IN SITU METABOLISM OF BENTHIC COMMUNITIES

by

Mario M. Pamatmat

Department of Fisheries and Allied Aquacultures, Agricultural Experiment Station, Auburn University, Auburn, Alabama 3*830, U.S.A.

Résumé

Etude in *situ* du métabolisme des communautés benthiques.

Il n'existe pas de méthode unique permettant de mesurer le métabolisme d'une communauté benthique de sédiment meuble. Le taux d'oxygène consommé par le sédiment de surface sous-estime d'autant plus le métabolisme communautaire que l'anaérobiose du sédiment sous-jacent prend une importance croissante tout le long de la colonne de sédiment. La calorimétrie directe de la chaleur dégagée par le métabolisme des organismes benthiques en place dans le sédiment peut être faussée par d'autres réactions thermo-chimiques concomittantes inconnues. L'entropie du système sédiment + organismes peut être le siège de poussées étonnamment fortes conduisant à une nette endothermie qui peut masquer complètement la production de chaleur biologique. Bien que tous les organismes emploient le même mécanisme de base pour extraire l'énergie utilisable le long de la chaîne respiratoire par oxydo-réduction, il semble douteux qu'une seule technique d'approche, basée sur des transporteurs ou des accepteurs artificiels d'électrons, puisse déterminer d'une manière également fiable l'activité métabolique de tous les organismes d'une communauté benthique. Le rapport variable entre l'activité de la déshydrogénase — ainsi qu'on la mesure par la réduction du chlorure de 2, 3, 5-triphényltétrazolium — et la concentration d'ATP dans les sédiments, suggère que ni l'ATP, ni la charge d'énergie ne peuvent être utilisés pour l'estimation du taux de métabolisme dans les sédiments.

Le taux annuel d'oxygène consommé par la surface du sédiment peut être considéré comme une approximation de la fraction annuelle de matière organique fournie au fond et oxydée au cours d'une année. Dans un bassin sédimentaire, si le taux de sédimentation de la matière organique et l'activité biologique de la communauté benthique dans cette colonne de sédiment sont stables, le métabolisme annuel de la communauté dans cette colonne de sédiment représente alors la fraction totale complètement oxydée chaque année. L'interprétation du métabolisme de la communauté benthique sous l'angle du flux d'énergie à travers le reste de l'écosystème, dépend de l'équilibre sédimentation-érosion du faciès dans lequel se situe la communauté.

Introduction

Community metabolism measurements are very desirable in the study of energy flow through ecosystems (Jansson, 1972). The biotic composition of ecosystems is so diverse and complex that it would be an immense task to measure the energy flow through every single population. The analytical approach is especially intimidating with respect to micro-organisms because of the possible problem of disrupting associative and antagonistic relationships (e.g. see Bryant et al., 1967, Cappenberg, 1974 a, b). The holistic approach of community metabolism measurements allows us to view an entire community in relation to other communities in an ecosystem, e.g. the role of the benthos in total energy flow through ecosystems.

Measurement of total community metabolism is made possible by the uniformity of bioenergetic mechanism across the phyletic spectrum in the

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community. There is no problem other than procedural when dealing with strictly aerobic communities, say of the plankton, whose members all use oxygen as terminal electron acceptor. In such a community, an amount of oxygen used by one species is equivalent to the same amount of oxygen used by another. This is the advantage of metabolic measurements over biomass and numbers of individuals; biomass or number of one species is not always equivalent to the same biomass or number of another.

Benthic ecologists are faced with serious theoretical and technical problems when dealing with the total metabolism of benthic communities. It is time to examine the methods available to us and determine exactly what it is that measurements applied to sediments mean.

Since von Brand (1946) reviewed the literature on anaerobic metabolism, there have been more reports indicating the likely importance of facultative anaerobic invertebrates and of strict anaerobes in benthic community energetics (Martin, 1961; Read, 1961; Theede et al., 1969; Fenchel, 1969; Fenchel and Riedl, 1970; Mangum, 1970; Mangum and van Winkle, 1973; Ott and Schiemer, 1973; Dries and Theede, 1974; etc.). Saz (1971), Hochachka and Mustafa (1972), Hochachka (1974) and others have endeavoured to explain the metabolic pathways in facultative anaerobes and show that anaerobic metabolism is much more efficient than formerly believed. The metabolism of anaerobic and fermentative bacteria (Stephenson, 1966; Doelle, 1969) have received much more attention and all the metabolic types have been found in sediments.

A typical benthic community, therefore, should be expected to include aerobic and anaerobic macrofauna, meiofauna, microfauna, bacteria and other prokaryotic microorganisms. This assemblage of organisms occupies a sediment layer that is oxygenated at the surface, anoxic but to some extent oxygenated in midlayer through mixing and ventilating activities of macrofauna, and permanently anoxic below a certain depth.

We then have the problem of finding the common bioenergetic mechanism among this complex assemblage of organisms on which to base a procedure for measuring their collective metabolic activity.

Oxygen uptake by the seabed

The rate of total oxygen uptake by the sediment surface has been assumed to be an integrated measure of metabolic activity in the sediment column, i.e. of the entire benthic community (Teal and Kanwisher, 1961). According to this assumption, all anaerobes produce reduced metabolic by-products which are oxidized in the sediment or diffuse to the surface where they are stoichiometrically oxidized by oxygen. For example, in the case of a sulfate reducer, such as *Desulfovibrio*

$$H_2SO_4 + 8\ H \longrightarrow H_2S + 4\ H_2O$$

and subsequently, H_2S diffuses to the sediment surface and reacts with oxygen

$$H_2S + 2O_2 \longrightarrow H_2SO_4$$

the two processes being at some equilibrium. It is clear that, in this case, the net reaction is the oxidation of the substrate by oxygen.

Unfortunately, sulfate reduction is only one part of the sulfur cycle (Kuznetsov, 1968) and, during the course of the measurement, H_2S may not be oxidized to SO_4 but to a lower oxidation state such as sulfite or thiosulfate (Cline, 1968). Furthermore, although sulfate reducers may indeed be an important group of anaerobes (Kanwisher, 1962; Jorgensen and Fenchel, 1974), denitrifiers are found to be important in other locations (Lathrop, 1970; Goering and Pamatmat, 1971;

Chen et al., 1972) and the latter's metabolic by-product is nitrogen gas. For *Thiobacillus denitrificans,* a chemolithotroph, the overall reaction (Zajic, 1969) is

$$5\ S + 6\ KNO_3 + 2\ H_2O \longrightarrow K_2SO_4 + 4\ KHSO_4 + 3\ N_2$$

This metabolic activity, as well as those of various fermentative bacteria and methane bacteria (Doelle, 1969), would never be detected in terms of oxygen uptake. Methane bacteria become more important in deeper sediment layers than sulfate reducers which predominate near the sediment surface (Cappenberg, 1974 a; Martens and Berner, 1974). For these reasons, and the fact that oxidizable metabolic by-products of anaerobic metabolism accumulate in subsurface sediments (Pamatmat, 1971 a, 1973; Pamatmat and Bhagwat, 1973), total oxygen uptake underestimates total benthic community metabolism. Direct estimates of anaerobic metabolism reveal how much inorganic chemical oxidation could underestimate anaerobic metabolism (Pamatmat and Bhagwat, 1973). In organically rich sediments of Lake Washington anaerobic metabolism was up to 39 times greater than indicated by the rate of inorganic chemical oxidation. The deeper the layer where anaerobes can exist and the more intense anaerobic metabolism is the more greatly it will be underestimated by chemical oxidation.

The shipboard technique of measuring oxygen uptake (Pamatmat, 1971b) may sometimes underestimate macrofaunal respiration. To estimate the fraction of total oxygen uptake which may be due to macrofauna, the latter's respiration has been computed from equations relating oxygen uptake to biomass and sometimes also temperature (Carey, 1967; Pamatmat, 1968; Smith, 1971; Banse et al., 1971; Nichols, 1972; Smith et al., 1972).

With this method, the results tend to show that where there are large burrowing macrofauna, such as *Brisaster* and *Molpadia,* the oxygen uptake by the macrofauna alone would account for the total measured respiratory uptake, leaving none for meiofauna, microfauna and bacteria which may be presumed to be present there (Nichols, 1972). I have also often measured the respiratory uptake of sediment cores which, at the end of the experiment, were found to contain deep-dwelling macrofauna, e.g. a Nemertean, *Brisaster, Travisia,* and the respiratory uptake of those cores was not any higher and sometimes even lower than the oxygen uptake of replicate cores that did not contain such animals. This raises the question of the extent to which these macrofauna are facultative anaerobes that show high metabolic rates during laboratory experiments in which their oxygen uptake is measured in oxygenated water. As anaerobes during the shipboard measurements, their metabolic activity in sediment would not be measured by the oxygen uptake method.

Consequently, there are two possible sources of error: 1) measured in *situ* rates of oxygen uptake could miss part of macrofaunal respiration if this is partly anaerobic, and 2) the computational method, based on results of unrealistic respiration experiments, could overestimate macrofaunal respiration. Both errors could lead to erroneously high macrofaunal respiration relative to apparent community metabolism.

Carbon dioxide evolution

Carbon dioxide evolution and oxygen uptake of an intertidal benthic community have been simultaneously measured (Teal and Kanwisher, 1961; Pamatmat, 1968). Teal and Kanwisher obtained respiratory quotients (RQ) ranging from 0.50 to 0.96, while Pamatmat's values ranged from 0.26 to 0.81 with one value of 1.35.

By comparison, the complete oxidation of fats and carbohydrates yields RQ values of 0.707 and 1.0, while RQ for protein varies somewhat according to the incompletely oxidized products and is commonly assumed to be 0.80 (Swift and Fisher, 1964). Actually measured respiratory gas exchanges in homoiothermic animals, however, have shown values much lower than 0.7 and much higher than 1.0 (Kleiber, 1962). Whether RQ of aerobic poikilotherms could also range beyond the limits of 0.7 to 1.0 remains to be seen.

RQ of benthic communities which are mixtures of aerobes and anaerobes, of heterotrophs and chemoautotrophs, does not mean the same thing as RQ of a group of aerobes alone. In the latter case, one compares the carbon dioxide evolution with the oxygen consumption of the same animals. In the case of benthic communities, the ratio of CO_2 production to O_2 consumption will depend on community composition. Part of the oxygen is consumed by chemical oxidation of H_2S and reduced metals (Teal and Kanwisher, 1961) without accompanying CO_2 evolution. At the same time, CO_2 could be liberated by the chemical action on carbonates by lactic acid and other organic acids produced by fermentative organisms. Chemoautotrophic bacteria take up from 3 to 90 percent of their carbon requirement from free CO_2 (Sorokin, 1969) ; thus O_2 uptake by some of these organisms would be accompanied by variable CO_2 evolution. On the other hand, some anaerobes, e.g. fermenters and methane bacteria (McCarty, 1964) produce CO_2 without consuming oxygen. Hence, one can expect benthic community RQ to be much more variable than the range of 0.7 to 1.0 of a typical aerobic animal. Assumed benthic community RQ (McIntyre et al., 1970; Pamatmat, 1971a; Hargrave, 1973) could be very different from the true value.

Measurements of CO_2 evolution do not appear to have any advantage over oxygen uptake; however, simultaneous measurements of both could be quite informative about benthic community composition and CO_2 measurements would be more accurately relatable than oxygen measurements to the carbon cycle.

Direct calorimetry

All living cells produce heat energy which is directly related to rate of metabolism (Walker and Forrest, 1964). The relationship between metabolic heat release, oxidation of carbohydrates, etc., oxygen consumption and carbon dioxide evolution has been well studied in homoiotherms (Brody, 1945; Kleiber, 1962). Direct calorimetry of

microbial metabolism dates back to 1856 (Winzler and Baumberger, 1938) but with the exception of the work of Prat (1956), direct calorimetry has rarely been applied to poikilotherms.

The advantage of applying calorimetry to mixed populations of microorganisms in the rumen, silage, soils, or sewage has been recognized by Forrest et al. (1961) who thought that the complexity of the microbial community and their natural habitat would not invalidate the measurement of overall catabolic reaction in terms of heat production. They cautioned, however, that unsuspected side reactions may considerably modify overall heat production. With this understanding, Doyle (1963) applied direct calorimetry to the study of anaerobic bacteria in sediments. Pamatmat and Bhagwat (1973) experienced difficulty in the routine use of their calorimeter and proposed as did Doyle, that its greatest usefulness would be for calibrating a chemical method.

Description of calorimeter and its principle of operation

There are different kinds of calorimeters (see Brown, 1969). The conduction type (Evans, 1969) represented by the gradient-layer calorimeter (Poppendiek and Hody, 1972) appears to be the simplest in principle and operation and the most suitable for investigation of benthic community metabolism.

The calorimeter that I am presently using is a double-walled, stainless steel cylinder enclosing a thermopile made of 12,000 junction sets of copper-constantan. It is 64 cm high overall. Its inner chamber is 6.6 cm in diameter and 41 cm deep. The inner dimension was dictated by the size of the sediment cores used in shipboard measurements of oxygen uptake (Pamatmat, 1971b) because our original intention was to measure the metabolic heat production of such intact cores.

The instrument setup (Fig. 1) shows the calorimeter buried in sand inside a polished, stainless steel, vacuum Dewar. The Dewar vessel is contained within a double-walled tank filled with water. In the early stage of my work (Pamatmat et al., 1973), water was circulated through this tank from an external water bath. Later the temperature control system was placed directly in this water-filled tank. In both setups, the calorimeter did not exhibit satisfactory baseline stability in variable ambient temperatures. Consequently, another annular water container made of fiberglass, was installed around the first water tank and the temperature control system was placed in this outer tank. In this last arrangement, a step change in temperature of the water in the outer tank causes a signal output change about 2 h later. With the two previous setups, the time lag following a step change was only about 10 min. The additional time lag in the present setup reflects the greater ability of the environmental control system to damp ambient temperature changes than before.

The principles of thermoelectricity, their application to the measurement of heat flux, and the theoretical background and problems of microcalorimetry are fully discussed by Calvet (1956).

Briefly, a thermal energy liberated by a sample inside the calorimeter flows through the calorimeter wall and the thermopile into the surrounding heat sink, a small temperature difference develops in the direction of heat flow, i.e. the inner wall becomes slightly warmer than the outer wall. This temperature difference is proportional to the rate of heat flow (Q) and the thermal resistance of the wall (R),

$$\Delta t = QR$$

R being equal to d/ka, in which d is wall thickness, k is thermal conductivity, and a is the inner wall area.

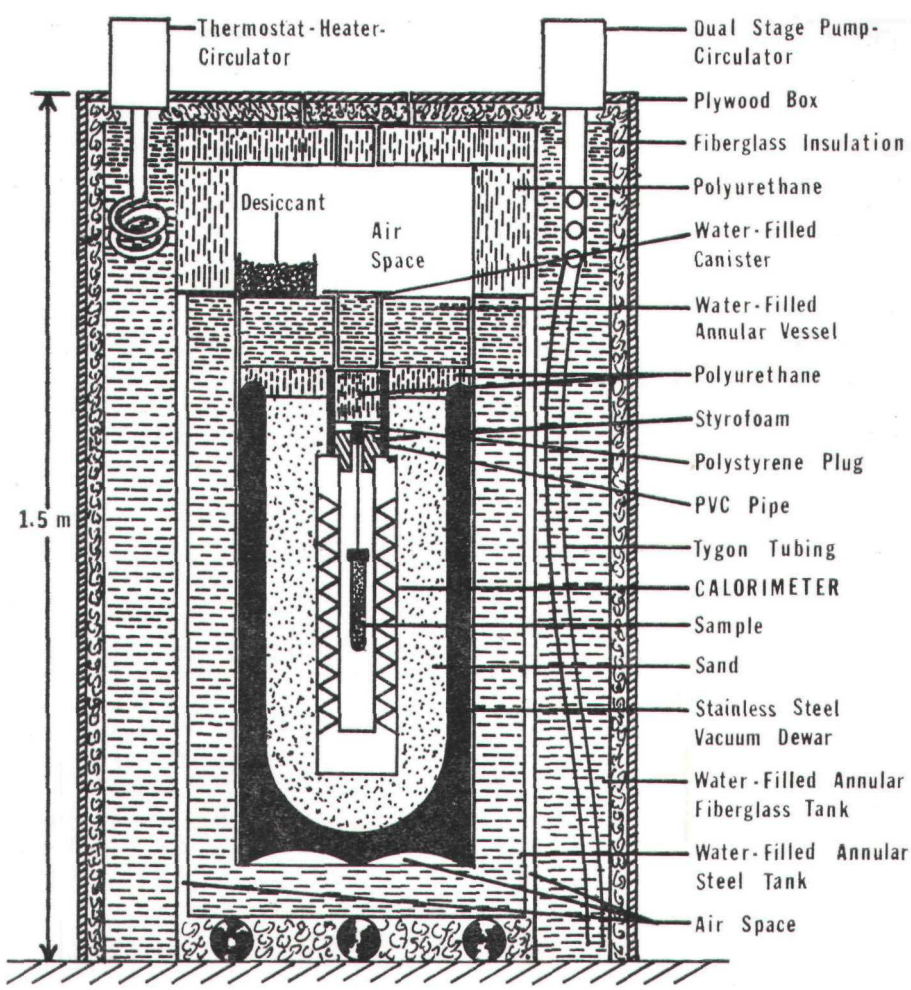

FIG. 1
Calorimeter setup showing a cross-section of the environmental temperature control system.

The sample is kept in the air space above the calorimeter to attain the temperature of the calorimeter as closely as possible before transferring inside; it is suspended inside the calorimeter by a thin monofilament nylon.

The sensitivity of the calorimeter is expressed in terms of the signal output (thermal e.m.f.) of the thermopile at a certain rate of heat flow,

$$S_c = \text{thermal e.m.f.}/Q$$

It may also be expressed as the reciprocal of S_c, in which case the units would be in cal/s per microvolt or their equivalent. S_c is most easily determined by passing a known electrical current (I) through a known resistance (R) inside the calorimeter chamber (Berger, 1969), in which case

$$Q = I^2R$$

or since voltage (E) equals IR,

$$Q = E^2/R$$

Thermal e.m.f. is a function of Δt and the sensitivity of the copper-constantan thermopile (S_s) :

$$\text{Thermal e.m.f.} = \Delta t S_s$$

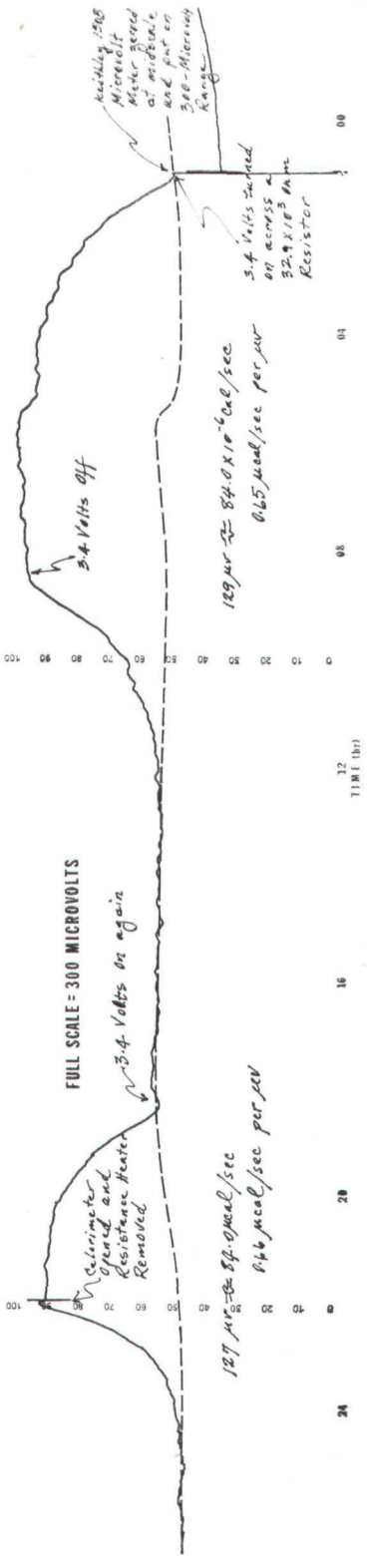

FIG. 2

Changes in thermal e.m.f. during calibration with an electrical resistance heater.

Removal from the calorimeter of the heater when a constant electrical current was flowing through it is identical to the procedure for measuring the steady metabolic heat release of a sample. The dashed line indicates the apparent fluctuation in the baseline.

removal varies from 1 to 5 h, probably depending upon the degree of thermal disturbance introduced to the system when handling and removing the successive thermal barriers and finally the calorimeter lid. This disturbance, however, causes only a transient change in c.m.f. and any drift in the baseline is readily determined by prolonged monitoring. Thus the difference in e.m.f. before and after sample removal represents a much more reliable estimate of heat flow.

The calorimeter is also subject to short-term instability caused by adiabatic heating and cooling of air in the chamber, during rapid barometric pressure changes (Fig. 3). According to Poisson's equation (Byers, 1959)

$$\frac{T}{T_o} = \left(\frac{p}{p_o}\right)^{0.286}$$

where T_o and p_o are the initial temperature in °Kelvin and pressure in millibars, T and p are the final values. The exponent 0.286 varies according to relative humidity and is a function of air mass and specific heat at constant pressure. Tremendous fluctuations in thermal e.m.f. accompany barometric pressure changes during a thunderstorm; however, the fluctuations are transient effects and dissipate rapidly. This source of

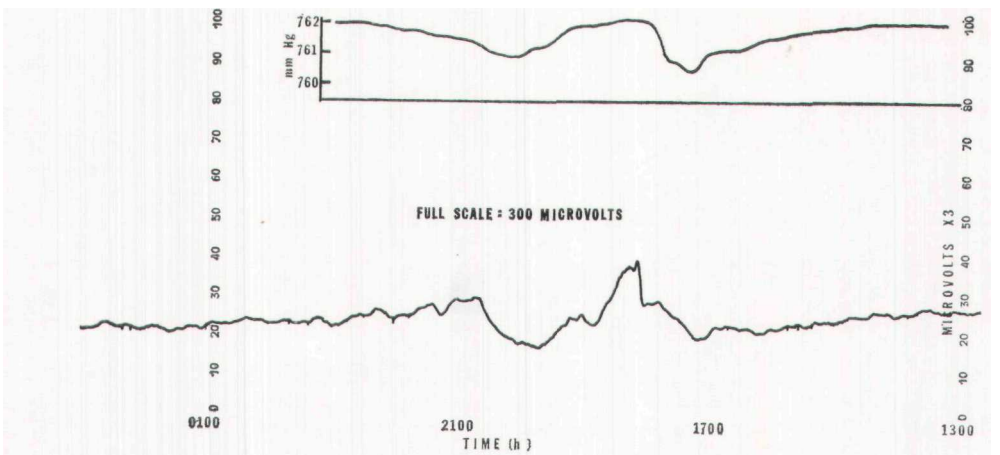

FIG. 3

Noise in the signal output of the calorimeter caused by adiabatic heating and cooling of the air inside the calorimeter due to rapid barometric pressure changes.

noise could be eliminated by an air-tight seal on the chamber but this would cause other technical difficulties. It is better to simply allow the atmospheric disturbance to pass before removing the sample from the calorimeter.

Since the resulting thermal e.m.f. after placing the sample inside the chamber has little to do with metabolic heat production (Fig. 4), it is not necessary to monitor the instrument until about 10 h later. The calorimeter is monitored continuously thereafter at the most sensitive range (e.g. 300-microvolt range) for at least 2 h until a definite steady state has been observed. If large fluctuations due to adiabatic heating or cooling are evident, the strip-chart recording is continued until the disturbance is well over. Then, the sample is quickly removed. The difference between the e.m.f. before and after removal of sample represents heat flow. If there is a slow drift in e.m.f. before sample removal, the instrument is monitored long enough to determine the drift trend after sample removal. The result could be ambiguous if the change in e.m.f. is small. The only recourse then would be to repeat the measurement. Under evidently good conditions, it appears that one could measure differences of as little as 20 microvolts (12.8 microcalories/s). Under seemingly adverse conditions, there could be an uncertainty of as much as 30 microvolts.

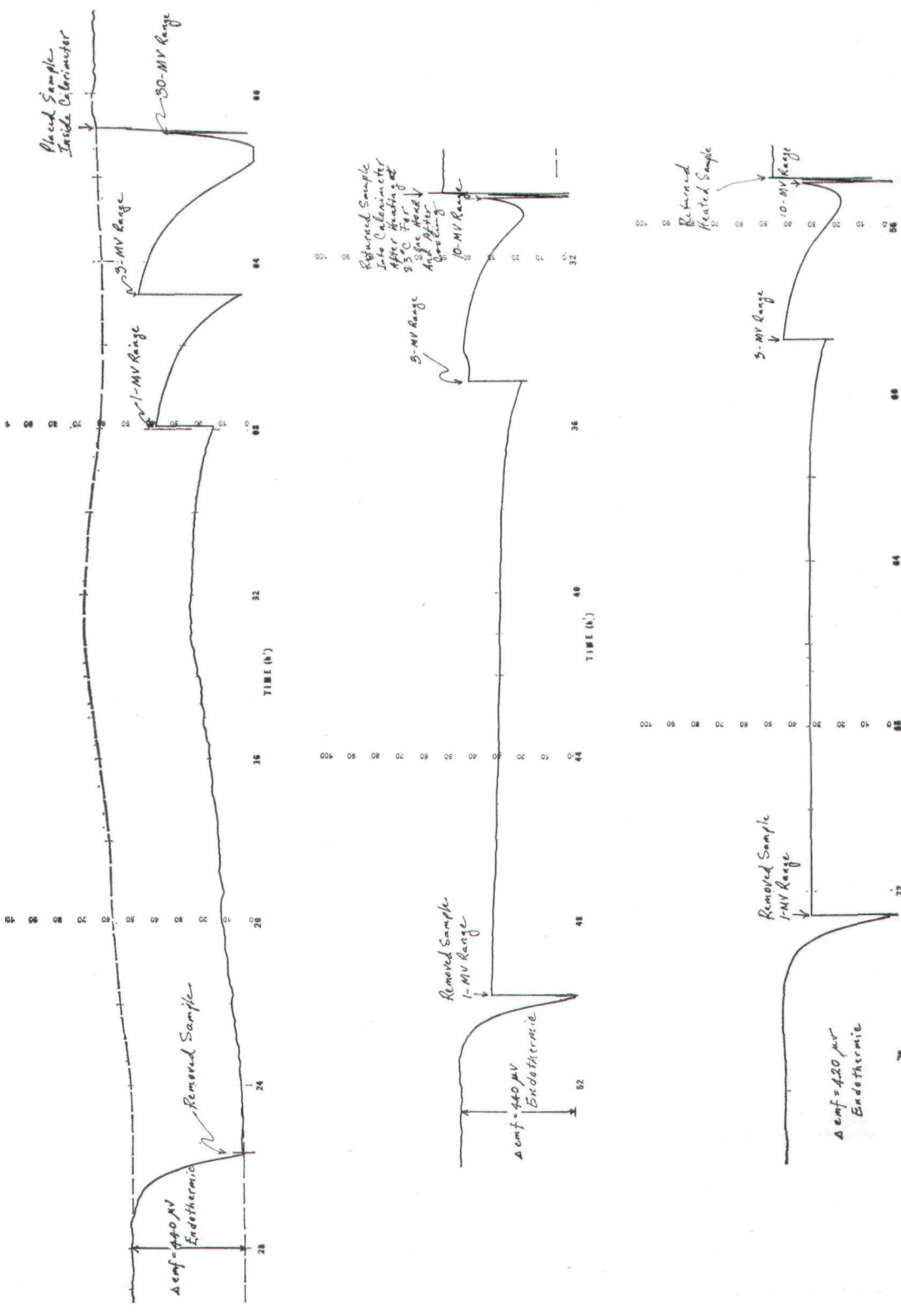

FIG. 4

Changes in thermal e.m.f. following introduction of a sample and its subsequent removal.

Thermal equilibrium is attained slowly by the sample and because of baseline drift of fluctuation, the difference between initial e.m.f. and e.m.f. at thermal equilibrium is not as reliable as the difference achieved following removal of the sample, when equilibrium is attained by the empty calorimeter much more rapidly. The sample was persistently endothermic from 21 June to 8 September. The dashed line indicates the apparent fluctuation in the baseline.

Endothermy in sediments

Sediment samples from the surface 2.5-cm of freshwater ponds in Alabama when sealed anaerobically in culture tubes absorb rather than produce heat (Fig. 4, Table 1). The rate does not appear to be constant but it is persistently high, 222 to 317 microcalories/sec in this one sample. Other samples have much higher rates, while others show decreasing rates with time.

According to the second law of thermodynamics

$$\Delta H = \Delta G + T \Delta S$$

where A G is change in free energy of the system (the culture tube containing sediment plus organisms, etc.), A H is enthalpy change or the heat transferred between the system and surroundings (the calorimeter), T is absolute temperature, and A S is entropy change of the

TABLE 1

Long-term endothermy at 25°C of a sediment sample from the top 2.5-cm layer of a freshwater impoundment.

Date	e.m.f. (microvolts)	Rate of heat absorption (microcalorles/s)
21 June	.480	307
22 June	.347	222
1 July	.495	317
7 Sept	.440 (a)	282
8 Sept	.440 (b)	282

(a) Immediately before heating for 1 h at 83°C.
(b) After heating for 1 h at 83°C.

system. Whatever process or processes that took place in the sealed sediment must have been spontaneous and irreversible, leading to decrease in free energy and increase in entropy. The absorption of heat by the system indicates that the decline in free energy was less than the increase in entropy (Lehninger, 1965). Entropy is ordinarily small in organisms as compared to free energy change and enthalpy change although Walker and Forrest (1964) thought that entropy changes could modify the actual amount of metabolic heat produced. It is obvious from the results obtained that entropy increase in some sediments, completely masks biological heat production.

Heating the sample at 83°C for 1 h to kill all organisms did not have any net effect on raté of heat absorption. It was thought possible that if heating and subsequent cooling did not affect entropy change but killed the organisms, the net result could be an apparent increase in the rate of heat absorption. The same rate of heat absorption after heating could mean no measurable metabolic heat was being produced, although processes bringing about increase in entropy might have simultaneously been affected.

When the sample was treated with acid dichromate, it showed a large heat production then gradually became endothermic again,

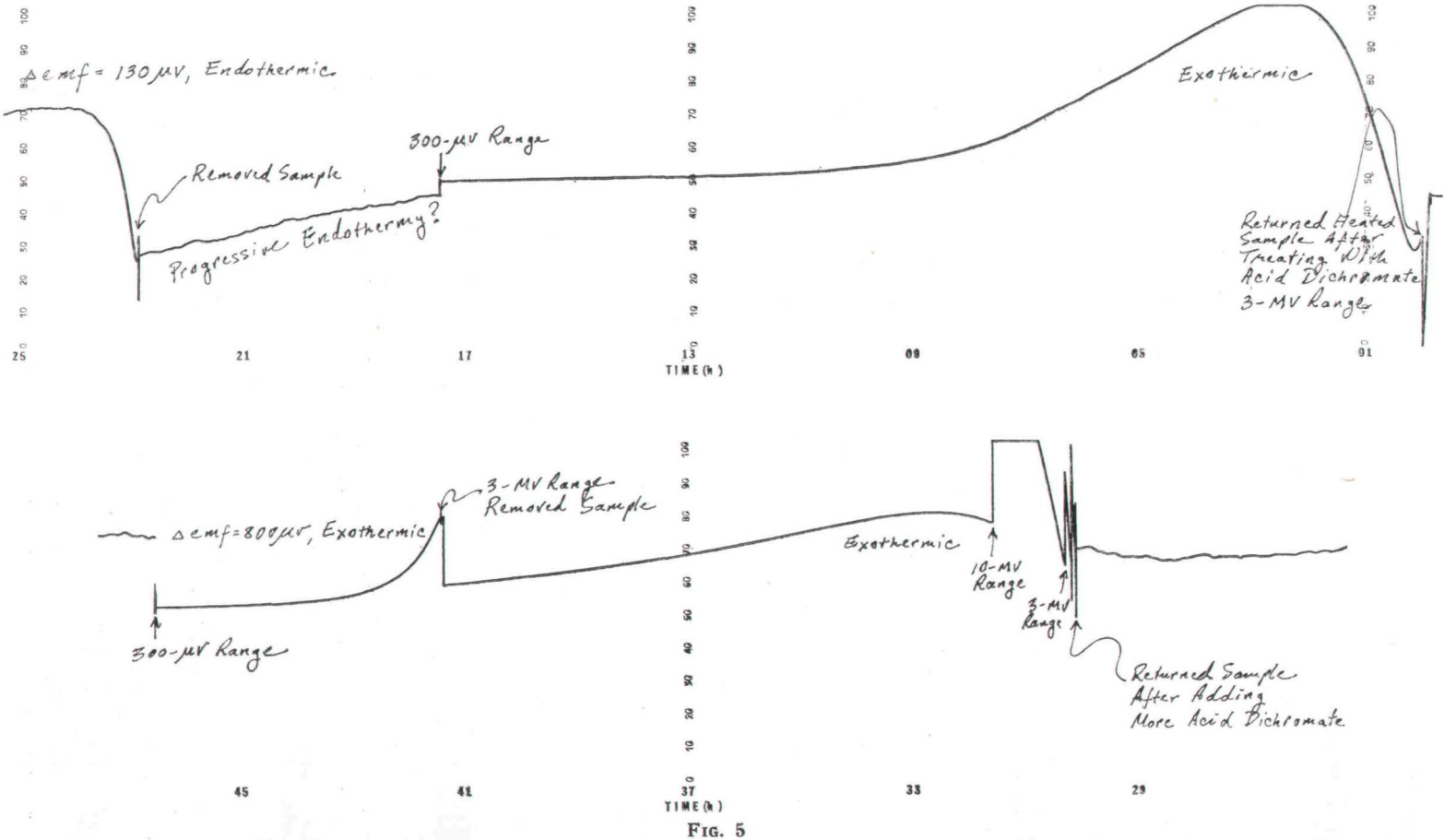

FIG. 5

Changes in thermal e.m.f. following introduction into the calorimeter and subsequent removal of the endothermic sample used in the previous experiment (see Fig. 4 and Table 1) after it was treated with acid dichromate.

The net reaction shifted from endothermy to exothermy but the latter gradually diminished to almost zero by the third day.

although less than before (Fig. 5). After the second dichromate treatment, it showed a persistent exothermy which gradually diminished to near zero on the third day.

Assuming that the measured rates of heat absorption were the total rates for the bottom mud (which are actually higher because deeper layers also absorb heat), they are equivalent to about 1 cal/cm^2 per 24 h. If such rate of heat absorption occurs in the top sediment in the deep sea, it could be one explanation for the existence of the conductive bottom layer at the sediment interface theorized by Wimbush and Munk (1970). Whether endothermy should be of concern also in the measurement of geothermal heat flux in the deep sea is unknown.

Direct calorimetry of a mixture of sediment and inhabiting organisms in a closed system measures only the net change in enthalpy of the system. Endothermy in the freshwater sediment studied was detected because it completely masked the low metabolic activity. The same degree of endothermic chemical reaction could be taking place while the sample is showing a net heat production, therefore leading to underestimation of metabolic heat production. The heat flow must always be assumed to be a net heat production or absorption from which metabolic heat production is to be determined. A control blank is needed from which metabolic activity has been eliminated without affecting other thermochemical reactions. A suitable technique for killing all organisms remains to be found.

The rate of heat absorption could be related to the mineral composition of the sediment. Dissolution of minerals and ionization are well known endothermic reactions. It appears that the sediment may not be presumed to be in equilibrium with respect to these processes.

Electron Transport

All organisms, aerobes, anaerobes and fermenters are alike, not only in producing metabolic heat, but also in the mechanism of biological oxidation. They all effect a successive dehydrogenation of a substrate catalyzed by dehydrogenase enzymes and transfer the hydrogen and electrons to a final hydrogen and electron acceptor forming high-energy ATP in the process (Lehninger, 1965; Peck, 1968; Doelle, 1969). There is more or less uniformity among aerobic Metazoa but a diversity of composition of the electron transport chain among the various metabolic types of bacteria. Furthermore, fermentative bacteria are regarded as not having an electron transport system (Cartwright, 1972). It appears that the common metabolic pathway for all organisms extends only to dehydrogenation. The dehydrogenase enzymes may transfer electrons to pyridinoprotein enzymes (NAD and NADP), to flavoprotein enzymes (FMN and FAD) or to cuproprotein enzymes. The prevalent oxidation-reduction reaction is catalysed by NAD and NADP which pass electrons on to a flavoprotein (FAD) or directly to a substrate in the case of fermentative bacteria. Oxidase, a cuproprotein enzyme, transfers electrons directly to oxygen. From NADH or NADPH, different metabolic types not only use different electron acceptors but also diverging pathways to whatever is their

terminal electron acceptor. Hence, total community metabolism, including that of macrofauna, meiofauna, microfauna and bacteria of all metabolic types, could be measured as the rate of transfer of hydrogen or electrons to the three electron carriers.

Artificial electron acceptors and electron carriers (Peel, 1972) have been used for measuring rates of electron transfer. 2,3,5-triphenyltetrazolium chloride (TTC) is such an artificial electron acceptor and its reduction to formazan has been regarded as a measure of total dehydrogenase activity (Lenhard, 1956). TTC reduction has been shown repeatedly to be an indication of biological activity because only viable cells appear to enzymatically reduce TTC (Mattson et al., 1947; Fred and Knight, 1949). Strauss et al. (1948) and Gunz (1949) reported concomitant TTC reduction during glycolysis. TTC has been used with positive results in soils (Stevenson, 1959; Casida et al., 1964), activated sludge (Lenhard et al., 1965; Ford et al., 1966), freshwater sediments (Lenhard et al., 1962; Edwards and Rolley, 1965; Pamatmat and Bhagwat, 1973) and in marine sediments (Pamatmat and Skjoldal, 1974).

The question remains whether TTC reduction is a measure of total dehydrogenase activity in all organisms regardless of metabolic type. TTC is believed to be reduced by various dehydrogenase enzymes that transfer electrons to NAD and NADP (Mattson et al., 1947; Jensen et al., 1951) and these coenzymes must be present for reduction to take place (Jensen et al., 1951). Kun and Abood (1949) reported reduction of TTC by succinic dehydrogenase which transfers electrons to a flavoprotein. Some dehydrogenase systems gave negative results (Jensen et al., 1951). Hence, there is the possibility that the same rate of TTC reduction, in different species or metabolic type, may not mean the same metabolic rate.

Other problems of using TTC for estimating *in situ* metabolism of microorganisms in sediments are discussed by Pamatmat and Bhagwat (1973). A recurring problem in our experience has been that of non-enzymatic reduction of TTC in reduced sediments. Reducing sugars which are ordinarily present in sediment form formazan at pH above 7 (Mattson et al., 1947). So does $NaHSO_3$ and probably other reduced compounds as well. We have previously failed to notice any chemical reduction of TTC by reduced sediments but, in recent experiments with different sediment samples from freshwater ponds, chemical reduction appears to be considerable (Fig. 6). Note that the poisoned replicates contain as much formazan after a 3-h incubation as the live ones. The reaction was very rapid, the sediment turning noticeably reddish after a few minutes. The reaction was essentially complete in about 15 to 30 min. The importance of pH to TTC reduction is also indicated; a difference in pH between the live assay and the control would mean a large difference in formazan production.

It seems doubtful that a single assay technique involving TTC reduction could be developed to measure dehydrogenase activity equally well in all organisms, and that the result could be directly translated into *in situ* metabolic activity. At best different assay conditions may be required for different metabolic groups. The condition of the sediment will be unavoidably altered in redox potential, pH, etc. and the measured activity must be different from

in situ activity, hence the need to calibrate such an assay. Direct calorimetry offered a means of calibration (Pamatmat and Bhagwat, 1973) but now the problem of how to determine biological heat production, when it is masked by endothermy, remains to be solved.

Many other problems remain to be worked out, but TTC reduction still looks like the most general method for measuring metabolic activity of a wide variety of organisms including microfauna and meiofauna. Any other method of assessing microbial activity in sediments (see Sorokin and Kadota, 1972) would be too selective.

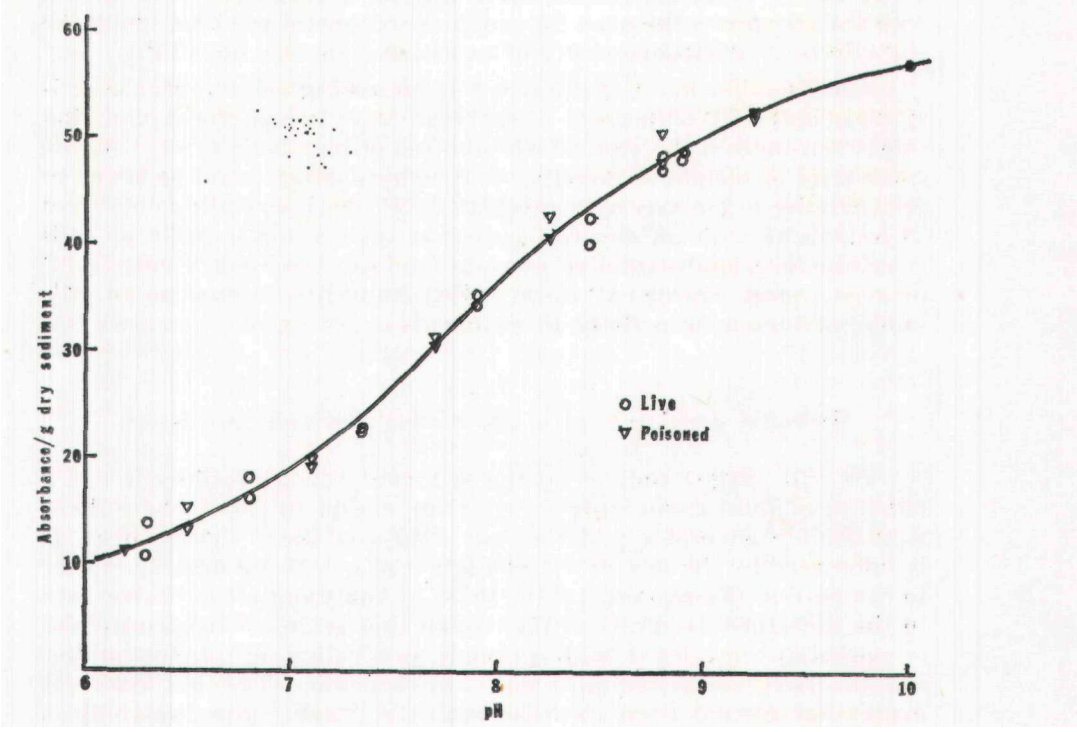

FIG. 6
Effect of pH on TTC reduction.

In the case of poisoned samples, the assay mixture was treated with formaldehyde prior to addition of the sediment. All mixtures were deoxygenated by sparking with nitrogen gas in a glove box. There is no measurable dehydrogenase activity and TTC reduction is evidently due solely to non-enzymatic chemical reduction.

Dehydrogenase activity and adenosine triphosphate in sediments

To further verify that TTC reduction by sediments is the result of dehydrogenase activity, the concentration of adenosine triphosphate (ATP) in replicate samples was determined by Pamatmat and Skjoldal (1974). Since ATP has a half-life of much less than 1 h in soils (Conklin and MacGregor, 1972), is found in living organisms only, and is a useful estimator of live biomass (Holm-Hansen and Booth, 1966), a positive correlation between TTC reduction and ATP concentration

seems good evidence that the former is a function of metabolic activity. The overall correlation coefficient of 0.62 (63 degrees of freedom) might have been higher if the ratio of dehydrogenase activity to ATP concentration did not increase with depth of sediment layer.

If the concentration of adenine nucleotides, and especially ATP in cells, regulates metabolic activity (Atkinson, 1968) similarly in all organisms, a more or less constant ratio of dehydrogenase activity to ATP concentration should be expected. Then ATP itself could be an estimator of metabolic activity. Holm-Hansen (1971) thinks that ATP, under uniform conditions, could be a measure of metabolic rate but recognizes the need for much experimental work to determine the effects of environmental and nutritional factors on ATP.

On the other hand, if the trend of increasing dehydrogenase activity per unit ATP with depth of sediment layer is real, the explanation might be found in the vertical distribution of metabolic types. At the same level of metabolic activity, ATP concentration could be lower in fermentative organisms with substrate-level phosphorylation only than in organisms with an electron transport system and capable of both substrate-level and oxidative phosphorylation. In such a case, ATP or even energy charge (Atkinson, 1968) could not be used as an estimator of metabolic activity in sediments.

Ecological significance of in situ benthic community metabolism

In situ oxygen uptake by the sediment surface, although not a measure of total community metabolism, except in cases where there is negligible anaerobic activity, has some ecological significance. It is influenced by the flux or sedimentation of oxidizable organic matter to the bottom (Pamatmat, 1971a, 1973). Analyzing all available data in the literature, Hargrave (1973) found that sediment oxygen uptake is positively correlated with primary production of phytoplankton and inversely correlated with mixed-layer depth. The mathematical expression derived from stepwise multiple linear regression analysis and relating oxygen uptake to primary production and mixed layer depth in effect formalizes the dependence of oxygen uptake on the sedimentation of oxidizable organic matter. The fraction of primary production oxidized at the sediment surface decreases with increasing primary productivity although the absolute amount of carbon respired increases. This indicates not only that total plankton metabolism becomes increasingly important with increasing primary productivity of the ecosystem but also probably results from increasing rate of burial of unoxidized organic matter, i.e. the total fraction of primary production that settles to the bottom does not decrease as much as the fraction wich is respired. Thus, with increasing primary productivity, it may be expected that anaerobic metabolism in the benthic community becomes more important than aerobic metabolism.

The annual oxygen uptake by the sediment surface is now regarded as a fair estimate of the fraction of annual supply of organic matter to the bottom that is respired during that year (Pamatmat and Bhagwat, 1973). The rest of the annual sedimentation is buried deeper every year. Anaerobic activity in deep sediment layers indi-

cates that, if that layer is 100 years old, organic matter slowly decomposes for at least 100 years. Of course, sediment mixing caused by deposit feeders and burrowing macrofauna (Young, 1971; Rhoads and Young, 1971; Nichols, 1972) complicates sediment stratigraphy but, on the average, an increasing age of deposit of sediment layer may be assumed.

We may further assume that, on the average, the fraction of a year's sedimentation that is oxidized each successive year decreases with time. The total fraction of a year's sedimentation that is finally completely oxidized is the sum total of this yearly oxidation up to 100 years or more as the case may be. If sedimentation rate and biological activity in the sediment column are at steady state, then annual benthic community metabolism in the sediment column represents the total fraction of each year's sedimentation that is finally oxidized. If sedimentation rate has been increasing as a result of eutrophication for instance, then annual benthic community metabolism would underestimate the fraction of the current year's sedimentation that would ultimately be oxidized. On the other hand, if sedimentation rate has been decreasing then annual benthic community metabolism would overestimate the fraction of the current year's sedimentation that would ultimately be oxidized.

There is a whole range of possible situations from one where the amount of sediment is essentially constant, to where the amount varies greatly from time to time due to massive horizontal shifting, to a strictly depositional environment where the sediment gradually thickens with time. The meaning of benthic community metabolism in the sediment column, with respect to energy flow through the rest of the ecosystem, will vary according to the depositional-erosional character of the area. .

Summary

A typical soft-bottom community comprises aerobic and anaerobic macrofauna, meiofauna, microfauna, bacteria and other prokaryotic microorganisms and occupies a relatively thick sediment layer that is oxygenated at the surface, is anoxic but may be occasionally oxygenated in midlayer through mixing and ventilating activities of macrofauna and is permanently anoxic below a certain depth. Benthic ecologists are faced with a difficult challenge of developing a method for measuring the collective metabolic activity of such diverse metabolic types.

The rate of total oxygen uptake by the sediment surface underestimates community metabolism by the magnitude of anaerobic metabolism in the deeper layers. Measurement of CO_2 evolution except for its obvious desirability in modeling the carbon cycle does not appear to have any advantage over measurement of oxygen uptake; however, simultaneous measurements of both could be quite informative about benthic community composition. Direct measurement of metabolic heat release by undisturbed benthic organisms in sediments may be confused by unknown concomitant thermochemical processes; biological heat production may be masked completely by a net endothermy due to a large increase in entropy of the system (sediment+organisms). There remains the possibility of measuring benthic community metabolism in terms of rate of hydrogen and electron transfer by using artificial electron carriers and acceptors such as TTC, but we do not know if TTC reduction could be a universal measure of dehydrogenase activity. In view of apparently variable relationship between dehydrogenase activity and ATP concentration in sediments, probably arising from differences in metabolic types in the samples, neither ATP nor energy charge seem to be promising estimators of metabolic rate in sediments.

The annual oxygen uptake by the sediment surface may be regarded as a fair estimate of the fraction of annual supply of organic matter to the bottom

that is respired during that year. In a depositional basin, if sedimentation rate and biological activity in the sediment column are at steady state, then annual benthic community metabolism in the sediment column represents the total fraction of each year's sedimentation that is completely oxidized. This fraction could be underestimated or overestimated depending on whether sedimentation rate has been increasing as a result of eutrophication or decreasing. The interpretation of benthic community metabolism, with respect to energy flow through the rest of the ecosystem, depends on the depositional-erosional balance in the site of the benthic community.

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