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Effect of Temperature on Heterotrophic Glucose Uptake, Mineralization, and Turnover Rates in Lake Sediments

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The V_{max} and turnover rates (TR) of [U-^{14}C]glucose uptake and mineralization of Lake Kinneret (Israel) sediment are temperature dependent. The following activation energies were determined: glucose uptake, ~15,000 cal (62,760 J); TR of glucose uptake, ~10,000 cal (41,840 J); glucose mineralization, 7,500 to 15,000 cal (31,380 to 62,760 J); and TR of glucose mineralization, ~15,000 cal. Q_{10} values varied as follows: glucose uptake, ~2.3; TR of glucose uptake, ~1.8; and glucose mineralization, ~2.5. $K + S_n$ values increased slightly with temperature and might reflect an increased K with increased temperatures. Glucose respiration/uptake ratios were low (9.5 to 12%) and were apparently not greatly influenced by the presence or absence of oxygen or by different assay temperatures. Aerobic or anaerobic sediments assayed under either aerobic or anaerobic conditions did not exhibit greatly different V_{max}, TR, or $K + S_n$ values.

The surface sediment of aquatic ecosystems is the site of intense microbial activity in many natural water bodies (7, 9). Some attempts have been made to determine the microbial activity of sediments (4, 6, 7, 9), but little information is available on the effect of temperature on sediment activities. An important influence of temperature on glucose uptake in marine waters was shown by Takahashi and Ichimura (12), and Wright (17) stressed the need to control the temperatures under which activity measurements are made. Delineation of temperature effects on sediment microbial activities would enhance the understanding of processes taking place in sediments of lakes where sediment temperatures vary throughout the year.

The kinetics of uptake of radioactively labeled organic compounds have been used extensively as a measure of microbial activity (17, 18). The use of the modified Lineweaver-Burk equation, i.e., $t/f = (K + S_n)/V_{max} + A/V_{max}$, where t is the incubation time (in hours), f is the fraction of available substrate used, K is a constant related to uptake, V_{max} is the maximum uptake rate, S_n is the natural substrate concentration (unknown), and A is the concentration of added substrate (see also reference 7), gives three important parameters, i.e., V_{max}, turnover time (T_t in hours), and in situ substrate concentration ($K + S_n$), assuming that $K \ll S_n$ (18). The inverse of turnover time has been called relative uptake rate (2), respiration rate when only $^{14}CO_2$ was measured (15), or turnover rate (TR) (10), which according to Wright (17) is less confusing and preferable. In determination of total uptake (8), the measurement of CO_2 released during incubation provides a measure of mineralization (7).

Using sediments from Lake Kinneret, Israel, we evaluated the effect of temperature on the V_{max}, $K + S_n$, and TR of glucose uptake and mineralization. Because we dealt with an anaerobic sediment, we first investigated the need to use strictly anaerobic conditions during the performance of assays.

MATERIALS AND METHODS

Sediment cores and pretreatment. The main features which characterize the sediments of Lake Kinneret are a high percentage of fine-grain material, an abundance of calcium carbonate, and a dominance of montmorillonite in the clay fraction (11). The in situ sediment temperature is in the range of 14 to 16°C.

Sediment cores were obtained with a Jenkin sampler, which takes a core of about 15 cm of sediment and 30 cm of overlying water, and the entire core was then transported to the laboratory. All assay procedures were started not more than 1 h after sampling. Aerobic sediment was collected from a littoral station (10-m depth), and anaerobic sediment was collected from a deep station (24-m depth). Generally, overlying water was carefully siphoned off, but in the experiments in which the need for applying anaerobic techniques was evaluated, about 4 cm of overlying water was left above the sediment; this water contained sulfide. Sediment (2 g, wet weight) was transferred by pipette to 1 liter of distilled water in a 2-liter Erlenmeyer flask made aerobic or anaerobic by flushing for about 30 min with air or nitrogen, respectively, before the addition of the sediment sample, and the sediment was thoroughly suspended by stirring with a magnetic stirrer. Dry weight was determined by drying samples in an oven for 24 h at 70°C.

In the aerobic-anaerobic experiments, each sediment was first treated anaerobically and then aerobically. Anaerobic and aerobic conditions were established in the sediment suspension by vigorous mixing for 20 min with magnetic stirrers, with nitrogen gas flushing for anaerobic conditions and with aeration for aerobic conditions. Winkler determinations indicated the absence of dissolved oxygen in the water overlying the sediment core and in the samples treated anaerobically. In the temperature experiments the suspension was divided into four subsamples, and these were equilibrated to the respective temperatures for 30 min.

Glucose uptake and mineralization measurements. Glucose uptake and respiration experiments were done in duplicate; generally, no more than a 20% difference was found between the flasks. Glucose uptake and respiration were determined by the kinetic method of Hobbie and Crawford (8). [^{14}C]glucose (0.1 μCi; 287 μCi mmol^{-1}) and unlabeled glucose (0 to 100 μg liter^{-1}) were added to 10 ml of sediment suspension in a 100-ml sterilized Erlenmeyer flask. The flasks were sealed with a serum stopper, through which a plastic cup containing a precombusted (450°C) folded glass fiber filter had been inserted. After incubation of the closed system in the dark at room temperature (22 ± 1°C) for 30 min, the reaction was stopped by injection of 0.5 ml of 6% trichloroacetic acid–0.05% HgCl in 40% Formalin, and 0.2 ml of Hyamine was injected on the filter wick to absorb $^{14}CO_2$. After a further incubation of 1 h with shaking, to allow for entrapment of $^{14}CO_2$, the wicks were removed and placed in liquid scintillation vials. The sediment suspension samples were filtered onto 0.45-μm membrane filters, washed with 10 ml of water, and also placed in scintillation vials. Five milliliters of scintillation fluid was added to both sets of vials, and radioactivity was counted in a Packard Tri-Carb scintillation spectrometer. For each duplicate flask, a poisoned control was run in which the 0.5 ml of 5% trichloroacetic acid–0.05% HgCl in 40% Formalin was added before the addition of the sediment suspension.

The data were corrected for the controls, and the *t/f* values were calculated and plotted against added substrate concentrations. Preliminary experiments have demonstrated linearity of the glucose uptake and respiration of the glucose for at least 1 h under these conditions. The percentage of $^{14}CO_2$ released during incubation was not influenced by the increased level of unlabeled glucose added. Anaerobic assays were carried out as described above except that after the addition of labeled and unlabeled glucose, a serum stopper was fitted to the flask and the flasks were flushed with a slow flow of nitrogen gas for 10 min. The anaerobic suspension (10 ml) was drawn into a 10-ml syringe, injected into a flask through the serum stopper, and then treated as described above.

Standard Arrhenius plots of the natural logarithm of the V_{max} or TR against the inverse of temperature (in degrees Kelvin) allowed the calculation of activation energies and Q_{10} values.

RESULTS

Comparison of aerobic and anaerobic conditions in the assay. An experiment in which aerobic and anaerobic conditions in the assay of glucose uptake and mineralization rate were compared is summarized in Table 1. The results show that the V_{max} of glucose uptake was similar when aerobic or anaerobic conditions were used in the assay procedure.

The V_{max} values of CO_2 evolution (glucose mineralization) ranged from 2.75 to 4.77 μg of CO_2 g^{-1} h^{-1} (Table 2). No consistent pattern was observed in glucose mineralization for the aerobic or anaerobic sediments when assayed aerobically or anaerobically. The variation for the same sediment sampled at different times was as great as or greater than the variation between anaerobic and aerobic assays. Good fits of data to the modified Michaelis-Menten equation were observed (r ranging for 0.98 to 1.00; n = 5). Therefore, we concluded that the CO_2 evolution kinetics of aerobic assays could be used to investigate the effect of temperature on glucose mineralization.

Influence of temperature on V_{max}, $K + S_n$, T_t, and TR. Sediment from a deep station (24-m depth) was used for the temperature experiments, and the temperature range used was 10 to 36°C. Temperatures were checked at the beginning and the end of all experiments, and deviations were within 1°. All temperature experiments were run under aerobic conditions.

TABLE 1. Kinetic parameters of glucose uptake by aerobic and anaerobic sediments under aerobic and anaerobic assay conditions[a]

Sediment	Assay	V_{max}[b] (μg g^{-1} h^{-1})	$K + S_n$[b] (μg g^{-1})	T_t (h)	TR[c] (h^{-1})	r[d] (n = 5)
Anaerobic	Aerobic	14.5	20.5	1.4	0.71	0.99
	Anaerobic	13.8	48.7	3.5	0.29	0.96
Aerobic	Aerobic	32.7	66.8	2.0	0.50	1.00
	Anaerobic	34.6	108.0	3.1	0.32	1.00

[a] Incubation temperature, 25°C.
[b] Glucose per gram of dry sediment.
[c] TR = $1/T_t$.
[d] Correlation coefficient for linearity of V_{max}, $K + S_n$, and T_t calculations. Mean values for each glucose concentration were used in calculations, and coefficients are presented to the nearest two decimal places.

TABLE 2. Kinetic parameters of mineralization of aerobic and anaerobic sediments under aerobic and anaerobic assay conditions[a]

Sediment	Assay	V_{max}[b] ($\mu g\ g^{-1}\ h^{-1}$)	$K + S_n$[b] ($\mu g\ g^{-1}$)	T_t (h)	TR[c] (h^{-1})	r[d] ($n = 5$)
Anaerobic	Aerobic	3.59	242.4	66.6	150	0.98
	Anaerobic	2.75	130.9	46.9	213	0.98
Aerobic	Aerobic	3.70	188.8	52.2	192	1.00
	Anaerobic	4.77	259.6	54.3	184	0.99

[a] Incubation temperature, 25°C.
[b] CO_2 per gram of dry sediment.
[c] $TR = 1/T_t$.
[d] Correlation coefficient for linearity of V_{max}, $K + S_n$, and T_t calculations. Mean values for each substrate concentration were used in calculations, and coefficients are presented to the nearest two decimal places.

As shown in Table 3, which represents two experiments carried out with two different sediment samples, the V_{max} of glucose uptake greatly increased and the turnover time (T_t) decreased correspondingly with increasing temperatures. Slighter increases with increasing temperatures were obtained for the $K + S_n$ values (from 10.2 to 16.4 and from 8.2 to 23.2 μg $liter^{-1}$ in experiments 1 and 2, respectively [Table 3]).

The same pattern as for glucose uptake was observed for glucose mineralization, i.e., increased V_{max} values and decreased T_t values with increased temperatures (Table 4). In contrast to the glucose uptake results, the $K + S_n$ values for glucose mineralization did not show any consistent trend with changes in temperature (Table 4), but covered a fairly narrow range of concentrations (33.0 to 84.7 and 14.7 to 23.1 μg of CO_2 $liter^{-1}$ in experiments 1 and 2, respectively).

The V_{max} and TR for glucose uptake and mineralization were dependent upon temperature. The Arrhenius equations indicated activation energies of approximately 15,000 cal (62,760 J) for glucose uptake and 10,000 cal (41,840 J) for the TRs of glucose uptake and mineralization (Table 5). The activation energies determined for glucose mineralization in the two experiments varied in the range of 7,570 to 15,255 cal (31,673 to 63,827 J; Table 5).

Q_{10} values for the ranges of 10 to 20°C and 20 to 30°C of the different parameters varied around a value of 2 (Table 5).

DISCUSSION

Temperature affects glucose uptake by lake sediments in a manner similar to that of marine waters (12) and cultured marine bacteria (5). However, the activation energy for glucose uptake in the lake sediments (ca. 15,000 cal) was lower than that of surface and mixed-layer ma-

TABLE 3. Summary of the glucose uptake kinetics of the temperature experiments

Expt	Temp (°C)	V_{max} (μg of glucose $liter^{-1}\ h^{-1}$)	$K + S_n$ (μg of glucose $liter^{-1}$)	T_t (h)	TR (h^{-1})	r ($n = 5$)
1	10	1.41	10.2	7.2	0.14	0.995
	19	5.09	12.0	2.4	0.42	0.998
	23.5	6.79	14.4	2.1	0.48	0.994
	35.5	12.18	16.4	1.4	0.71	0.997
2	10	0.84	8.2	9.7	0.10	0.983
	17.5	3.3	14.5	4.4	0.23	0.999
	26.0	7.1	22.1	3.1	0.32	0.999
	36.0	10.2	23.2	2.3	0.43	0.993

TABLE 4. Summary of the glucose mineralization kinetics of the temperature experiments

Expt	Temp (°C)	V_{max} (μg of CO_2 $liter^{-1}\ h^{-1}$)	$K + S_n$ (μg of CO_2 $liter^{-1}$)	T_t (h)	TR (h^{-1})	r ($n = 5$)
1	10	0.40	84.7	209.0	4,800	0.93
	19	0.55	43.3	81.2	12,300	0.96
	23.5	0.99	60.1	60.7	16,500	0.98
	35.5	1.17	33.0	28.6	35,000	0.99
2	10	0.18	23.1	117.1	8,500	0.95
	17.5	0.51	22.7	43.6	22,900	0.96
	26.0	1.32	14.7	11.1	90,100	0.99
	36.0	1.87	17.2	9.2	108,700	0.99

TABLE 5. Arrhenius equations, activation energies, and Q_{10} values for glucose uptake, mineralization, and turnover in lake sediments

Expt	Arrhenius equation	r (n = 4)	Activation energy (cal)[a]	Q_{10}	
				10–20°C	20–30°C
Glucose uptake					
1	V_{max} (μg of glucose g^{-1} h^{-1}) = 9×10^{11} $e^{-7,230/T}$	0.96	14,365 (60,103)	2.39	2.27
2	V_{max} (μg of glucose g^{-1} h^{-1}) = 1.7×10^{13} $e^{-8,204/T}$	0.95	16,301 (68,203)	2.69	2.52
1	TR (h^{-1}) = 2.08×10^{8} $e^{-5,522/T}$	0.94	10,972 (45,907)	1.94	1.86
2	TR (h^{-1}) = 9.09×10^{6} $e^{-4,758/T}$	0.97	9,454 (39,556)	1.78	1.71
Mineralization					
1	V_{max} (μg of C g^{-1} h^{-1}) = 3.17×10^{5} $e^{-3,810/T}$	0.83	7,570 (31,673)	1.54	1.58
2	V_{max} (μg of C g^{-1} h^{-1}) = 1.46×10^{11} $e^{-7,678/T}$	0.97	15,255 (63,827)	2.52	2.37
1	TR (h^{-1}) = 4.34×10^{7} $e^{-6,677/T}$	0.99	13,266 (55,505)	2.24	2.12
2	TR (h^{-1}) = 6.99×10^{11} $e^{-9,019/T}$	0.96	17,920 (74,977)	2.97	2.76

[a] Numbers given within parentheses are joules.

rine waters (22,000 cal [92,048 J]) (12) and of cultured marine bacteria (26,800 and 34,200 cal [112,131 and 143,093 J]) (5) but higher than that of deep stable marine waters (12). The suggestion of Wright (17) that the control of temperature during glucose uptake measurements is important and that the temperature at which activity measurements are made should be stated is supported by our data. Since the Q_{10} values for glucose uptake were approximately 2.3 (Table 5), a temperature differential of even 2 to 3°C during an assay can result in rate changes of up to 20 to 30%, additional to the variation inherent at a constant temperature.

TR is equal to $V_{max} \cdot (K + S_n)$. If $K + S_n$ is a constant, as predicted by theory, it follows that TR would be a function of temperature if V_{max} is a function of temperature. This was indeed the case (Table 5) for both glucose and mineralization.

In our experiments we noticed a small increase in the $K + S_n$ value with increasing temperatures (Table 3). We would expect to find a constant S_n value since the sediment suspensions used for the temperature experiments are subsamples of a single original sediment sample. The increase in the $K + S_n$ values must therefore represent increases in the affinity constant (K) with increasing temperatures. Similar results were obtained by Witzel, using water samples from the Saar River (16). Witzel (16) suggested that this decrease in the K values at low temperatures may indicate a successive population with higher affinity for glucose at low temperatures, but in our experiments this could not be the case because there was no time for the development of a successive population.

Harrison et al. (7) suggested that mineralization measurements can be helpful in understanding the activities and roles of sediment microbial communities. Our results indicated that glucose mineralization and TRs are a function of temperature (Tables 4 and 5) and that temperature must be an important environmental factor for the activities of sediment microbial populations. The activation energies for mineralization and turnover appear to be similar to that of glucose uptake. Our results also suggest that it would be feasible to use CO_2 evolution kinetics to obtain estimates of $K + S_n$ for glucose in sediments.

A number of researchers (9; L. W. Wood, Ph.D. thesis, North Carolina State University,

TABLE 6. Ratios of glucose mineralization and uptake by lake sediments under different environmental conditions

Sediment state	Temp (°C)	Assay	Mineralization/uptake (%)[a]	
			Avg[b]	SD
Aerobic	25	Anaerobic	10.64	3.23
Aerobic	25	Anaerobic	7.48	1.54
Aerobic	25	Aerobic	9.18	3.55
Aerobic	25	Aerobic	5.93	0.86
Anaerobic	25	Anaerobic	13.98	3.72
Anaerobic	25	Anaerobic	10.56	3.58
Anaerobic	25	Aerobic	7.91	1.59
Anaerobic	25	Aerobic	10.69	3.42
Anaerobic	10	Aerobic	3.83	0.77
Anaerobic	19	Aerobic	6.30	1.03
Anaerobic	23.5	Aerobic	5.75	1.01
Anaerobic	35.5	Aerobic	5.83	0.67
Anaerobic	10	Aerobic	9.91	3.22
Anaerobic	17.5	Aerobic	11.18	1.86
Anaerobic	26	Aerobic	16.34	2.47
Anaerobic	36	Aerobic	15.98	3.10

[a] Respiration (counts per minute)/total uptake (uptake and respiration [counts per minute]) × 100.

[b] Overall average, 9.47; standard deviation, 3.70.

Raleigh, 1970) have reported that the respiration of sediment microorganisms relative to glucose uptake (2 to 10%) is considerably lower than that of planktonic bacteria, which can respire up to one third of their total glucose uptake (8, 14). Our results (Table 6) support this finding and also indicate that there are no consistent changes in the respiration/uptake ratios under different environmental assay conditions such as the presence or absence of oxygen or different temperatures. This finding is in contrast to the findings of Tison and Pope (13), who reported increased CO_2 evolution with increased temperatures.

Our results further suggest that the ratio of respiration to uptake varies considerably with the time and place of sampling of sediments, as was also found by Wood (Ph.D. thesis). Wood suggested that such low respiration of sediment rates could be due to the release of fermentation products or the use of substances other than glucose or both. Since we obtained similar results in assays done under aerobic or anaerobic conditions, it seems unlikely that the release of fermentation products could be the reason for the low respiration values. The mineralization rates of a microbial community are dependent upon the metabolic capacity of that community for a given substrate. It is possible that the benthic microbial community differs from the planktonic community, and this difference is reflected in the lower values of mineralization in the sediment.

The fact that the V_{max} values for glucose uptake and mineralization were not apparently greatly affected by the presence or absence of oxygen was surprising. Since this was the fact for both aerobic and anaerobic sediments, it suggests that the majority of microbes which are active in metabolism of glucose in sediments are facultive anaerobic and can switch easily from aerobic to anaerobic metabolism. The obligate aerobic and anaerobic bacteria are probably killed or inhibited by transferring from oxic to anoxic conditions or vice versa.

Our assay procedure used disturbed sediments and thus differed from the methods used by Fleischer (3), Andersson et al. (1), and Meyer-Reil (9). However, for the purpose of investigating temperature effects, it is imperative that identical samples be used at all temperatures, a condition that can only be achieved by disturbing the sediment cores. Nevertheless, although the physical disturbances of this layer during pretreatment and assays could alter the quantitative results, the temperature response itself was probably not changed.

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