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Temperature Compensation of [U-^{14}C]Glucose Incorporation by Microbial Communities in a River with a Fluctuating Thermal Regime

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In summer, the river Saar in the southwest of Germany exhibits distinct temperature fluctuations from 8°C at the source to nearly 30°C in the middle region. Temperature optima for bacterial plate counts and the uptake velocity of [U-^{14}C]glucose by the natural microbial communities of different regions ranged from 20 to 30°C, which is significantly above the mean annual water temperature. A correlation between temperature optima and different seasons or habitats was not observed. Despite the relatively high temperature optima, the turnover time for glucose was shortest at temperatures around the mean annual water temperature, due to changes in the substrate affinity. At limiting substrate concentrations, the higher substrate affinity at lower temperatures may lead to a higher real activity at in situ temperatures, and a compensatory stabilization of uptake rates at fluctuating temperatures is possible.

In the temperate areas of the world, most microbial transformations in freshwater habitats take place at temperatures between 0 and 20°C. Temperatures exceeding 20°C are found only during short periods of the annual cycle. This temperature range should favor the selection of psychrotrophic, or, at stable low-temperature conditions, even of psychrophilic microorganisms.

The existence of psychrophilic and psychrotrophic microorganisms in permanently cold habitats of polar regions (14) and oceans (7, 20) has been well documented (2, 13, 15). The predominance of thermophilic microorganisms in extremely hot aquatic environments has also been demonstrated (24). In addition, much information is available on the temperature responses of many isolates, psychrophilic as well as mesophilic or thermophilic ones. Upper and lower temperature limits for the existence of microbial life (2, 18, 24) as well as some of the factors responsible for adaptation to both extremes of the temperature range (1, 13, 18) have been established in many reports. Changes within the composition (16, 19, 20) and activity (3, 26) of natural microbial communities due to temperature variations are well known, and many authors have pointed out that besides temperature, the concentration of available substrate is one important factor regulating the activity and distribution of microorganisms (2, 22). The mechanisms for temperature adaptation and competition between microorganisms at moderate temperatures, however, are widely unknown.

In an elegant study using continuous-culture experiments with an obligate and a facultative psychrophile, Harder and Veldkamp (8) demonstrated that successful competition between their strains at different temperatures depended on the substrate concentration. However, besides these initial and convincing experiments, no further studies on the interaction between temperature and substrate concentration in the regulation of microbial activity and distribution within natural environments have been reported.

In the course of extensive studies on the distribution and activity of heterotrophic microbial populations in a river with different thermal regimes, the question arose how these bacterial communities might effectively function at environmental temperatures which at times could be as much as 10 to 20°C below their optimum for growth. A comparison of the kinetic parameters of ^{14}C-labeled glucose uptake (maximum uptake velocity, V_m; saturation constant, K_s; turnover time, T_s) by natural microbial communities at different temperatures should help to solve this problem.

MATERIALS AND METHODS

Sampling. At different times throughout the year, water samples were taken with sterile glass bottles from about 20 cm depth of the river Saar at different sites, and from the outflow of a power station's cooling tower. A map of the sampling area is shown in Fig. 1.

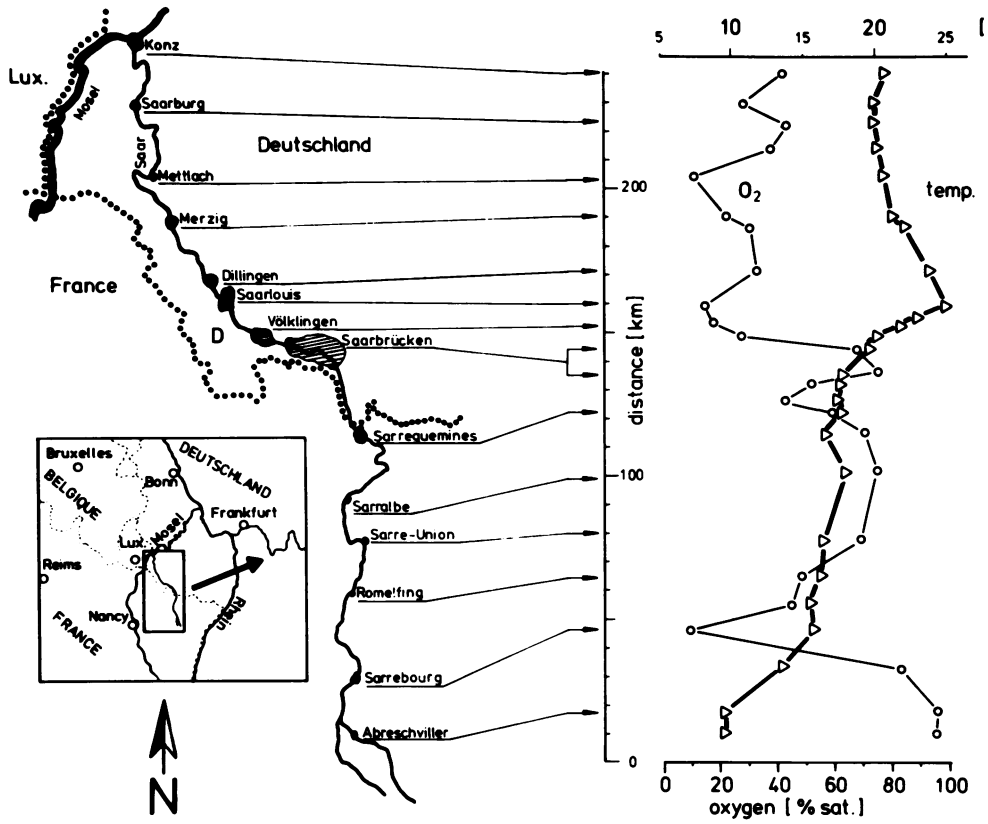

FIG. 1. *Map of the sampling area in the southwest of Germany, together with a typical summer profile of oxygen and temperature measurements.*

At each time and site, one sample of about 250 ml was taken, and this was then subdivided for further analyses in the laboratory. After sampling, the bottles were kept in a polystyrol bag to keep their temperature stable within 2°C during transport to the laboratory; temperature was controlled by measurements in the field and in the laboratory.

Plate counts. The samples were appropriately diluted with a sterile 0.9% (wt/vol) NaCl solution (temperature about the same as in the sample). Three subsequent dilutions were inoculated onto five replicate, precooled plates with nutrient agar (Difco) by the spread-plate technique. The plates were then incubated aerobically at different temperatures as indicated, and the colonies were counted after 7, 14, and 21 days. The two longer incubation periods were necessary at low temperatures for the slow-growing psychrotrophic organisms to form visible colonies. Counts were determined by selecting the set of five parallel plates that contained an average of 50 to 200 colonies per plate. The highest counts after any of the three incubation periods were then used.

Uptake experiments. Triplicate 10-ml amounts of a water sample were incubated in a shaken water bath at different temperatures as indicated. Water temperatures were maintained at ±0.1°C. They ranged from 5 to 40°C with 5°C intervals in the kinetic approaches, and from 4 to 45°C with 2 and 3°C intervals in the other experiments. Each sample was acclimatized at the experimental temperature for 15 min. Then, the experiment was started by the addition of different amounts of a glucose solution which contained 0.5 μCi of [*U*-^{14}C]glucose per ml (specific activity, 2.6 mCi/mmol). Final glucose concentrations covered the range from substrate limitation to saturation, corresponding from 0.02 to 1.9 μmol/liter. In those experiments where only one substrate concentration was applied, glucose was added to a final concentration of 1.9 μmol/liter. Preliminary experiments had shown that this concentration saturated the uptake systems at any temperature and did not cause substrate inhibition, so that the resulting uptake velocity approximated the maximum uptake velocity. The experiments were stopped after 30 min (some samples with low activity were incubated for 1 h) by direct filtration on membrane filters (Schleicher & Schüll BA 85/1) without prior fixation. The filters were then washed twice with distilled water. This procedure proved to be advantageous over fixation techniques because the time necessary for filtering the 10-ml sample was less than 1 min. For each substrate concentration and temperature, a blank was run in which the organisms had been killed with 0.2 ml of Lugol J/KJ solution per 10 ml, added 15 min before the substrate. The membrane filters (25-mm

diameter) were dissolved in 5 ml of Bray cocktail and counted in a scintillation counter (Isocap/300, Nuclear Chicago). Counts were corrected for the blanks and quenching and were standardized with [U-^{14}C]glucose added to the same cocktail. Net counts were used to calculate the kinetic parameters of uptake with a Telefunken TR-440 computer. The computer program, kindly provided by H. J. Krambeck, calculated maximum uptake velocity (V_m) and the sum of the saturation constant (K_s) plus the natural substrate concentration (S_n) according to the formula given by Wright and Hobbie (25). The turnover time T_s was then calculated manually using the relationship $T_s = (K_s + S_n)/V_m$.

RESULTS

Description of the sampling area. The studies described here were carried out with microbial communities from different habitats of the river Saar. This river is about 240 km long and drains an area of about 7,500 km^2 in the southwest of Germany and eastern part of France. With respect to water temperature, different characteristic zones can be distinguished (Fig. 1). At the source and just below, temperatures are permanently low and never exceed 10°C. In the subsequent part of the river, from about 20 to 120 km, water temperatures seasonally fluctuate between 0 and 20°C due to natural warming and cooling processes. In the middle region (120 to 170 km), the water is heated up by thermal loading, mainly from heavy industries and power plants. Here, water temperatures are permanently about 10°C higher than in the upper part. In summer time, they can rise up to nearly 30°C. In the lower part, temperature may fall again by a few degrees, due to natural cooling of the water.

Temperature optima for plate counts and uptake of [U-^{14}C]glucose. Changes in temperature optima of natural microbial communities corresponding to a fluctuating temperature regime have been demonstrated by Sieburth (20) and Bott (3). There are still several reports, however, that temperature optima in natural habitats are invariable and are several degrees above ambient. Because of its fluctuating temperatures, the river Saar seemed to be a good object to test this question. Plate counts on nutrient agar and heterotrophic activity with [U-^{14}C]glucose were determined at a broad range of incubation temperatures using water samples from different habitats and during different seasons.

In the river water, the temperature optima for glucose uptake were within a narrow range, from 24 to 32°C, and were obviously not influenced by different habitats or seasons (Table 1). In the outflow from a power station's cooling tower, with maximum water temperatures in the tower permanently above 30°C, the optima could be slightly higher, 28 to 32°C. Temperature optima for the uptake velocity of natural microbial communities in these water samples were never detected above or below this narrow range. This was confirmed by plate count determinations, where maximum numbers were found between 20 and 28°C (Table 2).

Thus, in the river at different habitats, the optima for uptake velocity as well as for plate counts were constantly about 10 to 15°C above the mean annual water temperature (which is in the middle region of the river around 15°C), and they even were significantly higher than the temperature maximum during the annual cycle at any station.

Influence of incubation temperature on V_m, K_s, and T_s. The microbial communities at the habitats studied seemed not to be fitted to the prevailing environmental temperature, when their uptake velocity at high substrate concentrations and the plate counts were taken as a criterion. It can be assumed, however, that the

TABLE 1. *Temperature optima of [U-^{14}C] glucose incorporation at different habitats and seasons*[a]

Sampling station	Optimum temperature (°C)			
	Spring	Summer	Autumn	Winter
Abreschviller	22 and 30[b]	24 and 28[b]	26	24
Oberstinzel	24	26	28–30[c]	28
Güdingen	20–26[c]	28	ND[d]	28
Saarbrücken	24	26	26–28[c]	22–28[c]
Völklingen	28	26–30[c]	30	ND
Saarlouis	24–32[c]	28	32	26–30[c]
Konz	28	28	30	28–30[c]

[a] Optima were taken from temperature-velocity curves between 5 and 45°C. Temperature intervals were 5°C from 5 to 15°C, 2°C from 18 to 36°C, and 3°C above 36°C. At each temperature, five replicates plus blank were incubated. Glucose added: 1.9 μmol/liter.

[b] Two distinct maxima.

[c] One broad maximum.

[d] ND, Not determined.

TABLE 2. *Plate counts (10^3/ml) on nutrient agar after incubation at different temperatures*

Sampling station	Water temp (°C)	Plate count (×10^3 per ml) at incubation temp:					
		4°C	15°C	20°C	28°C	37°C	45°C
Abreschviller	7.3	0.29	0.31	0.78	0.54	0.32	0.02
Sarreguemines	12.6	32	295	512	496	176	3.2
Saarbrücken	12.8	37	376	645	430	105	2.1
Völklingen	13.4	53	548	1130	987	273	5.7
Saarlouis	15.2	20	395	720	390	98	3.8
Konz	14.6	18.3	143	326	516	187	1.9
Outflow of a cooling tower	32.8	8.5	23.3	281	460	201	1.6

maximum uptake velocity will normally not be attained in the ecosystem itself. Under these conditions, the substrate affinity, which corresponds here to the saturation constant K_s, is a main factor regulating true uptake velocity. Therefore, it was interesting to test whether incubation temperature would influence the saturation constant K_s and the turnover time T_s, which is defined as the quotient K_s/V_m.

The results from one typical experiment with water from the river Saar at Saarbrücken (29 May 1978) are shown in Fig. 2. Here, the optimum temperature for V_m was 25°C; above and below this level, uptake velocity was limited by temperature. As the substrate affinity increased (lower K_s values) when the temperature was decreased within the range from 40 to 10°C, the uptake efficiency increased at lower temperatures. The optimum for an efficient uptake and turnover of the added substrate was at 15°C, which is about 10°C lower than that for the maximum uptake velocity. The water temperature of this sample was 13.8°C; the mean annual water temperature at that station was 14.6°C. At temperatures above 15°C the turnover time increased exponentially, so that at 25°C (highest V_m) it was by a factor of about 2 longer than at 15°C. Above 30°C, K_s values were above the range of natural substrate concentrations, and uptake in situ became ineffective. Together with the low uptake velocity, relatively long turnover times resulted. The same responses of K_s, V_m, and T_s to incubation temperature were obtained with other water samples (results not shown) from the river Saar at Saarbrücken (11 January 1977; 9 May 1978), Güdingen (13 March 1978), and Völklingen (19 April 1978). The absolute amounts of K_s, V_m, and T_s, however, could vary considerably between different samples.

The microbial community that was exposed to ambient temperatures permanently above 30°C, in the outflow of a power station's cooling tower, behaved in another way (Fig. 3). The optimum for V_m was again at 25°C, but the K_s-temperature curve displayed a maximum at

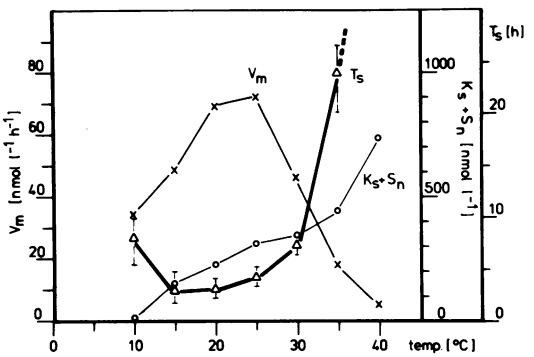

FIG. 2. *Influence of incubation temperature on the kinetic parameters of [U-^{14}C]glucose incorporation in a water sample from the river Saar at Saarbrücken (29 May 1978). Water temperature: 13.8°C; colony-forming units (28°C, 1 week): 675 × 10^3 ml^{-1}. Symbols: (×) maximum uptake velocity (V_m); (○) sum of the saturation constant (K_s) and the natural substrate concentration (S_n); (△) turnover time (T_s). Each value for K_s and V_m is calculated on the basis of three replicates plus blank at each of five different glucose concentrations; 95% confidence limits are indicated for T_s.*

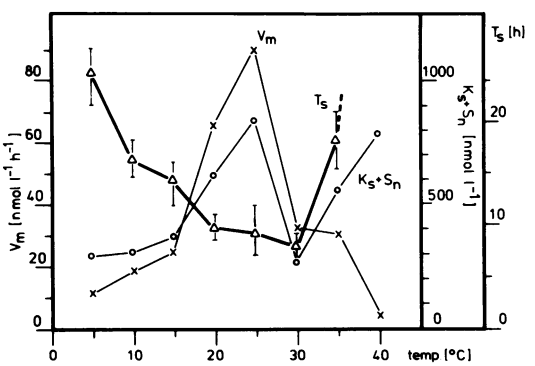

FIG. 3. *Influence of incubation temperature on the kinetic parameters of [U-^{14}C]glucose incorporation in a water sample from the outflow of a power station's cooling tower near Saarlouis (11 July 1978). Water temperature: 37.6°C; colony-forming units (28°C, 1 week): 375 × 10^3 ml^{-1}. Symbols and performance of the experiment were as in Fig. 2.*

25°C and a minimum at 30°C. Shortest turnover time, i.e., the highest uptake efficiency, resulted at 30°C, which is higher by 15°C than in the experiments with river water, but again close to ambient temperatures.

Using in vivo systems as complex as those we used here, one cannot always assume that substrate affinity is definitely measured by the apparent K_s value, because the influence of many variables cannot be reliably excluded. In the experiments described here, there is to be expected a falsification of K_s by an increased V_m at optimum temperatures. It is, therefore, important to prove directly the higher uptake velocity at low temperatures when the substrate concentration is kept low. In Fig. 4, the results of an experiment with water from the river Saar at Güdingen (13 March 1978), incubated at different substrate concentrations and temperatures, are shown. At substrate concentrations up to 90 nmol/liter above ambient, definitely more glucose was taken up at 15°C than at 20°C and above. At substrate concentrations between 90 and 275 nmol/liter above ambient, the community was in advantage at 20°C rather than at 15 or 25°C. Above 275 nmol/liter, the highest uptake velocity was gained at 25°C.

DISCUSSION

When determining the temperature responses of microbial freshwater communities, a separate investigation of the various elementary steps is necessary, because the functioning of a heterogeneous community in its ecosystem is a highly complex phenomenon. One such elementary step that can be studied under near in vivo conditions is the incorporation of ^{14}C-labeled organic substrate into cellular substances, apart from other processes involved in the transformation of organic carbon sources. Transport, respiration, or excretion of organic molecules might exhibit independent responses to temperature. Incorporation of the added substrate is comparable to growth.

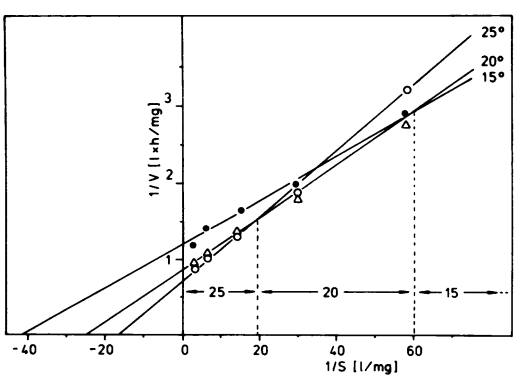

FIG. 4. *Lineweaver-Burk plot of [U-^{14}C]glucose uptake kinetics in a water sample from the river Saar at Güdingen (13 March 1978), incubated at different temperatures. Water temperature: 8.3°C; colony-forming units (28°C, 1 week): 647 × 10^3 ml^{-1}. Symbols:* (●) *incubated at 15°C;* (△) *at 20°C;* (○) *at 25°C.*

The equations that are currently used to describe the uptake kinetics of heterogeneous microbial communities in water samples (22) have been derived in principle from the kinetics of purified enzyme reactions. They have been proven by many authors to be valid in many instances for much more complex systems, like whole bacterial cells (17) or natural mixed populations in their heterogeneous environment (25). The kinetic parameters determined in the uptake experiments with natural communities are thus analogous to those of the reactions with purified enzymes; V_m is therefore a measure of the maximum uptake velocity when all transport sites are saturated, and K_s corresponds to the Michaelis constant and is equal to the substrate affinity when the dissociation of the enzyme-substrate complex is the rate-limiting step within the reaction scheme. The turnover time ($T_s = K_s/V_m$) is comparable to the reciprocal of the specificity constant (10) and is a single measure of the metabolic efficiency of the organisms when V_m is normalized to a parameter of active biomass. In those cases where the active biomass is kept constant throughout an experiment, as done here, T_s may be treated as the specificity constant, so that the problems and uncertainties about the significance of biomass measurements in aquatic environments may be circumvented.

The results presented in this paper indicate that temperature influences the activity of heterotrophic microbial communities from the river Saar in two ways. First, the reactivity of all metabolic processes will be increased by pure thermodynamic reasons, according to the Arrhenius equation. This will result in an increased rate of the biochemical reactions at unlimited substrate concentrations, as in the plate count determinations of ^{14}C-uptake rates at the high glucose concentrations applied. Second, parallel to this increased reactivity, and already at low temperatures, the higher orders of protein structure, membrane fluidity, and the weak interactions that stabilize the enzyme-substrate complexes are influenced (12). This may lead to the loss in substrate affinity (21) at higher temperatures that has been observed in the water samples. Even slight changes in enzyme conformation, especially in active side geometry, may severely perturb an enzyme's binding and catalytic abilities (12). A mathematical description

of the K_m-temperature relationships for reactions following the simple Michaelis-Menten scheme, and for some extensions of it, is given by Havsteen (9, 10). From a pure biochemical point of view, the changes in substrate affinity might be unimportant within certain limits. But, since the microorganisms in their natural environment are permanently competing for life-essential substances at suboptimum concentrations, an increase or decrease in substrate affinity may cause a distinct advantage of one over another organism. Harder and Veldkamp (8) demonstrated that a facultative psychrophile outgrew an obligate one in a continuous culture at 4 and 10°C due to a higher substrate affinity at these temperatures, and hence a higher growth rate at limiting substrate concentrations despite its lower maximum growth rate. Alterations of the substrate affinity by temperature and their role in temperature adaptation have been extensively studied with isolated enzymes from poikilotherms (21). There are still a few reports on changes of the saturating constant of whole bacterial cells growing at changing incubation temperatures (6, 11), but up to now, there is no information on how temperature influences the substrate affinity of natural microbial communities, and what consequences this might have for metabolic activity and competition in the ecosystem.

Thus, an increased substrate-binding ability at low temperatures might have an obvious survival value, as is shown in Fig. 4 by the actually higher uptake rates at low temperatures, when the substrate concentration is limiting. Moreover, it could lead to a stabilization of metabolic processes at an unstable temperature regime by compensating for the pure thermodynamic effects on metabolic rates. This may be of some importance in habitats like the river Saar, where in summertime the microbial communities may have to come through an increase in water temperature of up to 20°C when they drift from the source to the middle region of the river. The ability to compensate for thermodynamic effects of low temperatures may explain the discrepancy existing between the high temperature optima and the much lower habitat temperatures. In the river Saar at any time and habitat, the temperature optima for plate counts as well as for the maximum velocity of glucose incorporation were above the water temperature of the samples by 5 to 20°C. This is a phenomenon that has been previously observed by many others (3–5, 16, 23) and seems to be a characteristic feature of natural communities in many habitats. It seems reasonable that such organisms should have evolved mechanisms which enable them to compensate for the thermodynamic retardation of their metabolic activity at suboptimal temperatures by a higher substrate affinity. High temperature optima may then prevent the organisms from irreversible inactivation when the environmental temperature is fluctuating and could exceed the optimum.

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