Glucose Metabolism in Sediments of a Eutrophic Lake: Tracer Analysis of Uptake and Product Formation†

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The uptake of glucose and the formation of end products from glucose catabolism have been measured for sediments of eutrophic Wintergreen Lake with a combination of tritiated and 14C-labeled tracers. Time course analyses of the loss of [3H]glucose from sediments were used to establish rate constants for glucose uptake at natural substrate concentrations. Turnover times from these analyses were about 1 min for littoral and profundal sediments. No seasonal or site differences were noted in turnover times. Time course analyses of [U-14C]glucose uptake and 14C-labeled end product formation indicated that glucose mass flow could not be calculated from end product formation since the specific activity of added [14C]glucose was significantly diluted by pools of intracellular glucose and glucose metabolites. Mass flow could only be accurately estimated by use of rates of uptake from tracer studies. Intermediate fermentation end products included acetate (71%), propionate (15%), lactate (9%), and only minor amounts of butyrates or valerates. Addition of H2 to sediments resulted in greater production of lactate (28%) and decreased formation of acetate (50%), but did not affect glucose turnover. Depth profiles of glucose uptake indicated that rates of uptake decreased with depth over the 0- to 18-cm interval and that glucose uptake accounted for 30 to 40% of methanogenesis in profundal sediments.

Measurements of microbial heterotrophic potential, using the methods of Parsons and Strickland (21) as modified by Wright and Hobbie (26), have found wide acceptance among microbial ecologists. The parameters generated from these measurements (Vmax, Ks, and Tt) provide information that can be used to compare the responses of a system to temporal changes as well as changes in parameters such as temperature, organic inputs, pH, etc. However, assays of heterotrophic potential do not provide direct estimates of the flux of specific organics through the biota. Specific flux rates are often more desirable for comparative purposes than potential rates and are required for estimates of mass flow. To estimate specific rates of mass flow, two parameters are necessary: (i) the concentration of the substrate of interest and (ii) the extracellular turnover time (Tt) of the substrate of interest. Whereas there are numerous reports in which one or the other of the above parameters have been measured, relatively few reports exist in which both parameters have been measured simultaneously (2, 9, 17, 23).

We report here results from a study of glucose metabolism in eutrophic lake sediments, using [3H]- and [14C]glucose. The use of [3H]glucose uptake at natural substrate concentrations is described as an alternative to Wright-Hobbie methodology for measuring turnover times. Turnover times measured from [3H]glucose uptake, when coupled with glucose concentrations, provide an estimate of glucose mass flow and the relative importance of glucose carbon to total carbon flow in the lake sediments. Studies of product formation from [14C]glucose provide estimates of the extent of glucose mineralization and patterns of volatile fatty acid (VFA) production. The studies with [14C]glucose also indicate that the rate constants of extracellular and intracellular glucose pools differ markedly and that glucose mass flow cannot be determined from rates of end product (i.e., CO2) formation.

(Results from this study were presented previously [G. M. King and M. J. Klug, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, 12, p. 94].)

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MATERIALS AND METHODS
Surface sediments for glucose turnover studies were collected from the littoral and profundal zones of
eutrophic Wintergreen Lake, using an Ecken dredge. These sediments are highly organic (>20%) silts and have been characterized elsewhere (15, 16, 18, 19, 22). Samples were stored at ambient sediment temperature and processed within 24 h of collection. Glucose turnover times were measured with 5-cm³ aliquots of sediment contained in 10-ml Vacutainers with an O₂-free headspace of 100% N₂. Deionized water (0.5 ml) containing 2 μCi of [6-3H]glucose (14 Ci/mmol; New England Nuclear) per ml was injected into each Vacutainer by needle and syringe. The sediment mixtures were incubated at ambient field temperatures for intervals of up to 10 min. At appropriate times 1 ml of a 10% solution of glutaraldehyde was injected into each of triplicate samples to terminate glucose uptake. Triplicate samples injected with glutaraldehyde at T₀ served as controls. After addition of glutaraldehyde, the Vacutainers were centrifuged at high speed in an IEC clinical centrifuge. A 1-ml portion of the supernatant was subsampled and evaporated to dryness at ~50°C to remove ³H-labeled water. The residue was reconstituted in 2 ml of deionized water; 1 ml of this solution was washed through a column containing Dowex AG1 anion-exchange resin (Cl form) to separate unreacted glucose from anionic (or acidic) end products of glucose catabolism. Controls indicated that radiolabeled glucose could be quantitatively separated from anionic fermentation end products and recovered with a 10-ml rinse. Fermentation end products were recovered quantitatively from the anionexchange column by elution with 10 ml of 0.1 M NaCl. Residual [³H]glucose was counted with 10 ml of aqueous scintillating scintillator (Amershams Corp.).

Similar studies of glucose turnover and end product formation in profundal sediments were conducted with [U-¹⁴C]glucose. Aliquots of 0.5 ml of deionized water containing 3 μCi of [U-¹⁴C]glucose (250 mCi/ml; New England Nuclear) per ml were injected with a syringe and needle into Hungate pressure tubes (Bellco, Inc.) containing 10 ml of profundal surface sediment and an atmosphere of either O₂-free 100% N₂ or 100% H₂. Tubes were incubated as described above and 1 ml of 10% glutaraldehyde was injected at appropriate time points into each of triplicate samples to terminate biological activity. Unreacated [U-¹⁴C]glucose in the sediments was determined as above. ¹⁴CO₂ and ¹⁴CH₄ production from added [U-¹⁴C]glucose was measured by gas chromatography and gas-proportional counting as described by Lovely and Klug (16). Total ¹⁴C-labeled VFA production was determined from the eluant of the anion-exchange columns used to separate glucose and VFA (see above). The radioactivity associated with specific VFAs was measured in fractions eluted from an HPX-87 ion-exchange column (Bio-Rad Laboratories) with a high-pressure liquid chromatography system operated at ambient temperature with a solvent of 5 mM H₂SO₄ at a flow rate of 0.8 ml/min. Individual VFAs were detected by UV absorbance at 210 nm.

Depth profiles of glucose uptake in Wintergreen Lake sediments were determined on cores collected with 7.6-cm-inside diameter coring tubes by either gravity or SCUBA from the profundal and littoral zones. Subcores, 5 cm², were taken through ports in the coring tube at 3-cm intervals from 0 to 18 cm, using a cutoff 5-ml syringe, and injected into Vacutainers as described earlier. [6-3H]glucose was added as previously detailed. Triplicate samples for each depth were incubated for 30 s before addition of glutaraldehyde at T₀ to serve as controls. Uptake of [6-3H]glucose was determined as outlined above. To determine whether or not mixing of sediments in the Vacutainers had a marked effect on glucose uptake, a set of subcores from the profundal and littoral zones at 0- to 3- and 15- to 18-cm depth intervals was assayed by using a direct injection technique as described by Lovely and Klug (16). Briefly, 5-cm³ subcores were obtained from the 7.6-cm-inside diameter cores with minimal disturbance at the appropriate depths with cutoff 5-ml syringes sealed with septa. A 10-μl portion of deionized water containing 1 μCi of [6-3H]glucose was injected into each syringe through the septum sealing the cut-off end of the syringe. Triplicate samples at T₀ and at 30 s of incubation were “quick frozen” in liquid nitrogen. The frozen subcores were thawed rapidly in a solution of 10% glutaraldehyde and processed as above.

Depth profiles of glucose concentration in sediments of the profundal and littoral zones were measured by using a fluorometric technique with a detection limit of 10 μM (12). Interstitial water for the glucose assays was collected with dialysis samplers (19). All reagents and enzymes used for the glucose assays were obtained from Sigma Chemical Co. All glassware used for the assays was fired at 550°C for at least 3 h before use. Solutions of reagents and enzymes were made with water from a Millipore “Q” water system (Millipore Corp.) to minimize blank fluorescence. Interference from other sugars was minimal, as determined by the method of Hanson and Snyder (10) for selected samples.

RESULTS

Typical curves of [6-3H]glucose uptake during a 10-min incubation (Fig. 1) indicated very rapid glucose turnover in Wintergreen Lake surface sediments. The disappearance of [6-3H]glucose was exponential (r = 0.86 to 0.96) in form and was used to calculate glucose turnover times from the following relationships: A = A₀e⁻kt (equation 1), where A₀ and A are initial and time t [6-3H]glucose dpm, respectively, and k = apparent uptake rate constant; and T₁ = 1/k (equation 2). Since [6-3H]glucose recovery was >90% at T₀, little if any of the labeled glucose disappearance was due to absorption on the sediments. Coefficients of variation (n = 3) for each time point were <25% for both littoral and profundal surface sediments. Turnover times estimated from equations 1 and 2 for littoral and profundal surface sediments ranged between 0.6 and 1.4 min (Table 1). No significant site or seasonal differences were observed.

The rapid glucose turnover measured in Wintergreen Lake sediments was in marked contrast to the turnover of glucose observed for water column samples taken near the sediment water interface (Fig. 2). Disappearance of [6-3H]glucose was also exponential in nature, but much slower than that measured in sediments. The T₁ for hypolimnetic water sampled in April was 88

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The uptake of [U-14C]glucose (Fig. 1) in profundal surface sediments showed the same patterns as the uptake of [6-3H]glucose. Turnover times estimated from 14C-labeled glucose was 1.1 and 1.3 min for samples incubated with 100% N2 and 100% H2, respectively. Within 5 min, >90% of the added [U-14C]glucose was taken up by the sediment microflora. Production of the

FIG. 1. Uptake of [6-3H]glucose in littoral and profundal surface sediments of Wintergreen Lake during May 1981. Uptake is represented as percentage of added glucose remaining versus time.

FIG. 2. Uptake of [U-14C]glucose from hypolimnetic waters of Wintergreen Lake (6-m depth) during April 1981. Uptake is represented as percentage of added glucose remaining versus time.
initial end products of [14C]glucose catabolism in the sediments, 14CO2 and 14C-VFA, occurred without a lag (Fig. 3). However, only a small fraction (<30%) of the added 14C was recovered as glucose metabolites after 5 min, the point at which cells had taken up virtually all added glucose. 14CO2 production was significantly less and 14C-VFA production was greater during early time periods (<60 min) for samples incubated with a headspace of 100% H2. Incubation of samples for periods up to 60 min resulted in the recovery of approximately 60% of added label as fermentation end products in both treatments. Longer incubation periods resulted in increased production of 14CO2 and decreased production of 14C-VFA pools (Fig. 3 and 4). 14CO2 production was more nearly equal in the 100% N2 and 100% H2 treatments after 60 min. Significant 14CH4 production was also first noted after 60 min (Fig. 3). 14CH4 increased over time in both 100% N2 and 100% H2 treatments, though rates of production were greater with H2 (Fig. 3). Total label recovery as fermentation end products (including 14CH4) exceeded 80% for both H2 and N2 treatments after 34 h of incubation. Therefore, incorporation of added glucose into biomass was <20%.

After 1 h of incubation with [U-14C]glucose, acetate, propionate, and lactate accounted for 71, 15, and 9%, respectively, of the 14C-VFA produced in sediments incubated with 100% N2; these acids accounted for 50, 16, and 28%, respectively, of 14C-VFA production in sediments incubated with 100% H2 (Table 2). Only minor amounts of butyrates or valerates were produced in either N2 or H2 treatments.

Depth profiles of glucose (Fig. 5) and rates of glucose uptake (Fig. 6) near the end of summer stratification differed for littoral and profundal sediments. Glucose concentrations ranged between 96 and 339 nmol/liter in profundal sediments and between 316 and 858 nmol/liter in littoral sediments. Coefficients of variation for each depth (n = 3) were usually <10% at both sites. Rates of glucose uptake paralleled trends in glucose concentration (Fig. 6), with rates of uptake highest in surface sediments and decreasing with depth. Rates of uptake were greater in littoral than in profundal sediments. Coefficients of variation were <23% (n = 6 for each depth) and typically <15%. No significant differences were found for samples processed in Vacutainers or with a direct-injection in situ technique in 5-ml syringes (Fig. 6). Rates of glucose uptake integrated over the 0- to 18-cm interval were 74.5 and 13.2 mmol of glucose/m2 per day for littoral and profundal sediments, respectively.

**DISCUSSION**

In Wintergreen Lake sediments, concentrations of dissolved glucose are quite low (<1 μM). At these concentrations, tracer studies with [14C]glucose are precluded since the addition of isotope at levels sufficient for measuring end product formation can appreciably alter natural pool sizes. However, because of its high

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**TABLE 1. Average turnover times (Tc) for Wintergreen Lake surface sediments**

<table>
<thead>
<tr>
<th>Date</th>
<th>Littoral</th>
<th>Profundal</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 1981</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>July 1981</td>
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<td>0.6</td>
</tr>
<tr>
<td>January 1982</td>
<td>0.7</td>
<td>0.6</td>
</tr>
</tbody>
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**FIG. 3.** Uptake of [U-14C]glucose and 14C-end product formation in profundal surface sediments versus time. Symbols: •, samples incubated with a 100% N2 headspace; ○, samples incubated with a 100% H2 headspace. Note change in time scale at 60 min.
specific activity, [3H]glucose provides an alternative for measuring glucose uptake directly without altering in situ substrate concentrations.

Time course analyses of [6-3H]glucose uptake (Fig. 1) provided a relatively quick, simple method for determining glucose turnover times in profundal and littoral surface sediments of Wintergreen Lake. In the studies reported here, [6-3H]glucose was added to the sediments at tracer levels (added glucose, ≈3% of natural glucose concentrations), thus satisfying the requirements for determining turnover time by the tracer approach (27). However, turnover times in Wintergreen Lake sediments could not be calculated from the linear transformation suggested by Wright and Burnison (27). An exponential data transformation, as described by Fleischer (7), is more appropriate (see Fig. 1) and provides a more accurate estimate of $T_r$. Calculations based on a linear transformation can significantly overestimate $T_r$, with the magnitude of the error depending in direct proportion on the fraction of added label metabolized.

The rapid turnover times observed for both littoral and profundal surface sediments measured with [6-3H]glucose uptake are consistent with turnover estimates based on a modified Wright-Hobbie kinetic assay (26), using [1-14C]glucose, over a 50-fold range of added glucose concentrations (data not presented). $T_r$ values for Wintergreen Lake sediments based on both tracer and kinetic assays are among the most rapid reported for freshwater or marine sediments. Hall et al. (9) report $T_r$ values ranging from 3.7 to 24 min for surface sediments of undisturbed cores of Marion Lake; Wood and Chua (25) report a range of $T_r$ from 1.8 to 10 min for surface sediments of Toronto Harbor diluted 100-fold; Fleischer (7) and Meyer-Reil (17) indicate (but do not report) very rapid $T_r$ (<10 min) for surface lake and sandy beach sediments, respectively. Others (1, 4, 11, 23) have measured $T_r$ from minutes to >20 h for uptake experiments with a variety of sediments incubated under a variety of conditions. Because of the great variety of sampling techniques and incubation conditions (temperature, sediment dilution, anoxia, etc.) used in the reported studies, meaningful comparisons among systems may be limited.

Data for glucose turnover based on tracer studies (7) or kinetic studies of water column samples (e.g., 8) are much more numerous and variable than sediment data. $T_r$ values for water column samples range from <0.5 to >1,000 h. In general, however, water column $T_r$ values are greater than sediment $T_r$. Data from the study reported here are consistent with the observa-

FIG. 6. Depth profiles of rates of glucose uptake in profundal and littoral sediments during September 1981. Rates in micromoles of glucose per liter of sediment per hour. Rates from direct injection experiments are indicated by "x" at 0- to 3- and 15- to 18-cm depths.
tions on water column and sediment samples. Our work revealed a $T$, for surface sediments of ~1 min and a $T$, of >1 h for hypolimnetic water. An even greater disparity between sediment and water column $T$, has been reported by Wood and Chua (25). The measured differences between sediment and water column are undoubtedly due to the greater microbial biomass associated with sediment.

Although tritiated substrates are useful for determining in situ turnover times of compounds at low concentrations, studies with tritiated substrates provide relatively little information about intermediary metabolism. In this study, details of glucose metabolism are illustrated with data from additions of $[U-^{14}C]$glucose (Fig. 3). As used in this study $[^{14}C]$glucose would have increased ambient glucose concentrations. However, the rapid turnover of glucose would have resulted in a total mass flux in excess of the added substrate, thus minimizing any effects of temporarily increased pool sizes.

Time course analyses of $[U-^{14}C]$glucose uptake and $^{14}C$-end product formation indicate that the kinetics of glucose uptake differ substantially from the kinetics of end product formation. Although there is no lag in $^{14}C$-end product formation, the formation of $^{14}CO_2$ and $^{14}C$-VFA is much slower than $[U-^{14}C]$glucose uptake. The differences between uptake and end product formation are readily apparent from a comparison of the rate constants for each process. The rate constant for glucose uptake is determined from equation 1. The rate constant for end product formation is calculated from the sum of $^{14}CO_2$ and $^{14}C$-VFA production by making the following assumptions: (i) added $[U-^{14}C]$glucose is taken up instantaneously relative to $^{14}C$-end product formation; (ii) the specific activity of $[U-^{14}C]$glucose decreases exponentially with time during turnover of intracellular glucose pools; (iii) added $[U-^{14}C]$glucose is not incorporated into biomass. Based on these assumptions, the disintegrations per minute found in end products ($X$) at any time ($t$) is the difference between initial $[U-^{14}C]$glucose disintegrations per minute ($A_0$) and $[U-^{14}C]$glucose at $t$ ($A$): $X = A_0 - A$ (equation 3). Since the decrease in intracellular $[U-^{14}C]$glucose with time is described by equation 1, it follows that: $X = A_0 - A_0 e^{-kt}$ (equation 4) and $X = A_0 (1 - e^{-kt})$ (equation 5). Rearranging to solve for the rate constant of end product formation ($k_1$) gives: $k_1 = -\ln [1 - (X/A_0)]/t$ (equation 6). The absolute value of the rate constants for $[U-^{14}C]$glucose uptake and $^{14}C$-end product formation in profundal sediments incubated with 100% $N_2$ are 0.51 and 0.04 min$^{-1}$, respectively, as determined by equations 1 and 6.

The gross differences between rate constants for uptake and end product formation are not an artifact of the assumptions used to generate equation 6. Although $[U-^{14}C]$glucose uptake is not instantaneous, the error associated with the first assumption is minimized by calculating rate constants for end product formation from incubation points that are relatively long compared with the time course for $[U-^{14}C]$glucose uptake. Rate constants from 10- and 60-min incubation points are suitable since >90% of the added glucose was taken up within 5 min. Rate constants from these time points are equivalent. The second assumption has not been evaluated experimentally for this study; however, it has been validated in general for numerous other biochemical systems (3). The error associated with the third assumption is also minimal since incorporation into biomass can be corrected based on the percent recovery of added glucose as catabolic end products. In these studies, recovery of catabolic products after 1 h of incubation is 73%, and after 34 h of incubation it is 88%. Incorporation of added glucose into biomass is thus relatively small. For the rate constant calculations given here, incorporation is assumed to approximate 20%.

The observed differences between $[U-^{14}C]$glucose uptake and $^{14}C$-end product formation are most likely due to differences in specific activities of extracellular and intracellular glucose pools. The slower formation of $^{14}C$-end products can be readily explained by the transport of $[U-^{14}C]$glucose from extracellular pools to a relatively high specific activity to intracellular pools of a lower specific activity. This explanation is supported by the observation that the input to and output of glucose mass from fermenting bacteria appears near equilibrium as indicated by the low incorporation of $[U-^{14}C]$glucose into biomass. If the slower rates of $^{14}C$-end product formation were in fact an indication of only a small mass flow through catabolic pathways, mass balance constraints would necessitate a higher rate of incorporation into biomass.

The disparity between $[U-^{14}C]$glucose uptake and $^{14}C$-end product formation illustrates an aspect of kinetic experiments that is often ignored in ecological studies. If a reaction sequence contains one or more intermediates, mass flow through the sequence cannot be estimated by simply determining the specific activity of the initial reactant(s) and then measuring accumulation of label in the final product(s). Instead, the specific activity of the immediate precursor(s) of the final product(s) must be determined. Mass flow cannot be calculated without specifying precursor-product relationships in this manner since no corrections can be made for isotopic dilution by the pools of reaction intermediates. For example, total $CO_2$ pro-
duction from glucose in Wintergreen Lake surface sediments cannot be determined from rates of $^{14}$CO$_2$ production since the specific activity of intracellular glucose or any of the immediate precursors of CO$_2$ from glucose is not known. The use of $^{14}$CO$_2$ production rates to calculate total CO$_2$ production from glucose results in estimates $\pm 4\%$ of that expected from the observed glucose uptake (as moles of carbon) assuming fermentation of glucose to acetate and CO$_2$. Such low CO$_2$ production rates are clearly unreasonable in view of the low incorporation of glucose into biomass in Wintergreen Lake surface sediments.

The data presented here are consistent with data for adenine uptake and incorporation into RNA in water column samples (14). Karl et al. (14) state that RNA synthesis cannot be measured by adding [3H]adenine to exogenous pools and then determining the activity of 3H in RNA because it is ATP and not adenine that is incorporated into RNA. They (14) point out that the specific activity of ATP must be measured to calculate rates of RNA synthesis. The similarity between patterns of labeled glucose distribution in Wintergreen Lake sediments and labeled adenine uptake for incorporation in water column samples emphasize the general need for specifying precursor-product relationships in ecological studies of kinetics. Precursor-product relationships become especially important when examining reactions involving multiple intermediates and branched pathways.

An interesting consequence of unknown or unspecified precursor-product relationships concerns the calculation of percent mineralization or percent respiration indices. These indices are typically calculated from the fraction of the added label recovered as CO$_2$. In the case of glucose metabolism in sediments, percent mineralization is usually $<$20% for incubation periods up to 1 to 2 h (4, 9, 17, 23, 25), although Novitsky and Kepkay (20) have reported somewhat higher values for a 1:100 dilution of sediment in seawater. The low values may be accurate but they may also be the result of isotopic dilution as described above. The extent of mineralization calculated from $^{14}$CO$_2$ production in this study ranges from 18% at 10 min to 29% at 60 min and finally 67% at the termination of the experiment (34 h). The changes within the first hour of incubation are probably due to isotopic dilution, whereas the increase after 1 h is due to mineralization of fermentation end products. Clearly, the use of any one time point $<$1 h to calculate mineralization could provide rather misleading results. Since percent mineralization is usually reported for only one short-term time point, it is difficult to compare results from Wintergreen Lake sediments with other studies.

It is apparent, however, that previous reports of percent mineralization of glucose in sediments should be reevaluated with respect to effects of isotopic dilution.

Although mineralization indices can be calculated for sediment systems given appropriate incubation periods or corrections for isotopic dilution, such indices may not be a useful indicator of catabolism. Since anaerobic processes often play a significant role in the metabolism of lake sediments, the formation of fermentation end products must be measured to assess the extent of catabolism. In Wintergreen Lake sediments, where fermentation dominates anaerobic carbon flow (15, 16, 19), the production of organic acids represents a major fate for glucose carbon (Fig. 4). The catabolism of $^{14}$C-VFA to $^{14}$CO$_2$ and $^{14}$CH$_4$ in these sediments must be included with the initial formation of $^{14}$CO$_2$ and [U-$^{14}$C]glucose fermentation to calculate a realistic estimate of glucose mineralization. With the exception of those by Christian and Wiebe (4) and Novitsky and Kepkay (20), most reports of glucose mineralization in sediments have assumed that fermentation end products are unimportant (9, 17, 23). This assumption is tenuous since it is known that reduced microenvironments can exist in otherwise oxidizing sediments (13). Without actually measuring radiolabeled fermentation end products, one cannot categorically dismiss their significance or draw conclusions such as those of Toerien and Cavari (23) regarding the effects of oxygen availability.

In the experiments reported here for profundal surface sediments, $^{14}$C-VFA account for a major fraction of the end products of glucose catabolism after 1 h of incubation. The total activity of $^{14}$C-VFA at 1 h represents primarily the fermentation of glucose. Catabolism of fermentation end products is thought to be minimal at this time since only trace amounts of $^{14}$CH$_4$ are observed (Fig. 3). The appearance of $^{14}$CH$_4$ is an indicator of the point at which [$^{14}$C]acetate is beginning to be fermented and is also assumed to indicate the onset of the acetogenic fermentation of $^{14}$C-VFA other than acetate. The distribution of $^{14}$C among the various fermentation end products at 1 h (Table 2) suggests that glucose fermentation in undisturbed sediments was dominated by acetate and CO$_2$ production, in agreement with Lovely and Klug (16). Differences in the relative importance of lactate and propionate production from glucose (this study) and from the bulk organic matter catabolized in the sediment (16) are most likely due to differences in the fermentation patterns of carbohydrates, fatty acids, and proteins. Higher percentages of lactate and propionate from glucose fermentation are not unexpected since lactate and propionate are only minor products of fatty
acid and protein fermentations and since glucose contributes only a portion of total carbon flow.

Patterns of 14C-VFA production in the presence of 100% H2 differed from patterns found with 100% N2 (Table 2). In general, acetate decreased in importance and lactate increased. The changes in VFA patterns were undoubtedly responsible for the decreased production of 14CO2 in the presence of H2 (Fig. 3). Decreased 14CO2 production was probably not due to any effects of H2 on [U-14C]glucose uptake since the uptake of added label was similar in both H2 and N2 treatments. The phenomenon of isotopic dilution as discussed earlier was also evident in samples incubated with 100% H2; incorporation of glucose into biomass was relatively low as indicated by the recovery of 81% of the added 14C as 14CO2, 14CH4, and 14C-VFA at the termination of the experiment, and rates of CO2 production based on 14CO2 were much less than rates of glucose uptake.

The distribution of 14C-VFA in the presence of 100% H2 is consistent with previous studies of the effect of H2 on fermentation pathways (5, 24). High partial pressures of H2 are known to inhibit regeneration of NADH by hydrogenase and thus increase formation of reduced end products from pyruvate. It is interesting to note that, in spite of altering fermentation pathways (and presumably ATP yields), 100% H2 had no negative effect on [U-14C]glucose uptake in profundal surface sediments. The lack of any effect suggests that rates of glucose uptake may be independent of fermentation pathways.

The concentrations of dissolved glucose measured in Wintergreen Lake sediments, 96 to 858 nmol/liter, are within ranges observed by others (17, 25). The higher concentrations measured in littoral sediments are probably due to the influence of rooted vegetation. In addition, glucose concentrations in littoral sediments may have been elevated somewhat during the sampling period as a result of the die-off of a large bloom of a green filamentous algae (Rhizoclonium spp.) which settled onto the sediment surface before sampling.

Rates of glucose uptake in the sediment parallel glucose concentrations. The highest rates of uptake are found in the littoral zone, and rates decrease with depth over the 0- to 18-cm interval in both littoral and profundal zones. The decreasing rates of glucose uptake with increasing depth in the sediment are qualitatively similar to the patterns observed for sulfate reduction (15, 22), methanogenesis, and acetate turnover (16). Reasons for the decrease in rates of glucose uptake are not entirely clear, however, since total organic matter in the sediment does not decline at nearly as rapid a rate as glucose uptake (Klug, unpublished data). Rates of uptake measured with a direct injection technique are virtually identical to rates measured with sediments mixed in test tubes (Fig. 6). The agreement between techniques indicates that the uptake rates are probably reasonably accurate estimates. Others (9, 17) have observed, though, that significant changes in glucose turnover time or mass flux occur as a result of physical disturbance. Results from these studies may differ from the findings of this study since other studies involved significant dilution of sediment whereas Wintergreen Lake sediments were diluted slightly.

Rates of glucose uptake have been used to estimate the significance of glucose to methanogenesis in profundal sediments. In Wintergreen Lake, carbon flow is primarily through fermentation and methanogenesis (16, 19, 22); mass balance budgets indicate that ~40% of the organic input to the sediment is recovered as methane and 60% is recovered as CO2 (18). Carbon flow through glucose was determined by multiplying rates of uptake by the percent recovery of [U-14C]glucose, 14CO2, 14CH4, and 14C-VFA in the studies described earlier. The mean recovery from these studies is 80%. Total glucose uptake integrated from the depth profiles (Fig. 5) over 0 to 18 cm for the profundal sediments is calculated as 92.9 mmol of glucose-C/m2 per day (Table 2). Net carbon flow through glucose is, therefore, 92.9 × 0.8 = 74.3 mmol of glucose-C/m2 per day after correction for catabolism. Rates of methane production from glucose catabolism are calculated by assuming that all fermentation end products are converted to acetate and that acetate is converted to 60% CO2 and 40% methane (16). Estimated rates of methane production from glucose catabolism are 32.3 mmol of C/m2 per day. Total methane production for the time period in which glucose uptake was measured is calculated from rates of dissolved and gaseous methane flux (18). Of this total, 89.3 mmol of C/m2 per day, glucose catabolism accounts for approximately 36% (19). Based on the errors in both the estimate of glucose uptake rates and methane production (<20%), the fraction of total methane production accounted for by glucose catabolism ranges between about 30 and 40%. A similar fraction of total methane production from glucose catabolism is calculated from direct estimates of methane production in the 0- to 2-cm interval of the profundal sediments (16) and glucose uptake rates for the same interval (this study).

The fraction of carbon metabolism accounted for by glucose in this study cannot be compared with other systems since similar data are not readily available. It is also difficult to evaluate the significance of the observations for Wintergreen Lake sediments since carbohydrates ac-
counted for only 12% of the organic inputs to the sediment (18). That glucose should contribute more than 12% of total carbon metabolism seems to require that proteins, lipids, nucleic acids, etc., are degraded to a lesser extent than carbohydrates. Alternatively, glucose may be dissimilated to a greater extent than other forms of organic matter. A more detailed study of organic matter metabolism will be needed to test the observations made here.

In summary, very rapid turnover times ($T_r = 1$ min) for dissolved glucose in littoral and profundal surface sediments of Wintergreen Lake have been measured with [6-3H]glucose. Uptake of [6-3H]glucose provides a rapid, reliable method for determining $T_r$ without altering natural substrate concentrations. Time course analyses of [U-14C]glucose uptake and 14C-end product formation indicate that isotopic dilution of added label by intracellular pools of glucose and glucose metabolites can result in significant errors in interpretation of the extent of glucose metabolism or calculation of mass flow from rates of labeled product formation. Rates of mass flow are best calculated from uptake data from tracer studies and product formation data from long-term incubations. Studies with Wintergreen Lake sediments also indicate that the formation of VFA must be considered to accurately assess glucose catabolism. In Wintergreen Lake profundal sediments, acetate is the major VFA formed, with propionate and lactate accounting for ~21 and 12% of VFA production, respectively. Addition of H2 to sediments alters patterns of VFA formation but does not appear to affect uptake of added glucose. Concentrations of glucose in sediments and rates of glucose uptake before detratification of the water column are higher in littoral than in profundal sediments and decrease with depth at both sites. In the profundal zone, rates of glucose catabolism are estimated to account for 36% of total methane formation.

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LITERATURE CITED


