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Heat Production, ATP Concentration and Electron Transport Activity of Marine Sediments*

M. M. Pamatmat[1], G. Graf[2], W. Bengtsson[2] and C. S. Novak[3]**

[1] Tiburon Center for Environmental Studies, San Francisco State University, P. O. Box 855, Tiburon, California 94920, USA
[2] Sonderforschungsbereich 95, University of Kiel, D-2300 Kiel, Federal Republic of Germany
[3]Department of Fisheries and Allied Aquaculture, Auburn University, Auburn, Alabama 36830, USA

ABSTRACT: Sediments in the southwestern Baltic Sea showed variable correlations between heat production rate, adenosine triphosphate (ATP) concentration, and activity of the electron transport system (ETS). There was excellent correlation between heat flux and ATP for beach sand but none for subtidal sand. Heat production/ATP, heat production/ETS, and ETS/ATP ratios are 3.5, 2.8, and 1.8 times higher, respectively, for sandy beach sediments than for sandy subtidal sediments. Heat production/ATP ratio in a batch culture of the obligate anaerobic fermenter, *Bacteroides* sp. was relatively stable during growth phase but decreased greatly from stationary through senescent phases, indicating that at least part of the variability in the correlation between heat production rate and ATP in sediments might be due to differences in the physiological state of bacteria. Long-term storage of sediments resulted in decreases in both ATP concentration and rate of heat production, possibly due to exhaustion of food substrate, of electron acceptors, or changes in microbial composition. Inferring differences in metabolic rates of very different sediment samples from differences in ATP or ETS activity alone could be misleading. Direct metabolic rate measurements are essential in addition to ATP or ETS activity measurements for accurate understanding of the differences and changes in sediment metabolism. When dealing with unknown mixtures of metabolic types in sediments, direct calorimetry has the advantage of measuring equally well the rate of production of a common end-product of all kinds of metabolic activities.

INTRODUCTION

Overall sediment metabolism has been determined in terms of oxygen uptake, carbon dioxide production, enzyme activity, and heat production (reviewed by Pamatmat, 1977). Each technique for measuring total sediment metabolism gives results that tell us something different from the others; however, all of these measures should be somehow related. Each method is subject to some limitation or inherent difficulty, in procedure as well as interpretation. To learn the combined, undisturbed metabolic activity of all sediment organisms in nature it is necessary to find some agreement, or reasons for disagreement, among the different methods. The actual metabolic rates in natural sediments may be expected to lie somewhere in the area of convergence of various measures.

In this paper we report various cases and conditions under which three measures of metabolic activity, adenosine triphosphate (ATP), electron transport system (ETS), and heat production, have been conducted and how these measures compare with each other. Each of the present measurements of ATP, ETS, and heat production contributes to our understanding of sediment metabolism but using all of them together may help us to eliminate bias or inaccuracies that might result from the use of only one of the methods.

The concentration of ATP is commonly regarded as an estimate of living or metabolically active biomass (Ernst, 1970; Karl, 1978), but it has also been thought to be a measure of metabolic activity (Hobbie et al., 1972; Holm-Hansen and Paerl, 1972). If ATP concentration is an equally good measure of metabolically active biomass and metabolic rate, this would mean a constant relationship between ATP pool size and metabolic rate for all organisms under various conditions. The present paper will show that this is not true.

The activity of the electron transport system (Packard, 1971) has been increasingly widely used and applied to sediment organisms (Christensen and Packard, 1977; Olanczuk-Neyman and Vosjan, 1977; Wieser and Zech, 1976). The method determines *in*

* Contribution No. 1 Tiburon Center for Environmental Studies, P. O. B. 855, Tiburon, CA 94920, USA
** Present address: 35 Ward Street, Ashburton, Victoria 3147, Australia

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vitro respiratory enzyme activity and represents maximum potential metabolic activity. Certain assumptions and calibrations are required to convert ETS activity into respiration rate of living organisms. An analogous measure of respiratory enzyme activity is the total dehydrogenase assay (Lenhard, 1956; Pamatmat and Skjoldal, 1974) which is an *in vivo* method. Both methods use artificial electron acceptors which react with reduced substances in sediments other than respiratory co-enzymes, NADH, NADPH, and FADH.

The heat production by sediments has been regarded as a measurable output of natural and undisturbed metabolism by complex communities of organisms (Doyle, 1963; Pamatmat and Bhagwat, 1973), but there have been persistent technical difficulties (Pamatmat, 1975; in press). One problem is how to distinguish metabolic activity from possible heat effects of purely chemical reactions that might be taking place simultaneously (e. g. complexation and neutralization reactions). The use of poisons as commonly done in measuring sediment oxygen uptake (Teal and Kanwisher, 1961) does not work in direct calorimetry because of the long-lasting heat effects of unknown reaction between the poison and reduced compounds or other substances in the sediments (Pamatmat, in press a). There is accumulating evidence, however, that metabolic activity accounts for at least the first order of magnitude of sediment heat production (Pamatmat, unpublished). We think that direct calorimetry is uniquely valuable for understanding sediment metabolism and more workers will be drawn to its use in the future. The present paper is intended to show some positive results while drawing attention to attendant difficulties with the method.

MATERIALS AND METHODS

The first series of measurements was done as part of a group effort to determine day-night cycles in benthic biological activity over a delineated area for 2.5 d. The study site, at Bøoknis Eck, Eckernförde Bight (in the southwestern Baltic Sea), is 11 m deep and comprises a zone of red algae. This biotope has been described by Lüthje (1978). The bottom was poorly sorted coarse sand and gravel overlying glacial clay at about 4–5 cm below the surface. The sediment appeared to be well oxygenated above the glacial clay deposit; interstitial water sampled with a syringe contained from 2.0 to 7.1 ml O_2 l^{-1} (mean of 7 determinations over 2 d = 4.6 ml l^{-1}) without any apparent trend. The water temperature averaged 13.2 °C during the investigation.

In situ oxygen uptake was determined from changes in dissolved oxygen of the water in Plexiglas enclosures over the bottom. Random core samples (32 cm^2 in surface area) were taken by divers periodically from 1600 h on 19 September 1978 to 1200 h on 22 September. Replicate cores were taken independently for ATP, ETS, and heat-flux measurements. ATP was extracted immediately aboard ship, while samples for ETS and heat-flux measurements were carried back to the laboratory inside a cooler with a delay of 1 to 1.5 h.

Sediment layers (0–1.5, 1.5–3.0 and 3.0 to 4.5 cm) were scooped into 50-ml bottles for direct calorimetry. The sediment was exposed to air during transfer and some air bubbles were occasionally trapped in the bottles. Only the 0–1.5 cm layers could be run in the calorimeter immediately since each measurement lasted until the next sample was delivered, 8 to 12 h later. Hence the deeper layers, 1.5–3.0 and 3.0–4.5 cm, were not placed inside the calorimeter until later. They were stored at 8 °C to slow down the metabolic rate and the apparent rate of decrease of the metabolic rate with time until the time for calorimetry when they were warmed to 15 °C before being placed inside the calorimeter. The bottles were immersed in silicone oil in a metal canister during calorimetry, the oil serving as heat conducting medium.

Some of the bottled samples were stored at 8 °C after calorimetry and returned to the calorimeter from time to time for repeat measurements. After the last calorimetry determination, the sediment was analyzed for ATP. All of these sediment samples became anaerobic. Thus the Bøoknis Eck samples included one set of fresh initial and a later set of final anaerobic samples stored for heat flux and ATP comparison.

The rates of heat production were measured at 15 °C with a heat-flow double-twin calorimeter (Pamatmat, 1978; in press b). The instrument's calibration constant is 2.16×10^{-5} W μv^{-1}. One twin chamber was used to measure the heat flux from sediment samples while the other served to monitor, simultaneously and continuously, the baseline for any drift or fluctuation that might occur. This reference baseline showed no detectable drift most of the time; when drift appeared a corresponding correction was added or subtracted from the measured thermopile signal. With appropriate baseline corrections the relative errors in the heat flux measurements are estimated to be less than 10 %.

ATP was extracted from sediment samples (1 ml measured and dispensed with a cut-off syringe) with 50 ml of boiling 0.02 M TRIS buffer (pH 7.8) according to the method of Ernst (1970). The extract was stored at –20 °C until analyzed several days or weeks later. ATP was measured according to the method of Witzel (1979) using a JRB ATP-photometer and stripchart recorder. Recorded light scintillation peak heights were determined from samples and ATP standards. Based on the recovery of a known amount of ATP (833 ng of ATP as disodium salt in 50 μl of distilled water solution) that

was added to the sediment immediately before injecting the sediment sample into boiling TRIS, the recovery rate averaged 60 %. Replicate subsamples of mixed sediment showed ATP values with coefficients of variation of 10 to 15 %.

The ETS activity was measured according to the methods of Christensen and Packard (1977) and Kenner and Ahmed (1975a) modified as follows: 3-ml sediment samples were added to 9 ml of phosphate buffer (pH 8.0). The respiratory enzymes were liberated from the cells by ultrasonic treatment for 3 min (10–15 s on, 25 s off) while the container was immersed in an ice bath to prevent temperature increase above 15 °C. The samples were incubated for 20 min at 20 °C and then the reaction was stopped with 1 ml of 1.0 M H_3PO_4/$HgCl_2$ solution (1:1 proportion). After centrifuging for 10 min at 3500 RPM, sample absorbance was measured at 495 nm. As controls, to determine nonenzymatic reduction of the tetrazolium dye (INT), replicate samples that had been initially treated with the stopper solution were incubated like the experimental samples.

In the subsequent comparisons using sediment from the waterline at seven different beach locations (all samples taken in the morning, one sample per day), the sediment layers (0–1.5 cm only) were thoroughly mixed and transferred into 50-ml bottles for direct calorimetry while subsamples were taken for ATP extraction. All sediment samples were medium to coarse sand and gravel. Chemical and microbiological properties of these biotopes have been described in detail by Meyer-Reil et al. (1980). At the end of 6 to 8 h of calorimetry the sediment was emptied into a dish and quickly mixed again before subsampling for ATP extraction. Separate replicate cores were used for the assay of ETS activity. All sediments were dried at 95 °C to constant weight and all values are expressed per g of dry sediment.

Experiments were conducted to compare ATP and heat production in a batch culture of bacteria that was isolated from freshwater sediment. Under nitrogen atmosphere sediment was streaked onto plates of Brewer's thioglycollate medium (BTM) containing 2 % agar. The plates were incubated at 26 °C in a Gas-Pak incubator with an atmosphere of hydrogen and carbon dioxide. Isolated colonies were subcultured and maintained in BTM tubes. Cell morphology, motility, and Gram reaction were determined using 12-h cultures. Peptone-yeast glucose-extract agar tubes were used to determine gas production (Holdeman et al., 1977. Twenty biochemical reactions were determined using the Analytab Products Inc. sensitivity tests for anaerobic organisms (Shaw, 1979).

The bacterium was identified as *Bacteroides* sp. according to the descriptive keys in the Virginia Polytechnic Institute manual (Holdeman et al., 1977). This *Bacteroides* was a gram-negative nonsporeformer and an obligate anaerobic fermenter. It showed positive activity with glucose, lactose, saccharose, glycerol, cellobiose, and trehalose, among others, and negative activity for urease, catalase, gelatin, arabinose, and sorbitol.

A 500-ml volume of Brewer's thioglycollate medium was inoculated with the *Bacteroides* culture. A 50-ml aliquot was transferred under nitrogen atmosphere into a sterile polyethylene bag which was heat-sealed and placed inside an anoxic aluminum canister for calorimetry at 27 °C. The remainder of the inoculum was kept at the same temperature and subsampled at various stages in the growth cycle, as indicated by the thermogram of the 50-ml aliquot, for ATP determination and cell counts.

RESULTS

Eckernförde Bight Experiment

Comparisons Between Heat Production, ATP and ETS Activity

The heat flux of all samples invariably decreased with time. Typically, but not always, heat flux leveled off near the beginning, following return to thermal equilibrium of the calorimeter after the disturbance of opening and introducing a sample inside, then decreased with time and later leveled off again (Fig. 1). Sometimes heat flux decreased continuously from the beginning before leveling off. For comparison with ATP and ETS in the first experiment we used the highest recorded rates of heat production after the

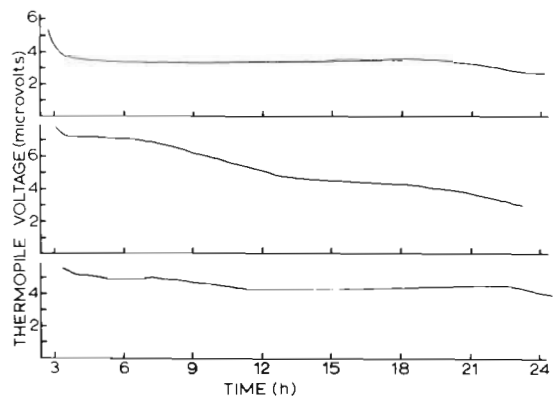

Fig. 1. Actual thermograms of three samples from Bøoknis Eck, Eckernförde Bight, southwest Baltic Sea, at 11 m depth, showing a tendency to stabilize after the calorimeter regained thermal equilibrium (about 4 h after introducing the sample). However, different samples show variable long-term trends in the decrease of heat-production rate. Calorimeter constant = 2.16×10^{-5} W μv^{-1}

Table 1. Heat production, ATP, and ETS activity of sediment (0–1.5 cm layers at 11 m depth in Bøoknis Eck, sampled repeatedly from 19 to 22 September 1978). All values expressed per g dry sediment

Sampling time	Heat production ($W\ g^{-1}$)	ATP concentration (mole g^{-1})	ETS activity ($\mu l\ O_2\ h^{-1}g^{-1}$)	($W\ g^{-1}$)
Sept. 19, 16^{00}	1.49×10^{-6}	1700×10^{-12}	3.32	1.85×10^{-5}
Sept. 20, 8^{00}	2.09	1360	2.67	1.49
Sept. 20, 16^{00}	1.86	912	–	–
Sept. 20, 22^{00}	1.79	784	2.64	1.47
Sept. 21, 8^{00}	1.99	843	2.58	1.44
Sept. 21, 16^{00}	1.90	687	2.75	1.54
Sept. 21, 22^{00}	1.41	1100	2.32	1.30
Sept. 22, 12^{00}	2.10	2130	3.57	1.99
Mean	1.83×10^{-6}	1190×10^{-12}	2.84	1.58
Standard deviation	0.26×10^{-6}	506×10^{-12}	0.44	
Coef. of variation	14 %	43 %	16 %	
Mean ratio of heat production/ATP		1770 W $mole^{-1}$		

instrument had regained thermal equilibrium (4 h after placing the sample inside).

The surface 0–1.5 cm layers from Bøoknis Eck (Table 1) showed rates of heat production ranging from 1.41×10^{-6} W g^{-1} dry sediment on the first sampling to 2.10×10^{-6} W g^{-1} on the last sampling 2.5 d later. The mean rate was 1.83×10^{-6} W g^{-1} with a standard deviation of 0.26×10^{-6}.

The deeper layers which were run several days later had a mean rate of 0.86×10^{-6} W g^{-1} and standard deviation of 0.46×10^{-6}. Repeat measurements several weeks later indicated a continuous decline in metabolic rate (Table 2). After several days inside the bottle, the sediment became black in spots indicating sulfide formation. Many black spots showed the remains of tiny amphipods, Harpacticoids, Tanaids, etc.

Table 2. Heat production rates of coarse sandy sediment from Kiel Bight, showing variable decrease in rates over periods of days and weeks

Sample No.	Layers (cm)	Date	Heat production rate ($W\ g^{-1}$)
1	0 –1.5	Sept. 19	1.49×10^{-6}
		Oct. 11	0.49
2	1.5–3.0	Sept. 27	0.41
		Sept. 28	0.54
		Sept. 29	0.49
		Oct. 17	0.16
3	3.0–4.5	Sept. 27	1.32
		Oct. 17	1.26
4	1.5–3.0	Sept. 25	0.91
		Sept. 26	0.44
		Oct. 22	0.26
5	3.0–4.5	Sept. 25	0.64
		Oct. 20	0.51
6	0 –1.5	Sept. 21	1.90
		Sept. 22	1.22
		Sept. 23	1.19
7	0 –1.5	Sept. 21	1.41
		Oct. 19	1.15

The ATP concentrations (Table 1) in the surface layers ranged from 687×10^{-12} mole g^{-1} dry sediment to 2130×10^{-12} (mean of 1190×10^{-12}).

ETS activity expressed in terms of equivalent oxygen consumption rate (Table 1), using the conversion factor of Kenner and Ahmed (1975a), ranged from 2.32 $\mu l\ O_2$ h^{-1} g^{-1} dry sediment to 3.57 (mean = 2.84, standard deviation = 0.44 $\mu l\ O_2\ h^{-1}\ g^{-1}$).

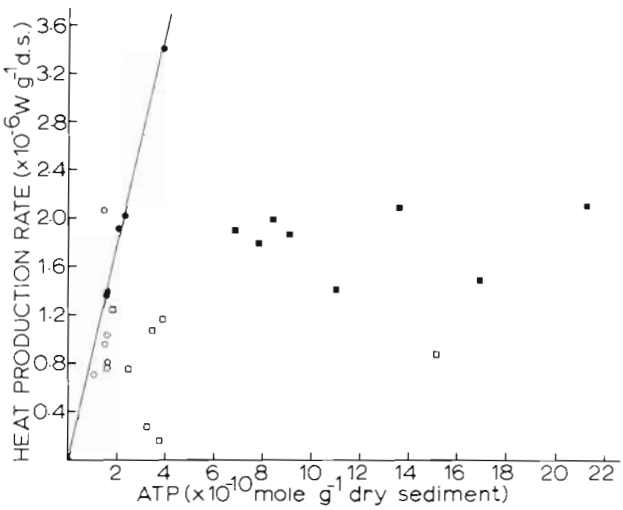

Fig. 2. Relationship between heat-production rate and ATP concentration in sandy beaches (circles) and Bøoknis Eck subtidal sediment (squares). Closed symbols: initial rates after thermal equilibration of calorimeter; open symbols: final rates after 1 or more d. Initial and final beach samples were all aerobic. Subtidal samples depleted their oxygen supply and were anoxic at the end. The regression equation for initial beach samples ($r^2 = 0.987$, 3 df) is $Y = 8.710X - 3.65 \times 10^{-8}$, where Y is heat flux in W g^{-1} dry sediment and X ATP in moles g^{-1} dry sediment. Mean heat flux/ATP ratios are 8.07×10^3 for the combined initial and final beach samples, and 2.29×10^3 for the combined initial and final subtidal samples

Table 3. Heat production, ATP concentration and ETS activity of beach sediments at beginning and end of calorimetric measurements. All values expressed per g dry sediment

	ATP concentration (mole g^{-1})		Heat production (W g^{-1})		ETS (W g^{-1})
	Beginning	End	Beginning	End	Beginning
Hindenburgufer-Falkenstein (combined)	160×10^{-12}	158×10^{-12}	1.36×10^{-6}	0.95×10^{-6}	5.39×10^{-6}
Mönkeberg	393	149	3.40	2.07	7.06
Strande		111	0.99	0.71	4.17
Laboe	209	169	1.91	0.81	6.61
Surendorf	164		1.38	0.97	4.67
Heidkate	235	164	2.02	1.03	4.78
Mean	232	150	1.84	1.09	5.45

Mean ratios of heat production/ATP concentrations: Beginning, 8660 W $mole^{-1}$; end, 7470 W $mole^{-1}$; combined, 8070 W $mole^{-1}$

There was no correlation between heat production rate and ETS ($r^2 = 0.05$), or between heat production and ATP ($r^2 = 0.0078$) but there was a statistically significant correlation between ATP and ETS ($r^2 = 0.67$) of aerobic surface 0–1.5 cm samples. The relationship remained the same whether ATP values were corrected for recovery rate or not.

Using Ivlev's (1934) oxycalorific coefficient of 4.8 cal ml^{-1} O_2 consumed, the average ETS activity is equivalent to 3.79×10^{-6} cal $s^{-1}g^{-1}$ dry sediment ($= 1.58 \times 10^{-5}$ W g^{-1}). Comparing this value with the average measured heat flux of 1.83×10^{-6} W g^{-1}, the ratio of respiration to ETS is about 0.12. This is a little lower than the respiration: ETS ratio of about 0.16 to 0.17 for phytoplankton organisms (Kenner and Ahmed, 1975b; Christensen and Packard, 1979) and about the same as that of substrate-limited bacteria in senescent stage (Christensen et al., 1980).

On the average, the ratio of heat production to ATP concentration was 1.77×10^3 W $mole^{-1}$ ATP, which is not significantly different ($t = 1.27$, 14 degrees of freedom) from that of 8 samples that became anaerobic (Fig. 2) with a mean of 2.82×10^3 W $mole^{-1}$ ATP. The combined initial and final anaerobic samples from Bøoknis Eck show a significant correlation between ATP concentrations and rate of heat production ($r^2 = 0.33$, 14 df), with a mean ratio of 2.29×10^3 W $mole^{-1}$ ATP.

Comparison Between *in situ* Oxygen Uptake and Integrated Heat Production

Measurements of *in situ* oxygen uptake by the sediment surface over the entire 2.5-d period gave an average value of 15 ml O_2 $m^{-2}h^{-1}$ (Schramm, personal comm.). Using Ivlev's (1934) oxycalorific coefficient of 4.8 cal ml^{-1} O_2 ($= 20.1$ joules ml^{-1}), this oxygen uptake is equivalent to 300 J $h^{-1}m^{-2}$ ($= 84 \times 10^{-3}$ W m^{-2}). Direct calorimetry of surface plus subsurface layers, on the average, gave us an integrated value for the 4.5 cm thick sediment layer of 280×10^{-6} W $core^{-1}$, or 87×10^{-3} W m^{-2}.

Sand Beach Experiment

Both ATP and heat flux decreased from beginning to end (Table 3). There is a highly significant correlation between heat production rate and ATP for the beginning ($r^2 = 0.996$, 3 df) but not the end samples, evidently as a result of a disproportionate drop in ATP and heat production rate of the Mönkeberg sample. The ratio of heat production to ATP averaged 8.66×10^3 W $mole^{-1}$ ATP in the beginning and dropped to an average of 7.48×10^3 at the end. The difference between these ratios is not statistically significant ($t = 0.73$, 8 df). The overall mean for the combined data is 8.07×10^3 W $mole^{-1}$ ATP.

Unlike the Bøoknis Eck data, there is no significant correlation between ETS activity and ATP concentration ($r^2 = 0.49$, 3 df).

Unlike the subtidal data, the beach data show a significant correlation between ETS and heat production ($r^2 = 0.65$) with a mean ratio of 0.33 when both processes are expressed in W g^{-1} dry sediment, that is, ETS activity is converted to W g^{-1} dry sediment using the factor of 1 μl O_2 = 1.42 $A_{1\,cm}^{490\,nm}$ (Kenner and Ahmed, 1975a) and 1 ml O_2 = 20.1 J of heat energy released (Ivlev, 1934).

Comparison between Bøoknis Eck and sand beaches. The initial Bøoknis Eck subtidal samples had higher ATP content than the beach samples (average of 1.19×10^{-9} versus 2.32×10^{-10} mole g^{-1} dry sediment) but generally the same or lower rates of metabolic heat production (Fig. 2). The ETS activity of the beach sediments averaged 3.4 times that of the subtidal sediments. The heat production/ATP, ETS/ATP, and heat production/ETS ratios are all significantly higher for the beach samples than for the subtidal sediments ($t \geq 3.50$, 10 to 24 df, Table 4).

Table 4. Ratios of the different measures of metabolic activity in subtidal and beach sediments in the southwestern Baltic Sea

	Heat production/ATP (W mole^{-1})	ETS/ATP (W mole^{-1})	Heat production/ETS
Bøoknis Eck (subtidal)			
Beginning	1.77×10^3	1.45×10^4	0.12
End	2.81		
Combined	2.29		
Sandy beaches (waterline)			
Beginning	8.66	2.64×10^4	0.33
End	7.48		
Combined	8.07		
Sandy beaches/Bøoknis Eck ratios	$\frac{8.07 \times 10^3}{2.29 \times 10^3} = 3.5$	$\frac{2.64 \times 10^4}{1.45 \times 10^4} = 1.8$	$\frac{0.33}{0.12} = 2.8$

Laboratory Experiment with Bacteroides Culture

The thermogram, ATP, and cell counts depict the characteristic growth cycle of a bacterial batch culture (Fig. 3). However, the shapes of the three curves vary and the thermogram peaked 1.2 h before the cell counts and ATP reached their highest values. ATP tended to level off while cell counts were decreasing, presumably as cells lysed, indicating that the ATP content per cell was increasing during this particular growth phase. This is in agreement with Forrest (1965) who observed that when no growth can take place (either as a result of inhibiting metabolic waste accumulation or exhaustion of substrate) catabolism of available energy gives rise to increase in the ATP pool level. Finally, metabolic heat production decreased steeply while ATP concentration changed little and cell counts decreased as the culture completed its growth cycle. The changing ratios of heat flux/ATP (Table 5) clearly showed that if heterogeneous samples containing different physiological states of organisms were pooled together there would be a poor or no correlation between heat flux and ATP.

Table 5. Ratios of heat production rate to ATP concentration (W mole^{-1}) during growth cycle of a batch culture of anaerobic *Bacteroides* sp. isolated from freshwater sediment

(1) Early exponential	34.9×10^3
(2) Mid exponential	50.1
(3) Late exponential	50.7
(4) Stationary	13.3
(5) Early death	4.34
(6) Mid death	1.68
(7) Late death	1.62

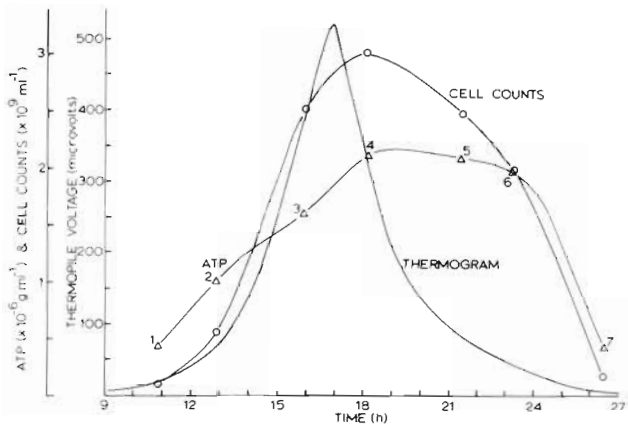

Fig. 3. Continuous measurement of heat flux and periodic determinations of ATP concentration and cell counts of an anaerobic batch culture of *Bacteroides* sp. in Brewer's thioglycollate medium. The sample (a 50-ml aliquot) was placed inside the calorimeter at time zero. The remainder, outside the calorimeter, was subsampled at the successive times indicated

DISCUSSION

It is clear that a disadvantage of direct calorimetry with the present instrument and technique is the length of elapsed time (about 4 h) before useful heat-flux measurements could be obtained. Obviously, for the instrument to be of use in determining natural metabolic rates metabolic activity should not change significantly during the thermal equilibration period. We think that the beach-sand samples maintained their natural activity long enough but it could be questioned whether the subtidal samples from Bøoknis Eck also did.

The measured 'initial' heat production of Bøoknis Eck sediment occurred after a delay of nearly 6 h, of which 4 h were after the bottle had been sealed. The important question is, by how much did the Bøoknis Eck sediment's metabolic rate decline during this period? The close agreement between independent estimates derived from calorimetry and from *in situ*

oxygen uptake indicates that the level of metabolic activity inside the bottle after thermal equilibration was still practically the same as *in situ* activity. This might seem surprising in view of the decrease in oxygen tension during calorimetry. One sample had 1 μl O_2 ml^{-1} of interstitial water after 12 h. Because of the different metabolic rates and the different amounts of air bubbles sometimes trapped in the sediment during transfer, some samples probably depleted their oxygen faster than others but all of them probably had relatively low oxygen tension by the time heat flux could be measured. A steady calorimeter signal of 5 microvolts, assuming fully aerobic metabolism, is equivalent to a rate of oxygen consumption of 5.4×10^{-3} μl O_2 s^{-1} and the aerobes would have used up all the avaibable oxygen in less than 4 h. The maintenance of metabolic rate with decreasing oxygen tension is well known, but a substained metabolic activity to the point of anoxia could also mean the relative importance of anaerobes in the sediment. A predominance of oxyregulators and facultative anaerobes in Bøoknis Eck is possible if the sediment naturally experiences low oxygen tension. Seven samples of interstitial water from time to time over a 2-d period showed 2.0 to 7.1 ml O_2 l^{-1}. Dissolved oxygen could have been lower at other times.

In the case of beach samples, the sediments were only moist with pellicular water and hence the bottles contained much more oxygen. Assuming that total air volume inside the bottles was 10 %, there would have been 10^3 μl O_2 available (vs about 40 μl in the subtidal samples) and O_2 supply would have lasted at least 10 times longer than in the Bøoknis Eck samples. There is no doubt that the beach samples remained fully oxic.

Since the initial heat flux values for Bøoknis Eck sediment appear reasonably realistic, we may compare initial heat flux, ATP, and ETS for Bøoknis Eck and the beach samples. These comparisons show marked differences in the following respects:

(1) Excellent correlation between heat flux and ATP for the beach samples and lack of correlation for the subtidal samples; (2) significant correlation between ETS and heat production for the beach but not the subtidal samples; (3) significant correlation between ATP and ETS for the subtidal but not the beach samples; (4) consistently higher absolute values of the various ratios for the beach samples than the subtidal samples; (5) five times higher mean ATP concentration in Bøoknis Eck but the same mean heat flux as in the beach samples. This last difference signifies lower turnover rates of ATP in Bøoknis Eck than in the beach samples.

The evident variability in the correlation between heat flux and ATP could be related to different species or metabolic types of organisms, to different physiological states of bacteria, or different stages of growth as shown by *Bacteroides* batch culture experiment. The ratios of heat production rate to ATP concentrations appear relatively constant during growth (Table 4) but decline rapidly during senescence. All of the beach samples may have contained actively growing bacterial populations while each of the Bøoknis Eck samples contained microorganisms in various stages of growth as well as mixtures of macrofauna and meiofauna. The lower heat flux/ATP ratio for subtidal samples than the beach samples further indicate active growth in the latter in contrast with the former. Likewise, the higher heat production/ETS and ETS/ATP ratios indicate higher turnover rates of ATP for the beach samples than the subtidal samples.

The difference between the subtidal and the beach samples with respect to the heat-flux-ATP correlation indicates that the use of ATP alone as a measure of metabolic rate is subject to error. ATP assays determine pool size whereas metabolic activity should be proportional to turnover rate of ATP. There is no fixed relationship between pool size and turnover rate, as the *Bacteroides* experiment shows, although under certain uniform conditions there may be an excellent correlation between them, as shown by the beach samples. When comparing ATP concentrations in different geographical areas, different seasons, different depths, different temperatures, different food supply conditions, different oxygen conditions, etc., it will be well to keep in mind that lower ATP values may not mean lower metabolic rates.

A decline in oxygen uptake/ETS activity ratio of bacterial cultures from growth to senescence has been noted by Christensen et al. (1980). It is interesting that in spite of the expected variability in respiration rate/ETS activity ratio arising from differences in physiological state of natural populations Christensen et al. (1980) noted good predictability of respiratory rates by natural plankton communities from measurements of their ETS activity. On the other hand, our data show that for sediments neither measures of ETS activity nor ATP would be reliable estimators of actual metabolic rate without correction for other factors. The apparent difference between planktonic and sediment communities implies a higher degree of homogeneity among planktonic organisms than sediment organisms, which is not surprising in view of the co-occurrence of aerobes, and facultative and obligate anaerobes of various kinds in sediments. The discounted importance of microbial senescence as a source of variability in the plankton may also indicate that microbial communities in the plankton are more at steady state than those in sediments. Plankton fallout, physical disturbance of sediment layers through bioturbation, etc., random availability of dead mac-

rofauna, are some events that could disturb otherwise steady state conditions in the sediments.

Both the beach and subtidal sediments showed one trend in common: they all decreased in ATP content and heat production rate with time. To what extent the decrease was due to exhaustion of food substrate or electron acceptors we do not know. It must be assumed that the microbial composition of the stored sediments, especially the Bøoknis Eck samples, changed with time. Thus the decrease in heat production rate must have been the combined effect of several changes that occurred. In any case, the decrease in ATP and heat flux shows that the decrease in heat production was related to a decrease in activity of living organisms. It is interesting, however, that although the ATP concentrations of the subtidal sediment dropped to the level of the beach samples the final heat production of the beach samples remained consistently higher than that of the former. We do not know if this could be attributed largely to the beach samples being still oxic at the end while the subtidal samples were definitely anoxic.

The lack of correlation between heat flux and ATP for the initial and final Bøoknis Eck samples, taken separately, could be viewed as an indication that heat flux by sediments is largely due to some other chemical reactions besides those involved in metabolism. Just how much of the measured heat flux was due to metabolic activity and how much to possible extracellular chemical reactions remains unknown. There is good agreement between oxygen uptake and heat flux measurements indicating that the latter represents metabolic activity (Pamatmat, 1978, in press b, and comparison with *in situ* oxygen uptake reported here), but anaerobic heat production, at least of macrofauna, cannot be fully explained by thermodynamics of known chemical reactions in intermediary metabolism (Gnaiger, 1980). Equivalence between heat flux and pure metabolic activity will be even more difficult to show for mixed microbial populations in sediments. Anoxic sediments, both live and poisoned, after extended isolation in a sealed glass container ultimately show no detectable heat production (Pamatmat, unpublished). This indicates that non-metabolic or extracellular heat production is tightly coupled to the metabolic activity of living organisms, i. e. death of the organisms ultimately leads to disappearance of measurable non-metabolic heat. If this is the case, we cannot experimentally partition total heat flux and directly measure the heat flux from natural extracellular chemical reactions and it must be estimated indirectly from known specific chemical reactions that result from excreted by-products of metabolism.

In spite of the present difficulties with direct calorimetry, we continue to believe that the technique is indispensable for the full understanding of sediment metabolism. No other single technique appears to be capable of measuring total undisturbed metabolism of mixed metabolic types. One Joule of heat produced by aerobes is equivalent to 1 J produced by fermenters, sulfate reducers, or any other type. However, different metabolic types will have degraded different kinds and amounts of substrate to different kinds and amounts of end-products in the course of producing the same amount of heat. Ultimately, we will want to determine the relative contribution of different metabolic types and this task will require a combination of other techniques. Calorimetry will then be necessary to show that the integrated activity of all metabolic types, each measured by a different technique and all results converted to their energy equivalents, will agree with direct measurements of total community metabolism. Any discrepancy should be helpful in tracking down possible sources of error in our methods.

We may not now be able to distinguish heat of extracellular chemical reactions from metabolic heat, but total heat flow from sediments should, nonetheless, be an important parameter in studying the energetics of ecosystems. Our present working hypothesis is that measured heat flux of both fully oxic and fully anoxic sediments is due to metabolic activity. However, heat flux of anoxic sediment receiving a supply of oxygen, from the sediment surface, from pockets of air bubbles or oxygenated water in burrows, tubes, etc., will inevitably include considerable heat from oxidation of sulfides and other reduced substances. Heat of extracellular chemical reactions as a fraction of total heat evolution by undisturbed sediments seems even more difficult to determine directly than uptake of oxygen by inorganic chemical oxidation as a fraction of total oxygen uptake (Pamatmat, 1977). The two processes should be equivalent, however, and experiments are needed to show this.

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