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BACTERIAL PRODUCTION, HEAT PRODUCTION AND ATP-TURNOVER IN SHALLOW MARINE SEDIMENTS *

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SUMMARY

Seasonal variation in bacterial numbers and biomass, ATP concentration, heat production, bacterial production and basic sediment chemistry was followed in shallow marine sediments off the Forsmark Nuclear Power Plant on the Swedish Baltic coast. Production and specific growth rate of bacteria were strongly temperature dependent although the sedimentation of diatoms in the spring caused a significant increase in microbial activity in spite of a low temperature. *In situ* bacterial doubling time and ATP-turnover values indicated that a large portion of the sediment bacteria was "dormant" or non-growing at all seasons, even in summer. Average growth yield of the bacterial community, calculated from the bacterial production and heat production measurements, varied between 17 and 40 % .

INTRODUCTION

The complex structure of marine sediments, with a high diversity of species and an irregular distribution (in space and time) of different functional groups of bacteria, has made them difficult to study on a community level. Therefore, studies have traditionally been indirect and concerned only specific groups of organisms or a certain metabolic process. The activity of benthic bacteria has been concluded from changes in concentration or turnover rates of inorganic or organic chemical parameters (ref. 1). In accordance, the activity of certain functional groups of bacteria, microfauna, meiofauna etc. has been neglected (ref. 2). The introduction of microcalorimetry into sediment studies has given an opportunity to estimate the total metabolic activity of the benthic community independent of the type of metabolism that is prevailing (ref. 3). Although the use of calorimetric methods in ecological studies of microorganisms has increased during recent years, studies on sediment ecosystems have often concerned the effect on microbial activity of substrates or toxicants added to the sediments, and there are still relatively few studies in which heat production measurements have been performed as a part of regular field studies of "natural" sediment ecosystems. The application of calorimetry in terms of heat production measurements in ecological research has been reviewed by Gustafsson (ref. 4) and Widdows (ref. 5).

Heat production values can be used to estimate carbon and energy budgets of a system by assuming certain combustion values of e.g. sedimenting organic matter (refs. 6-7) or using caloric equivalents for different metabolic processes (refs. 8-9).

To optimize the ecological information from microcalorimetry, however, heat production measurements should be combined with other parameters of microbial biomass and activity (refs. 4,

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10). ATP determinations combined with direct calorimetry have been used to calculate the relative rate of ATP-turnover in marine sediments under varying environmental conditions (refs. 6-7). Using a caloric equivalent for dissipative ATP-turnover, reasonable estimates of ATP-turnover time can be calculated (ref. 10). ATP-turnover time can be used as a sensitive indicator of metabolic state or growth phase especially in sediment communities dominated by bacteria. Parallel measurements of potential respiration, measured as electron transport system activity (ETS activity), and heat production can give information on the relative contribution of fermentative bacteria to the overall metabolism of the sediment community. Fermentative processes are included in the heat production measurements but not in the ETS assay (ref. 10). In this paper we present simultaneous measurements of heat production and bacterial production estimated from incorporation of ^{3}H-thymidine into DNA. Assuming that heat production primarily reflect catabolic processes these data can be used to estimate the average growth yield of the sediment bacteria, indicating partly the dominating type of mineralization and the proportion of actively growing cells.

The data presented in this paper derive from a field study performed in surface sediments of the recipient of heated cooling water from the Forsmark Nuclear Power Plant on the Swedish Baltic coast. The study included one site affected by the cooling water discharge and a reference site not affected by the cooling water plume. The overall purpose of the studies was to follow the effect of temperature on microbial processes and to study the regulation of the seasonal variation of microbial activity in these shallow marine sediments. The sampling programme included determinations of bacterial numbers and biomass, ATP concentration, heat production, bacterial production, redox potential and basic sediment chemistry. The present paper focuses on methodological aspects and information that can be gained by combining different biomass and activity parameters. Therefore, data presentation will be restricted to one sediment layer at one sampling site (0-2 cm layer at the reference site).

MATERIAL AND METHODS

Sediment samples were collected using a plexiglass core sampler with a diameter of 6.5 cm. The sediment cores were carefully sectioned into 0-2 and 2-5 cm layers and placed in plastic beakers. Samplings were performed on 9 occasions from January to November 1989. The sampling sites represented soft bottom areas and water depth at the "reference" site was about 8 m. After *in situ*-measurements (see below) the sediments were transported to the laboratory, normally within 2 hours. During transport the samples were kept at a temperature close to the *in situ* temperature. Preservation of sediments for microscopic analysis and drying of sediments for water content determination and chemical analyses were performed immediately upon arrival to the laboratory.

Numbers and biomass of bacteria were estimated on samples preserved with formaldehyde (final concentration 4%). Epifluorescence microscopy was used (Zeiss filters KP 490, Rfl 510, LP 520) after appropriate dilution of the sediment samples, sonication in an ice bath at 20 kHz and 100 W for 1 min., staining with acridine orange (Merck) to a final concentration of 0.005 %, and filtration onto 0.2 µm Nuclepore filters that were prestained with Sudan black (Sigma). 400 cells in at least 20 random fields were counted. 100 cells from each sample were measured and the average

volume was used in biomass calculations.

ATP extractions were performed according to Tobin *et al.* (ref. 11) immediately upon arrival to the laboratory. ATP analysis was made using a luminometer (LKB-Wallace; 1250 Luminometer) and the output signal monitored on a potentiometric recorder. A monitoring reagent (LKB 1243-200) and an ATP standard (LKB 1243-201) were used.

Heat production measurements were carried out using a heat conduction multichannel microcalorimeter (Thermal Activity Monitor 2277, Thermometric). Two channels were used, each containing a measuring and a reference cell (ref. 12). Glass ampoules with a working volume of approximately 3 ml were used. Prior to incubations a redox profile was determined on a core parallel to the core sectioned in the field. Incubation of sediments were arranged in order to simulate natural conditions as close as possible with regard to redox condition (by varying the sediment/water ratio and by using a supernatant with controlled oxygen content etc.). Regarding the surface sediment samples from the "reference" site used in this study, c. 1 g of sediment was incubated with c. 2 ml filtered (0.2 μm) aerobic seawater on all occasions. The reference ampoule contained 3 ml distilled water. The output signal was recorded on a two-channel potentiometric recorder (LKB 2210). The base-line fluctuations were less than 0.25 μW over 24 h in the 30 μW range used. Surface sediment incubations were started on the day of sampling. All measurements were performed at 20 °C. Heat production values were taken from the recordings after 5 hours of equilibration.

Bacterial production was determined from incorporation of [methyl-^{3}H]-thymidine into bacterial DNA according to Bell and Ahlgren (ref. 13). Incubations were performed in triplicates *in situ*, at the other sampling site (at 2.8 to 9.0 °C higher temperature) and in the laboratory at 20 °C in order to establish the temperature dependence of the process on each sampling occasion. 0.5 g of wet sediment was incubated in 10 ml Oak Ridge centrifuge tubes with c. 40 μCi of ^{3}H-thymidine (45-55 Ci mmol^{-1}, Amersham) between 30 and 60 minutes depending on the incubation temperature. To calculate the degree of participation of ^{3}H-thymidine (isotope dilution), unlabelled thymidine on a few occasions was added in different concentrations to additional parallel tubes together with sediment and labelled thymidine according to Pollard and Moriarty (ref. 14) and Bell (ref. 15). Bacterial production was calculated using a conversion factor of $0.44*10^{18}$ cells mole^{-1} assuming a DNA content of 2.5 fg cell^{-1} (ref. 16) and corrected for the average measured degree of participation (50 %). Cells produced were converted to carbon units by applying the factor $2.2*10^{-13}$ g C μm^3 (ref. 17).

Water content was determined by drying at 105 °C for a period of 24 hours. Carbon (and nitrogen) content was determined on dry sediment using a Carlo-Erba CHN-Elemental Analyser.

RESULTS AND DISCUSSION

The seasonal variations of temperature, water content, and carbon content, biomass and activity parameters, expressed on a dry weight basis, in surface sediments at the "reference" site are given in Table 1. Data on water content and carbon content indicate that the physical and chemical properties of the sediments were fairly constant between sampling occasions. They also indicate a moderate

TABLE 1

Temperature, water content, and carbon content, ATP concentration, bacterial production, specific growth rate and heat production expressed on a dry weight basis in the 0-2 cm sediment layer at the reference site.

Date	Temp.	Water content	Carbon content	ATP conc.	Bacterial biomass	*In situ* bacterial production	Bacterial production (20°C)	*In situ* specific growth rate	Heat production
	(°C)	(%)	(mg g^{-1})	(µg g^{-1})	(mg C g^{-1})	(ng C g^{-1} h^{-1})	(ng C g^{-1} h^{-1})	(d^{-1})	(µW g^{-1})
24.1	0.6	86.7	48.0	6.8	1.07	21.0	730	0.000472	32.0
2.3	3.3	85.2	44.0	5.5	1.59	27.6	960	0.000416	24.6
5.4	2.7	88.9	50.1	9.5	3.19	37.8	970	0.000284	40.9
26.4	5.3	85.7	41.4	7.8	0.97	282	—	0.00700	32.9
1.6	13.5	92.9	48.2	5.9	1.44	444	1200	0.00742	67.0
26.7	14.6	87.0	48.4	6.8	1.50	446	680	0.00712	15.4
22.8	16.1	87.9	50.2	7.7	1.65	668	1000	0.00974	21.8
4.10	9.4	86.0	48.2	6.8	0.99	230	470	0.00558	27.8
21.11	3.8	82.3	40.8	4.3	0.57	15.0	610	0.000628	20.8

horizontal variation in sediment characteristics at this sampling site. (Data from the other sampling site indicated that sediment characteristics have a large influence on microbial activity. Seasonal trends in microbial activity may therefore to a large extent be obscured if sediment characteristics are too variable between sampling occasions).

ATP concentration and bacterial biomass varied over the year without any clear seasonal trends and especially for ATP concentration within a quite narrow range of variation (Table 1). *In situ* bacterial production and specific growth rate (bacterial production/bacterial biomass; both expressed in carbon units), however, exhibited a clear seasonal pattern and appeared to be strongly temperature dependent. Specific growth rate (μ) as a function of temperature is shown in Fig. 1a. The relation could be described by the equation:

$$\mu = 0.00038 * 1.25^{T} \quad (R^2 = 0.729) \qquad (1)$$

On April 26, bacterial production was high in spite of a low temperature. This sampling occasion coincided with the sedimentation of the spring diatom bloom, indicating that the temperature dependence may be obvious only if substrate availability (and probably electron acceptor availability) remains fairly constant. If April 26 is excluded from the data set, the correlation to temperature improves (Fig. 1b):

$$\mu = 0.00026 * 1.27^{T} \quad (R^2 = 0.916) \qquad (2)$$

Expressed as Q_{10} values for the interval 0.6 to 16.1 °C the values are 9.14 or 10.9 if April 26 is excluded. The data indicate that a larger proportion of cells are dormant or non-growing at lower temperatures. A strong temperature dependence was also observed on single sampling days during winter (Q_{10}-values ranged between 6.3 and 9.9 from *in situ* temperature to 20 °C) indicating that sub-populations shift from non-growing to active growth if removed to a significantly higher temperature (20°C) (Table 1).

Different types of temperature rules have been proposed for biological systems. Equations (1) and (2) follow the general formula $y = a * B^{T}$ that initially was derived from chemical processes (ref. 18). Belehradek (ref. 19) suggested another formula based on temperature dependence of physical processes such as diffusion and viscosity (important for biological processes on the microscale): $y = a * T^{B}$. In fact, applying the data set to that equation (dashed lines in Fig. 1a and b) resulted in slightly improved correlation coefficients ($R^2 = 0.794$ and 0.966, respectively). The temperature dependence of biological systems on a community level such as marine sediments is extremely complex, however, and need not follow any of the relationships established for individual physical and chemical processes, enzymatic reactions or cellular systems. These aspects will be discussed more thoroughly elsewhere (Ahlgren, Boström and Törnblom in prep.).

The heat production measurements were all performed at 20 °C. Thus, any seasonal trends due to the temperature can not be revealed. The peak value recorded on June 1st coincided with a high water content of the sediments and a high redox potential (>300 mV) throughout the 0-2 cm layer.

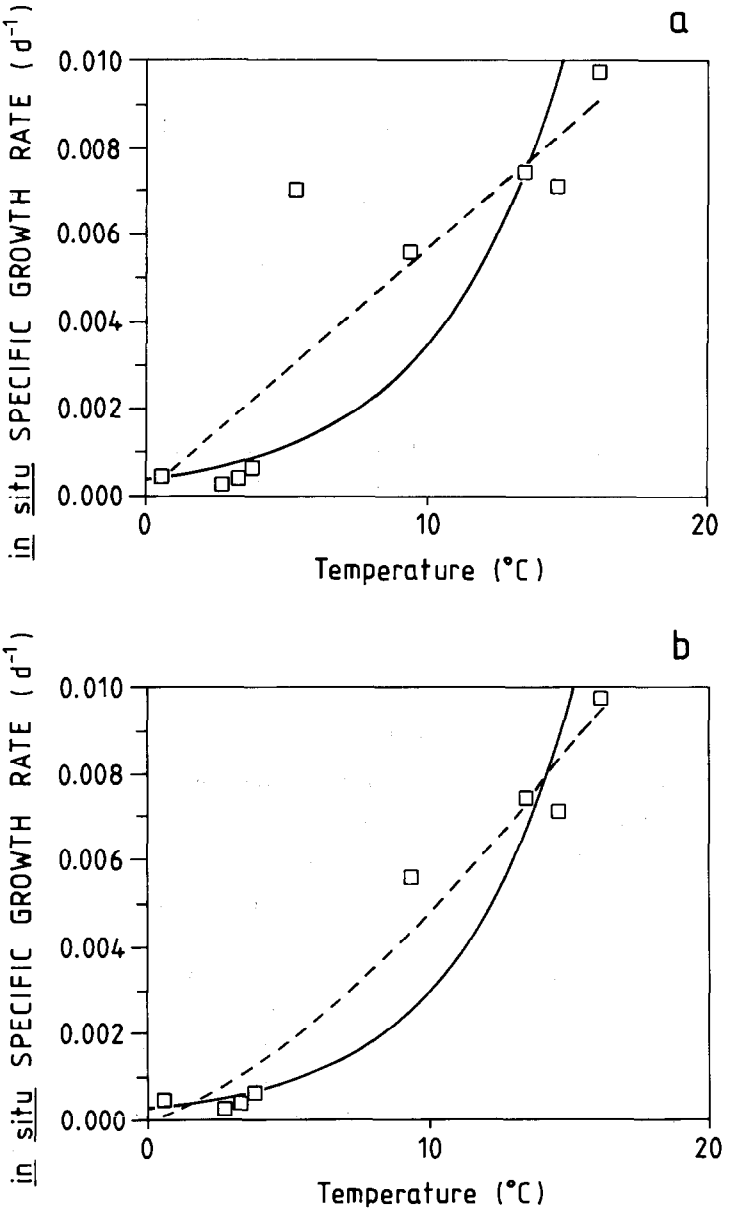

Fig. 1. *In situ* specific growth rate of sediment bacteria (0-2 cm) as a function of temperature. Solid lines represent equations according to Berthelot (ref. 17). Dashed lines represent equations according to Belehradek (ref.18). a) = all data, b) April 26 excluded. For a full explanation see the text.

Notably, the bacterial production at 20 °C also peaked at that date.

The ATP-turnover time can be calculated as the ATP/heat production ratio from heat production expressed in J s^{-1}, ATP concentration and applying a caloric equivalent ($\Delta_k H_{\infty ATP}$) expressed in J mol^{-1} ATP for aerobic dissipative ATP-turnover.

ATP concentration	*	$\Delta_k H_{\infty ATP}$	/ heat production	=	ATP turnover time	(3)
(mol ATP g^{-1})		(J mol^{-1} ATP)	(J s^{-1} g^{-1})		(s)	

Assuming that an average of 24 moles of ATP per mol of glucose (as a model substance) is formed in a mixed microbial community, the caloric equivalent $\Delta_k H_{\infty ATP}$ = -120 kJ mol^{-1} ATP can be applied (ref. 9). To calculate the ATP-turnover time at *in situ* temperature we assumed that Q_{10} for heat production was the same as the Q_{10} for bacterial production determined on each sampling occasion. This assumption may not be fully correct considering maintenance metabolism of non-growing cells, yet the error is probably smaller than applying a theoretical relation of temperature dependence of metabolic processes to a mixed microbial community. The unit for ATP-turnover time is seconds. In Fig. 2, ATP-turnover time at *in situ* temperature is compared to the *in situ* bacterial doubling time (the inverted specific growth rate) expressed in days. Bacterial doubling time varied from several thousand days in winter to 100-200 days from April to October, while ATP-turnover time at *in situ* temperature varied in the range 57 s, in June, to ≈ 2000 s in November. These data indicate that a large portion of the sediment bacteria was "dormant" or non-growing at all seasons, even in summer. The average bacterial doubling times and

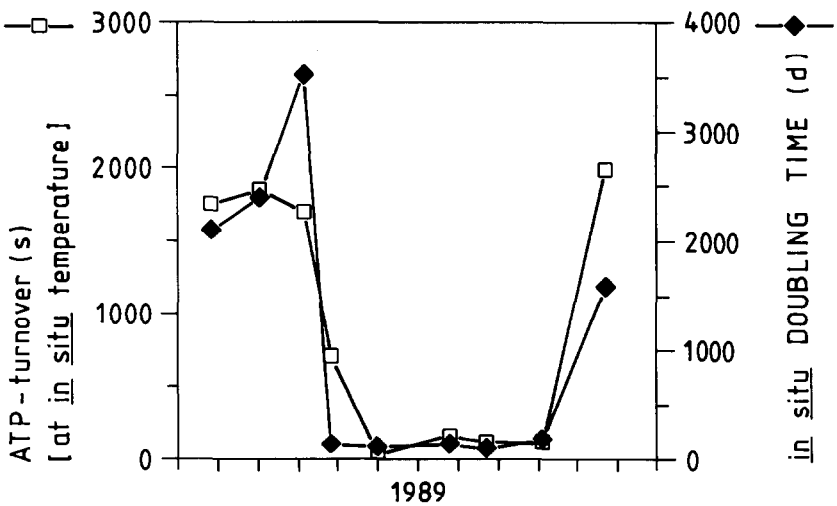

Fig. 2. Seasonal variation in ATP-turnover time (at *in situ* temperature) and *in situ* doubling time of sediment bacteria in the 0-2 cm layer at the "reference" site.

ATP-turnover times are longer than most values reported in literature. Moriarty *et al.* (ref. 20) report doubling times of 1-5 days in sediments of seagrass beds at temperatures between 20 and 25 °C. In the sediments of highly eutrophic Lake Vallentunasjön (c. 100 km from Forsmark, thus in the same climate regime), bacterial doubling time values ranged from 2 days at 20 °C (ref. 13) to more than 1000 days in winter (unpubl. results). Graf and Bengtsson (ref. 10) report ATP-turnover times of 4 to 20 s in sandy sediments of the southern Baltic Sea, assuming a heat release of 50 kJ mol^{-1} ATP. This value appears to be too low and is possibly based on the enthalpy of ATP hydrolysis rather than the catabolic enthalpy equivalent of ATP-turnover at steady state (Gnaiger pers. comm.). Using the caloric equivalent for aerobic dissipative ATP-turnover employed in this study the values correspond to ATP-turnover times in the range 10 to 50 s. In laboratory cultures of actively growing bacteria, ATP-turnover times from 0.1 to a few seconds are normal (ref. 21).

The low specific activity of bacteria in these sediments explains why seasonal patterns in activity are not reflected in the biomass parameters. The low specific activity of bacteria in sediments may be the result of a longer survival time compared to pelagic bacteria (ref. 22) e.g. due to a lower mortality enforced by grazers. Steep gradients in chemical conditions in space and time may also be important in this context by limiting the opportunities for optimal growth of the different populations of the microbial community in the sediments.

Assuming that the heat production measurements primarily reflect aerobic catabolic reactions, the average growth yield for the bacterial community (production/production+respiration) expressed in carbon units can be calculated. Bacterial production expressed in carbon units was estimated as described above. Heat production was converted to carbon units assuming RQ = 1 and using the aerobic catabolism of glucose as a model process assuming $\Delta_k H°_{O2}$ = -469 kJ mol^{-1} O_2 (ref. 9). The calculations are based on measurements performed at 20 °C. The contribution of bacteria to the total sediment respiration was calculated from the bacterial biomass/total biomass ratio assuming a C:ATP weight ratio of 250 in living biomass (ref. 23). The bacterial biomass/total biomass ratio varied from 0.50 on April 26 (during the sedimentation of the spring diatom bloom) to 0.86-0.97 in summer and (over) 1 in late winter. The late winter values indicate a C:ATP weight ratio above 250 within the relatively inactive bacterial community during that period.

TABLE 2

Average growth yield (expressed in percent) of the bacterial community in the 0-2 cm layer at the "reference" site.

Date (1989)	24.1	2.3	5.4	26.4	1.6	26.7	22.8	4.10	21.11	
Growth yield (%)	28.3	29.8	20.4	39.7	16.8	35.3	36.7	24.0	37.6	x = 29.8

The average growth yield for the bacterial community varied in the range 17 to 40 % over the season with a mean value of 30 % (Table 2). Considering all conversion factors employed, the values agree remarkably well with theoretical values ranging from 20 % (fermentative processes) over 30-40 % (anaerobic respiring bacteria) to 50 % (aerobic bacteria) given by Blackburn (ref. 24). The values also agree with those recorded for sediment bacteria at 20 °C in Lake Vallentunasjön (ref. 13). Based on bacterial production estimated from thymidine incorporation into DNA and ETS activity or Cartesian diver microrespirometry as measure of community respiration, Bell and Ahlgren (ref. 13) reported average growth yield values in the range 18 to 30 % .

When interpreting data on average growth yield of bacterial communities, maintenance metabolism of non-growing cells should be considered, as well as the fact that some heterotrophic bacterial species or functional groups of bacteria lack the ability to incorporate thymidine into DNA (ref. 25). Larger data sets including measurements in different sediment layers are needed in order to evaluate the extent to which growth yield data give reliable information about the types of mineralization processes which prevail in sediments.

The heat production values expressed in carbon units were also used to estimate the annual sediment respiration at *in situ* temperature in the 0-2 cm layer (assuming that Q_{10} for heat production was the same as determined for bacterial production on each sampling occasion). Using time-weighted averages, the annual benthic respiration in the 0-2 cm layer was estimated to be 12.8 g C m^{-2} y^{-1}. This value can be compared to an annual primary production in the water column at this site of circa 17 g C m^{-2} y^{-1} (Willén, pers. comm.). It is hard to estimate the accuracy of the calculated annual sediment respiration from this comparison; such an estimation would require information on the pelagic mineralization, the sedimentation and the lateral sediment transport pattern in the area.

Overall, there are good agreements between different measured and calculated activity parameters, and the calculated values of average bacterial growth yield and benthic respiration appear to be reasonable. We conclude that the methods employed can be used to gain reliable information in studies of the microbial community in marine sediments.

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