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Influence of Temperature and Substrate Concentration on Bacterial Growth Yield in Seine River Water Batch Cultures†

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The growth of natural bacterial assemblages was monitored in 3-liter reactors under various temperature and substrate concentration conditions. The oxygen concentration was continuously monitored, and subsamples were taken at short time intervals to determine bacterial number and biomass. The rate of bacterial mortality was also determined. Bacterial growth yield was calculated as the ratio of net production (increase in biomass corrected for mortality) to gross production (net production plus oxygen consumption expressed in carbon units). Averaging 33%, the growth yield did not show any trend with temperatures in the range of 8 to 25°C but increased with the concentration of dissolved organic carbon in the range of 2 to 12 mg of C · liter^{-1}.

For the last 10 years, investigations have focused on the role played by the microbial food web as a sink or as a link for higher consumers (2, 9, 12, 22, 23, 29–31, 34). Although the widely used method for calculating the rate of thymidine incorporation into bacteria leads to an estimation of the production of biomass, a value of the growth yield (GY; net production over gross production) of 50% (2) is generally adopted to estimate the total uptake of substrate by bacteria. However, depending on the method of determination, values of the GY are reported in the range of 2 to 70% (5–7, 13, 18). Further, the GY has been shown to depend on the nature of substrates (21, 33) or on the ecosystem under study and the time of the year (25). Therefore, the significance of heterotrophic bacteria in the utilization of organic matter in aquatic environments can be understood only if the different aspects of bacterial dynamics, including GY, are taken into account.

As a part of a comprehensive study of the River Seine in France that explicitly takes into account bacterial dynamics (16, 17, 28) to establish a model of oxygen balance and organic-matter degradation, the bacterial GY has been assessed by measuring oxygen consumption and bacterial biomass production under controlled temperature and substrate conditions. Indeed, the conclusion that emerges from the literature is that temperatures and substrates are important in the control of the GY. Therefore, it is the purpose of this paper to investigate the effects of these two factors on the GY. Further, the model of organic-matter degradation will become more realistic with better knowledge of the bacterial GY in a system strongly affected by allochthonous organic matter, in which the amount of oxygen often decreases to critical values.

Experiment design. Batch culture experiments were conducted according to the method described by Ammerman et al. (1). The inoculum, which consisted of river water (see below), was filtered through a 2-μm-pore-size Nuclepore polycarbonate membrane filter to eliminate grazers and was diluted fivefold into 0.22-μm-pore-size (cellulose acetate; Sartorius)-filtered river water. The cultures were grown with river water (River Seine) that originated either from a station characterized by a low concentration of dissolved organic carbon (LDOCM [low DOC medium]) or from a more organically polluted station downstream from Paris, where the effluents of the Achères sewage treatment plant are discharged into the river (HDOCM [high DOC medium]). The cultures were incubated in darkness in PVC reactors (3 liters). An oxygen electrode (YSI), inserted airtight into the reactor, was connected to a recorder. A tubing allowed subsampling from the reactor without air bubble formation. All joints were Teflon. The cultures were kept homogenous with magnetic stirrers. Each reactor was immersed in a thermoregulated water bath to keep the temperature of the experiment stable throughout the incubation period (about 24 h).

A river water culture was brought to a chosen temperature within 1 h after collection in a water bath. The experiment started (zero time) when the cultures had reached the expected temperature. Most experiments were performed at 20°C with LDOCM or HDOCM, the temperature of the water in the river ranging from 8 to 22°C when taken.

To investigate the effects of temperature, the behaviors of bacterial populations in LDOCM and HDOCM were monitored in parallel at four different temperatures (in situ temperature of 8°C and also 15, 20, and 25°C).

The influence of the substrate was tested at 20°C through the experiments conducted with LDOCM (station upstream from the Achères plant), and HDOCM (station downstream from the Achères plant) at different occasions when the substrate concentrations in the river ranged from 2.4 to 9.7 mg of C · liter^{-1}. Further, the influence of the substrate concentration as well as its quality was investigated in more detail with a set of experiments, conducted at 20°C, in which the same inoculum of bacteria was added (i) to natural water (HDOCM; 7.9 mg of C · liter^{-1} [control experiment]); (ii) to natural water (HDOCM; 7.9 mg of C · liter^{-1}) enriched with 3.9 mg of C · liter^{-1} of artificial substrates, which increased the natural concentration to 11.8 mg of C · liter^{-1}; and (iii) to natural water (HDOCM; 7.9 mg of C · liter^{-1}) enriched with

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x2.4 mg of C · liter^{-1} of phytoplanktonic lysate, which increased the concentration of HDOCM to 10.3 mg of C · liter^{-1}. The artificial substrate was composed as follow: glucose, 400 mg · liter^{-1}; galactose, 400 mg · liter^{-1}; Casamino Acids, 1,500 mg · liter^{-1}; acetate, 150 mg · liter^{-1}; and $(NH_4)_2KPO_4$, 250 mg · liter^{-1}.

Measurement and analyses. Oxygen concentrations were continuously recorded. Preliminary tests showed that the consumption of oxygen by the oxygen electrode itself was not detectable in sterilized water throughout the duration of the experiment.

Subsamples were taken every 4 h for the determination of bacterial abundance and biomass. Microscopic preparations were performed with preserved samples (formalin at 2% final concentration) according to the method described by Porter and Feig (24) with a direct epifluorescence microscope (Diaplan; Leitz). Determination of bacterial shapes and sizes (17) allowed estimation of the biomass with the biovolume-dependent conversion factor described by Simon and Azam (32).

Incorporation of [^{3}H]thymidine (87 Ci · mmol^{-1}; Amersham) was also measured every 4 h at saturating concentrations according to the method described by Fuhrman and Azam (14, 15) on 10 ml of water samples incubated for 0.5 h.

The rates of bacterial mortality were estimated for the duration of the experiment for LDOCM and HDOCM samples at different temperatures. Each estimate is the rate of decrease in radioactivity of the DNA for a [^{3}H]thymidine-labeled natural assemblage of bacteria after all added thymidine has been exhausted from the medium (26, 27).

DOC was determined at the beginning of each experiment on a carbon analyzer (model 700; Bioritech). In the set of experiments with added substrates, samples were taken throughout the experiment for determinations of the amounts of DOC, dissolved proteins, and mono- and polysaccharides. Dissolved proteins were analyzed with the Pierce Kit (micro BCA Protein Reagent), and the amounts of dissolved saccharides were measured according to the method described by Johnson and Sieburth (19) as modified by Dawson and Liebezeit (11). The data were converted to C units by using the following ratios: C:protein = 0.5; C:saccharide = 0.4.

GY calculations. Bacterial GY was calculated as the ratio of net production (increase in biomass [ΔB] corrected for mortality [ΔM]) to gross production (net production plus respiration [ΔR], i.e., the overall oxygen consumption expressed in carbon units). A stoichiometric ratio was used as respiratory quotient, i.e., a mole of O_2 consumed corresponding to a mole of C respired (8):

$$GY=(\Delta B+\Delta M)/[(\Delta B+\Delta M)+\Delta R].$$

Methodological problems which inevitably arise from determinations of bacterial GY must be mentioned. Although the amount of oxygen can be measured with enough precision compared with the total consumption throughout the experiment, a conversion factor is needed to calculate the amount of respired carbon. We used a constant respiratory quotient of 1; a quotient of 0.85 or 0.77, also mentioned in the literature, would have led to higher values. Regarding bacterial biomass, microscopic counting according to size and shape, and conversion of biovolume into biomass by the size-dependent factor described by Simon and Azam limited biases.

Typical trends in bacterial biomass and oxygen concentration for the LDOCM and HDOCM samples at 20°C were characterized by linearity throughout the experiments, suggesting that the experiment does not modify the behavior of the bacterial population.

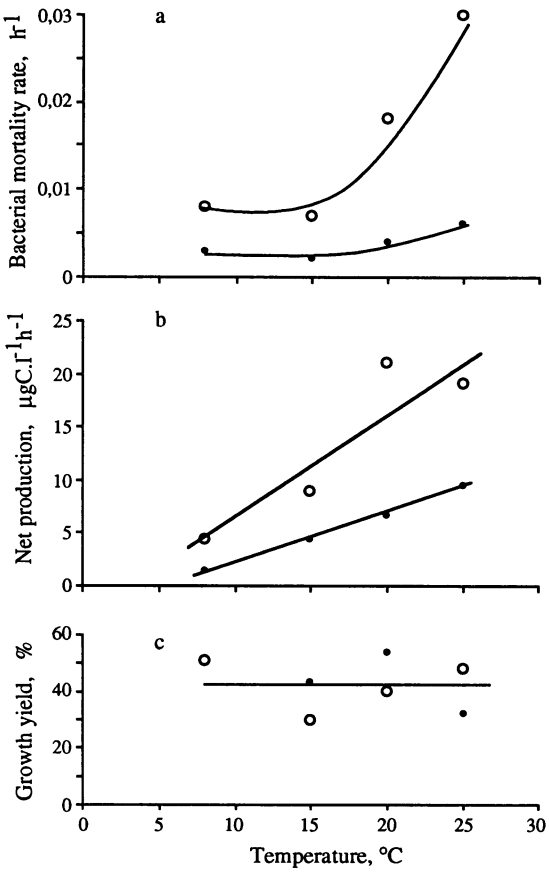

FIG. 1. Influence of temperature on bacterial mortality (a), bacterial net production (b), and bacterial GY (c) for a LDOCM (●) and for HDOCM (○).

Effect of temperature. The rate of bacterial mortality increased with increases in the temperature for both LDOCM and HDOCM but was much higher for HDOCM (0.03 h^{-1} versus 0.006 h^{-1} at 25°C, for example [Fig. 1a]). Rates of bacterial-biomass production corrected for mortality are given. Net bacterial biomass production also increased with temperature, from 1.35 to 9.4 µg of C · liter^{-1} · h^{-1} in LDOCM and from 4.33 to 19.1 µg of C · liter^{-1} · h^{-1} in HDOCM for temperatures varying from 8 to 25°C (Fig. 1b). A clear increase of thymidine incorporation rates in both cases (LDOCM and HDOCM) was also evident with increases in the temperature (Table 1). The response of bacterial activities to the different temperatures tested did not seem to correspond to a change in morphology, since the carbon content per cell did not differ significantly (Table 1). On the other hand, no temperature effect was observed either in HDOCM or LDOCM for bacterial GY (Fig. 1c), which is in contrast to the results described by Bjørnsen (5), indicating a negative correlation of GY to temperature (three experiments carried out in different seasons). As mentioned by Bjørnsen, several other factors might have influenced the GY, which is not the case with the present experiments; indeed, four temperatures were tested for the same medium-inoculum association. The lack of

TABLE 1. Influence of temperature on bacterial-biomass production, on the [^{3}H]thymidine incorporation rate, and on the content of carbon per cell in different substrates

Temp (°C)	Value for LDOCM			Value for HDOCM		
	Bacterial-biomass production[a]	[^{3}H]thymidine[b]	Carbon content[c]	Bacterial-biomass production[a]	[^{3}H]thymidine[b]	Carbon content[c]
8	1.35	0.055	12.39 ± 2.38	4.33	0.225	17.43 ± 4.96
15	4.4	0.13	15.72 ± 3.31	8.88	0.655	20.36 ± 4.57
20	6.6	0.35	17.35 ± 2.70	20.93	1.04	19.80 ± 4.95
25	9.4	0.46	16.20 ± 3.01	19.09	1.43	18.70 ± 4.00

[a] Values are micrograms of carbon per liter per hour.
[b] Values are nanomoles per liter per hour.
[c] Values are femtograms of carbon per cell. Confidence interval, ± 95%.

temperature effect on GY shows that all metabolic activities (assimilation, mortality, and respiration) followed the same relation to temperature. A Q_{10} of about 3 was found for the range 8 to 25°C. Considering all of the results from 18 experiments (13 were performed at 20°C), an average GY was found to equal 33% ± 6%, ($P < 0.05$). This average value agrees well with most values which were not derived from radiotracer methods based on the incorporation of simple organic molecules, with the exception of the values obtained with the method described by Billen et al. (4; see other references in reference 5).

Influence of substrate concentration. Experiments conducted at different occasions with LDOCM and HDOCM showed that production of bacterial biomass significantly ($r = 0.89$; $P < 0.05$) increased in relation to the concentration of DOC (in the range of 4.5 to 25 μg of C · liter^{-1} · h^{-1}, for DOC concentrations varying from 2.5 to 12 mg of C · liter^{-1} [Fig. 2a]). Similarly, the GY increased linearly with the concentration of DOC, although a weaker relationship was found ($r = 0.65$; $P < 0.05$) (Fig. 2b). The increase in the GY with the substrate concentration showed that relatively more biomass was produced in enriched systems. This is consistent with the relationship established for a variety of ecosystems by Cole et al. (10), who reported an increase in bacterial biomass along a trophic gradient (gradient of chlorophyll *a* concentrations). However, our results seem to contradict those found by Bauerfeind (3) showing, in four experiments, a similar GY for a range of small dissolved organic compounds (free dissolved monosaccharides and amino acids) ranging in concentration from 3 to 0.07 mg of C · liter^{-1}. Otherwise, it is generally stated that the bacterial GYs notably depend on the structural complexity of the substrates (21), since the conversion of organic carbon into biomass decreases with the increasing complexity of the substrates. The results presented here are consistent with such a statement, since a relative constant fraction of dissolved biodegradable organic matter (55%) is found in the river, whatever the level of dissolved organic matter (28).

The set of experiments performed with concentrations of substrates in the range of 7.9 to 11.8 mg of C · liter^{-1} did not show basic differences in the composition (dissolved proteins and total saccharides), except for monosaccharides, which were in higher proportion when artificial substrates were added (Table 2). Similar enhancements of bacterial biomass and oxygen consumption are shown when either artificial or natural substrate was added (Fig. 3). Without any addition of substrates, the bacterial activity was due more to the amount of saccharides, which decreased greatly in

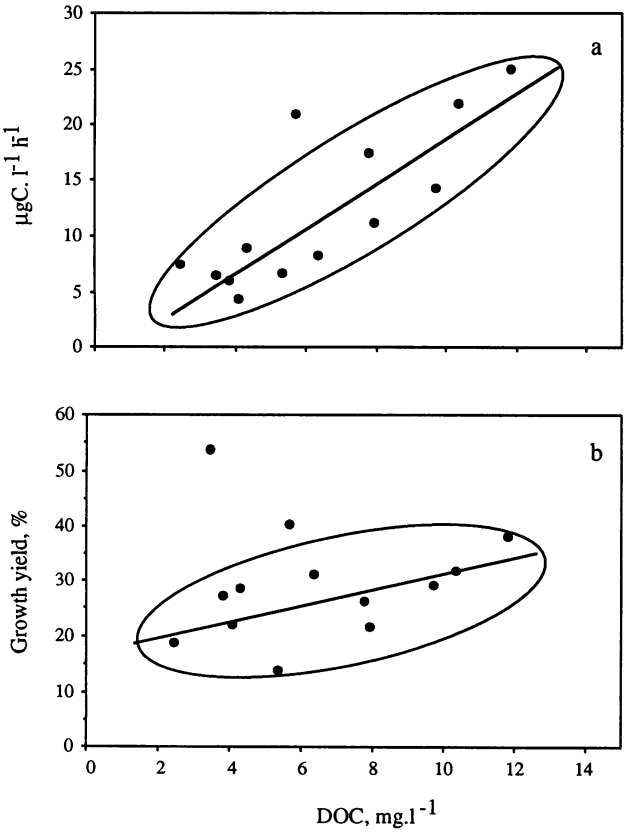

FIG. 2. Relationship between bacterial production of biomass ($r = 0.89$; $P < 0.05$) (a) and bacterial GY ($r = 0.65$; $P < 0.05$) (b) and increasing DOC concentrations (all experiments conducted at 20°C). Regression lines are drawn.

TABLE 2. Percentages of organic carbon present at zero time in various fractions in the set of experiments with addition of substrates

Substrate condition (at 20°C)[a]	Dissolved proteins (%)	Total dissolved saccharides (%)	Dissolved monosaccharides (%)	DOC (mg of C · liter^{-1})[b]
HDOCM	38	16.5	6.3	7.9
HDOCM + As	36.4	23.7	16.1	11.8
HDOCM + Lys	42.7	24.3	9.7	10.3

[a] As, artificial substrate; Lys, phytoplanktonic lysate.
[b] Amounts are at zero time.

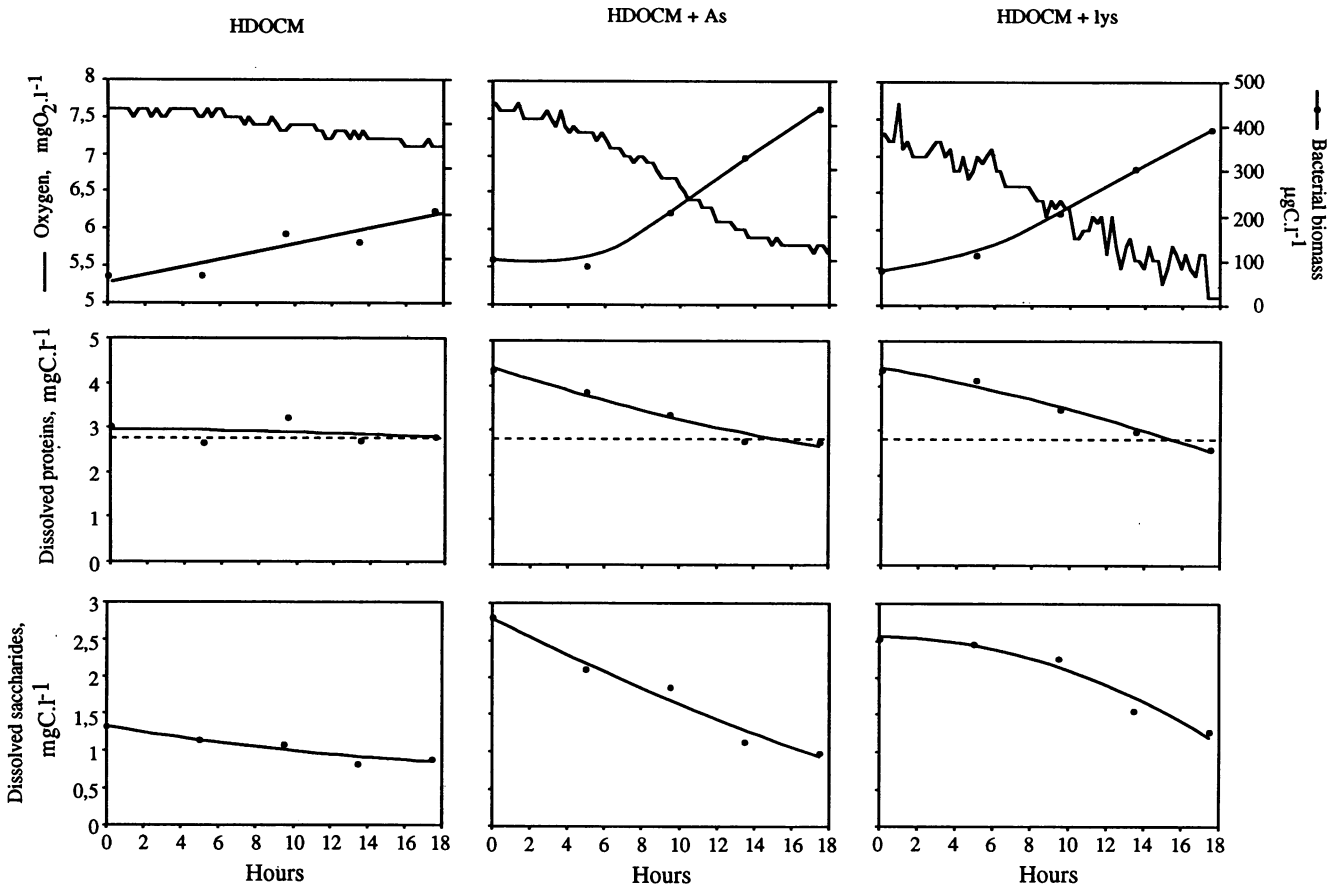

FIG. 3. Oxygen consumption and bacterial-biomass increase, consumption of dissolved proteins, and consumption of dissolved saccharides throughout the duration of the experiment with the HDOCM (control) and the HDOCM enhanced with artificial substrate (HDOCM + As) or phytoplanktonic lysate (HDOCM + lys).

concentration, than to that of proteins, which decreased in concentration only slowly (Fig. 3; Table 3). However, added proteins appear to be particularly degradable, since they completely disappeared; since the amount of proteins of the river water (HDOCM) decreased slightly more than those of the control (<2.8 mg of C · liter^{-1}), added proteins could act as cosubstrates, favoring the degradation of more-complex molecules present in the system. According to Keil and Kirchman (20), added proteins could act as fresh rapidly assimilated proteins, whereas proteins found in the system could be modified proteins, more slowly utilized. The amounts of total saccharides and monosaccharides decreased at the same rates in the control, suggesting that only monosaccharides were taken up. Compared with proteins, saccharides were preferentially taken up in the control (at a rate twice that for the proteins), whereas no difference was found when artificial substrates were added. However, as shown by determinations of V_{max} (maximal potential uptake rates) for ^{14}C simple organic molecules (Table 3), amino acids are (compared with glucose) preferentially taken up by

TABLE 3. Initial and final values of dissolved oxygen, dissolved proteins, total dissolved saccharides, and dissolved monosaccharides, along with maximal potential uptake rates[a]

Substrate condition[b]	O_2			Dissolved proteins			Total dissolved saccharides			Dissolved monosaccharides			$V_{max_{aa}}$	$V_{max_{Glu}}$
	Initial value (mg/liter)	Final value (mg/liter)	Rate of consumption[c]	Initial value (mg/liter)	Final value (mg/liter)	Rate of consumption[c]	Initial value (mg/liter)	Final value (mg/liter)	Rate of consumption[c]	Initial value (mg/liter)	Final value (mg/liter)	Rate of consumption[c]		
HDOCM	7.6	7.1	28	3.0	2.8	11	1.3	0.9	22	0.5	0.1	22	17.8	7.7
HDOCM + As	7.7	5.8	107	4.3	2.7	91.4	2.8	1.0	101.4	1.9	0.3	90	177.4	131.8
HDOCM + Lys	7.3	5.1	124	4.4	2.6	102.9	2.5	1.3	67.8	1.0	0.4	33	97.3	91.4

[a] The duration of the experiment was 17 h and 45 min. Maximal potential uptake rates were determined at the end of the experiment according to the method described by Wright and Hobbie (35, 36) with a ^{14}C-amino acid mixture ($V_{max_{aa}}$) and ^{14}C-glucose ($V_{max_{Glu}}$).

[b] As, artificial substrates; Lys, phytoplanktonic lysate.

[c] Values are for observed rates of consumption, expressed in micrograms of carbon per liter per hour.

bacteria with or without added substrates, suggesting a hierarchy in bacterial utilization of organic molecules in the following order: amino acids > monosaccharides ≥ fresh proteins > modified proteins ≥ polysaccharides. However, more research is still needed to understand both the qualitative and the quantitative roles of substrates as control factors of the GY.

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