Others (3, 9, 32) have reported even higher levels of biosynthesis than observed here; however, these studies have typically involved short-term incubations (e.g., <1 h) and are subject to isotopic dilution errors. Work by Cahet et al. (3) is exceptional in that the authors used incubation periods of up to 24 h. However, their work is not directly comparable to this or most other studies since they used deep-sea sediments and oxic conditions.

Organic acid analysis. Acetate was the only consistently detectable SCFA end product from glucose catabolism (Table 2). Others have also documented generally the significance of acetate in marine sediments (6, 37, 42, 44). In contrast, King and Klug (24) reported that acetate dominated SCFA end products from glucose in a lake sediment but that lactate and propionate were important as well.

Two observations affected interpretation of the results

reported here. First, some unidentified, radiolabelled glucose metabolites coeluted with lactate and pyruvate during the HPLC analysis; these compounds were most likely other intracellular metabolites. The use of an enzymatic technique provided a means of positively identifying specific SCFAs and distinguishing their radioactivity from that of coelutants. Previous studies (24) have only identified radiolabelled SCFAs on the basis of HPLC retention times. As a result, the importance of acetate may have been underestimated. Others have simply extracted pore water with solvents to recover presumed fermentation end products (8, 9). Novitsky and Kepkay (34) attempted to identify the compounds produced during glucose fermentation by using thin-layer chromatography, but their attempts were unsuccessful because of the heterogeneity of their extracts.

A second important observation concerns the method(s) used to terminate incubations. In this study, significant cell lysis appears to have resulted from the use of a heat "kill" (Table 2). Lysis most likely accounts for the presence of pyruvate as an apparent extracellular end product since a more gentle procedure for termination (e.g., centrifugation) yielded no extracellular pyruvate. The presence of pyruvate as well as that of a number of other low-molecular-weight intracellular metabolites are, of course, entirely consistent with known pathways of fermentation (27). Cell lysis as a result of the technique used to terminate an incubation can therefore give a misleading picture of the metabolites that are excreted by undisturbed cells. Unfortunately, Novitsky and Kepkay (34) have also observed lysis from the use of 1 N H_2SO_4 . Thus, it is not likely that some of the more traditionally used methods are acceptable for end product analysis. The effects of "quick freezing" and fixatives (formaldehyde or glutaraldehyde) are not certain but should be examined. Low-speed centrifugation offers an alternative but may not be adequate for studies requiring precise timing and short incubation periods.

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