Microbial Activity in Lake Sediments with Particular Reference to Electrode Potential Gradients

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Microbial activity was determined along electrode potential gradients within sediments, and along sediment surfaces in a stratified eutrophic lake. There was evidence of a change from a tricarboxylic acid cycle-based metabolism to a fermentative one with decreasing electrode potential. A more detailed examination of the stratification of the microbial community showed that the activities of enzymes associated with energy metabolism (in this case electron transport) were highest on electrode potential gradients. This was observed within a sediment core on a millimetre-depth scale, as well as over several metres at the surface of sediments, on a transect which ran from oxic littoral muds to the anoxic profundal zone. In contrast, microbial hydrolytic enzymes, such as protease and amylase, were most active at the sediment surface, where the highest concentrations of substrates might be expected.

INTRODUCTION

The bacterial populations in the water column of a lake fluctuate in response to a number of variables. Correlations with phytoplankton populations in the epilimnion (Overbeck, 1968; Jones, 1972) and, in shallow eutrophic lakes, with increasing nutrient concentration in the anoxic hypolimnion (Jones, 1977) have been demonstrated. Peaks in the bacterial population have also been observed in the metalimnion, the zone of temperature gradient between the surface water and the deeper cooler water in a stratified lake. In shallow eutrophic lakes this zone also comprises the oxycline and chemocline (gradients of dissolved oxygen and certain inorganic nutrients) particularly during late summer. High plate counts of bacteria in this region were attributed by Collins & Willoughby (1962) to populations carried by inflowing streams in spate, whose temperatures often corresponded to that of the metalimnion. Overbeck (1975) considered the metalimnion to be a region of high metabolic activity, possibly related to decomposition of the remains of primary producers sedimenting out of the epilimnion. Certain specific groups of bacteria may also find optimum conditions for growth on the chemocline (e.g. iron bacteria; see Jones, 1978) where there is sufficient oxygen from the water above and mineral nutrients from the anoxic water below. The depth of the growth zone at any given time will depend on the degree of turbulence in the water.

Bacterial numbers and activity in lake sediments are usually about three orders of magnitude higher than in the water column and their activity is such that its influence is detected in the overlying water. The electrode potential (E_h) discontinuity layer gradually moves upwards as the sediments of eutrophic lakes become anoxic (Mortimer, 1941) and eventually enters the water column. This, in turn, will affect the distribution and activity of bacteria such as methanogens and sulphate-reducers in the sediments. Microbial communities in sediments have been sampled at comparatively broad depth intervals (e.g. Johnston & Cross, 1976; Vosjan & Olanczuk-Neyman, 1977; Wieser & Zech, 1976) and a steadily

decreasing activity and population size with depth has been observed. Vertical stratification of populations such as denitrifiers, sulphate-reducers and methanogens within the microbial community has, however, been observed and the importance of E_h gradients as sites of high metabolic activity has been recognized (Fenchel & Jørgensen, 1977). The purpose of this study was to examine the metabolic activity of micro-organisms along E_h gradients in more detail.

METHODS

Sampling. Sediment samples were taken from the North Basin of Windermere and from Blelham Tarn with a Jenkin surface mud corer and were sub-sampled on extrusion using the device described by Jones (1976). This allowed samples to be taken at 2 mm depth intervals. Temperature and dissolved oxygen were recorded in the field on a combination thermistor/oxygen electrode (Mackereth, 1964). The electrode potential of the sediment in the core was measured at 1 mm depth intervals with a bright platinum electrode and a fast-flow calomel reference electrode. The platinum electrode was constructed from 0.46 mm diam. wire which was flattened at the tip to produce a horizontal blade approximately 1.5×4 mm. The wire was inserted into the tip of a Pasteur pipette and held in place by epoxy resin (Araldite, Ciba-Geigy). Contact between the platinum and a copper lead was via a mercury plug in the pipette. The electrode blade was polished with fine alumina (about $0.05 \mu\text{m}$ grain size) and the couple was tested in ZoBell's solution (3.3 mM-potassium ferro- and ferricyanide in 0.1 M-KCl) before use. The electrodes were clamped so that the reference electrode was just immersed in the overlying water and the platinum electrode was at the sediment-water interface. The sediment was then extruded upwards at 1 mm intervals and electrode potential readings were taken after 1 min at each depth.

Extraction of enzymes from sediment. For electron transport system (ETS) activity, a dilution of sediment in cold extraction solution was sonicated (MSE Ultrasonic Disintegrator) at an amplitude of $12 \mu\text{m}$ for 2 min in an ice bath. The extraction solution contained 1.7 % (v/v) Triton X-100, 0.15 % (w/v) polyvinylpyrrolidone and $75 \mu\text{M}$ - MgSO_4 in 50 mM-Tris/5 mM-EDTA buffer, pH 7.4. The sample was centrifuged at 4000 g for 10 min at 0°C and the supernatant (enzyme extract) was then removed. All solutions were kept in ice/water baths and all manipulations before incubation were performed at a similar temperature. For malate dehydrogenase and lactate dehydrogenase, EDTA was omitted from the extraction solution and the Triton X-100 concentration was reduced to 0.02 %. For the protease and amylase assays, 0.1 M-phosphate buffer, pH 7.5, was used in the solution and the Triton X-100 concentration was 0.2 %.

Electron transport system (ETS) activity. This was used as a measure of microbial energy-yielding metabolism in the sediments. The method used was that of Owens & King (1975) as modified by Jones & Simon (1979) to ensure optimum conditions for extraction of enzymes from freshwater sediments.

Malate dehydrogenase (EC 1.1.1.37) and lactate dehydrogenase (EC 1.1.1.27). These enzymes were determined by measuring the decrease in NADH fluorescence in the assay mixture. This contained 98 vol. enzyme extract (from a 5×10^{-3} dilution of sediment), 1 vol. NADH solution (1.5 mM in Tris/EDTA buffer) and either 1 vol. 120 mM-oxaloacetate (for malate dehydrogenase) or 1 vol. 120 mM-pyruvate (for lactate dehydrogenase). Test solutions were read against controls for sediment extract fluorescence and NADH fluorescence at the initial concentration (λ_{ex} 340 nm, λ_{em} 450 nm).

Protease. Protease activity was determined by incubating 2 ml of enzyme extract with 0.5 ml of an Azocoll suspension (0.5 %, w/v; Calbiochem) at 35°C for 3 h. The reaction was stopped by centrifuging at 4500 g for 15 min and the absorbance of the supernatant was read at 520 nm.

Amylase. The amylase assay was based on that of Rinderknecht *et al.* (1967). The substrate was amylopectin azure (Calbiochem) and the volumes, concentrations and conditions were as for the protease assay. The reaction was stopped as above, but 0.025 ml of glacial acetic acid was then added to 2 ml of the supernatant (final pH 3 to 4) and the absorbance was read at 595 nm. All absorbance values were corrected for separate substrate and enzyme controls.

Direct counts of bacteria. Counts were performed by epifluorescence microscopy on black membrane filters using the procedure of Jones & Simon (1975). Sediment samples were diluted 10^{-3} with membrane-filtered water before staining with acridine orange at a final concentration of 10 mg l^{-1} .

ATP. Microbial biomass was estimated by the firefly luciferin-luciferase bioluminescence method. Increased sensitivity was obtained by adopting the procedure of Jones & Simon (1977). Extractions were performed by injecting 0.5 ml of a 5×10^{-3} dilution of sediment into actively boiling Tris buffer at pH 7.8. This procedure was sufficiently rapid to ensure that the adenylate energy charge was not altered significantly and that ATP content could be used as a biomass indicator.

Fig. 1. Examples of E_h gradients in a shallow eutrophic lake (Blelham Tarn). (a) E_h gradients in sediment cores in winter (—) and in summer (---) when the hypolimnion is anoxic. (b) Cross-sectional transect of a shallow lake showing the dissolved oxygen concentration in the water column and the E_h at the sediment-water interface.

RESULTS AND DISCUSSION

Some typical E_h gradients from a shallow eutrophic lake (Blelham Tarn) are shown in Fig. 1. In sediment cores, E_h decreased rapidly with depth (Fig. 1a) but the depth of the discontinuity layer depended on the season and the site at which the core sample was taken. As anoxic conditions develop in the hypolimnion the discontinuity layer in profundal sediments moves closer to the mud surface (Fig. 1a) and occasionally into the water column (Mortimer, 1941, 1942). The E_h values obtained in this study are in good agreement with those of Mortimer (1941, 1942) and with other more recent studies on temperate freshwater sediments. On a whole-lake scale, the E_h at the sediment-water interface will reflect the conditions in the overlying water. Thus a transect across Blelham Tarn (Fig. 1b) demonstrates the oxidizing nature of surface sediments in the littoral zone, an E_h gradient in the region of the metalimnion and more reducing surface sediments in the profundal zone. The depth of both types of gradient (i.e. within the sediment and at the sediment surface across a lake transect) will depend on the degree of turbulence in the overlying water. The discontinuity layer within the sediment is deeper in the littoral zone because of greater turbulence and surface sediment disturbance; similarly, the depth-range of the gradient at the sediment surface in Fig. 1(b) will be affected by internal water movements (seiches). I have avoided the use of the term redox (and therefore the connotation of associated specific redox couples) because the E_h measurements in the sediments represent a range of oxidation-reduction reactions, and with a system such as that described in this paper, measurements are likely to be accurate only to within 50 mV. It has been demonstrated, however, that such measurements may be of value in understanding the microbiology (Cappenberg, 1974; Fenchel & Jørgensen, 1977; Jørgensen, 1977a) and chemistry (Whitfield, 1969) of sediments.

It was possible to demonstrate changes in the activities of certain indicator enzymes with increasing depth and decreasing E_h in individual sediment cores. Although it was not possible to obtain complete recovery of added dehydrogenase enzymes from sediments, the

Table 1. *Enzyme activities in Blelham Tarn sediment samples from different depths and having different electrode potentials*

Sample		Lactate dehydrogenase*	Malate dehydrogenase
Depth (mm)	E_h (mV)	($\mu\text{mol NADH}$ oxidized h^{-1})	Lactate dehydrogenase
0-5	+375	6.3	2.2
5-10	+180	5.0	2.6
10-20	+90	7.6	1.4
60-70	-70	7.9	1.3
0-10	+460	0.7	13.0
25-35	+340	1.5	9.4
40-45	+270	4.3	2.6
45-50	+150	6.1	1.1
55-60	+140	6.3	1.5

* Values are corrected for NADH oxidation.

Fig. 2. Energy metabolism along an E_h gradient in sediment from the North Basin of Windermere. (a) Electron transport system activity. (b) Electrode potential.

yield did not change in a systematic manner with E_h , and would not, therefore, account for the results obtained. The methods of enzyme extraction used were chosen after testing several combinations of buffer and extraction solution concentrations (particularly detergent and EDTA) and were found to give greatest recoveries of each enzyme. The actual values for dehydrogenase enzymes varied from site to site but the most consistent trend observed was a steady increase in lactate dehydrogenase activity and an attendant decrease in the malate dehydrogenase:lactate dehydrogenase ratio with decreasing E_h (Table 1). The enzyme activities indicated a switch from an aerobic, tricarboxylic acid cycle-based metabolism to a fermentative one. These particular enzymes were chosen because pure culture studies had demonstrated considerable changes in their concentration in response to oxygen tension and E_h (Gray *et al.*, 1966; Wimpenny, 1969). Such enzyme assays may, however, only be used to indicate general trends, largely because of the difficulties involved in obtaining reliable results from heterogeneous material such as sediment. Background levels of u.v.-absorbing materials could account for as much as 70% of the signal if the NADH was determined spectrophotometrically. This background was reduced to about 15% if the more specific spectrofluorimetric procedure was used, but this advantage was often counterbalanced by the degree of quenching which was observed, particularly in the reducing sediments. A range of known concentrations of NADH was therefore added to the samples to act as internal standards and to ascertain linearity of response. In addition, each sample was incubated with NADH as sole substrate to correct for NADH oxidation.

Fig. 3. Microbial activity, ATP content and bacterial numbers along an E_h gradient in sediment from the anoxic hypolimnion of Blelham Tarn. (a) Electron transport system activity. (b) Direct counts of bacteria. (c) ATP content. (d) Electrode potential.

Fig. 4. Microbial activity, ATP content and bacterial numbers in surface sediment on a transect of Blelham Tarn from oxic littoral sediments to anoxic profundal sediments. (a) Temperature (●), dissolved oxygen concentration (▲) and ATP content (○). (b) Direct counts of bacteria (●) and electron transport system activity (▲).

An attempt was then made to obtain a general estimate of the enzymes involved in microbial energy metabolism in the sediment by measuring the electron transport system (ETS) activity. Although microbial activity, biomass and numbers of most micro-organisms generally decreased with depth in Windermere sediment, it was possible to demonstrate peaks of activity associated with the E_h discontinuity layer (Fig. 2). These peaks were observed

Fig. 5. Activity of hydrolytic enzymes and numbers of bacteria along an E_h gradient in sediment from Windermere. (a) E_h (—) and direct counts of bacteria (---). (b) Protease activity (—) and amylase activity (---).

at 2 to 4 mm, 6 to 8 mm and 10 to 12 mm as E_h changed from +150 to -100 mV. Such pronounced peaks were not observed when the E_h gradient was near the sediment surface, as in Blelham Tarn towards the end of summer (Fig. 3). The decrease in activity with depth (Fig. 3a) did not always correspond to decreases in bacterial numbers (Fig. 3b) or ATP content (Fig. 3c). Although all three variables decreased with depth, the activity per bacterium or per unit ATP biomass was not constant. The ETS activity observed was two to three orders of magnitude greater than that in marine sediments (Wieser & Zech, 1976; Vosjan & Olanczuk-Neyman, 1977) but small differences in analytical technique can considerably affect the sensitivity of the assay and such comparisons may not, therefore, be valid.

It was also possible to demonstrate the importance of E_h gradients as sites of increased microbial activity on a whole-lake scale (Fig. 1b). Analyses from a transect of Blelham Tarn during late summer showed that the dissolved oxygen concentration fell from 8 to 0 mg l⁻¹ in the metalimnion (Fig. 4a) and that in this region peaks of bacterial numbers, ATP and microbial activity were observed in the surface sediment (Fig. 4b). Once again the ratio of activity to biomass or numbers was not constant. The results confirmed earlier observations on the occurrence of large crops of filamentous sulphur bacteria and cyanobacteria in this zone.

Microbial hydrolytic activity, however, (e.g. the hydrolytic cleavage of proteins and polysaccharides) was not particularly associated with the E_h discontinuity layer. Protease and amylase activity were maximal at the surface of the Windermere sediment and decreased steadily with depth (Fig. 5b) even though bacterial numbers showed no such rapid decline (Fig. 5a). Microbial hydrolytic activity, in contrast to energy metabolism, appeared to be greatest at the sediment-water interface, i.e. at the point where the substrate concentration, in the form of detritus and sedimented algal remains, might be expected to be highest.

It has already been suggested that E_h gradients provide conditions conducive to microbial growth and activity (Fenchel & Jørgensen, 1977). Karl (1978) has demonstrated peaks of biomass and higher adenylate energy charge values at the O_2 -sulphide interface in the water column, as well as sub-surface peaks in the sediment of the Black Sea. This paper demonstrates that, in contrast to the conventional picture of a steadily decreasing microbial population and activity with depth of sediment, energy metabolism in particular is associated with, and maximal on, sub-surface E_h gradients. This has been shown over several metres, on a whole-lake transect of surface sediments, as well as over a few millimetres in a sediment

profile. Such gradients may occur on an even smaller scale. Jørgensen (1977*b*), for example, has shown that obligate anaerobes such as *Desulfovibrio* can grow within sediment pellets (50 to 200 μm diam.) in the oxidized surface layers. The H_2S produced by them diffused into the interstitial solution and was oxidized by sulphide oxidizers (such as *Beggiatoa* spp.) before any detectable accumulation took place. Although attempts to measure such fine E_h gradients with microelectrodes failed during this study, it is possible that histotopochemical techniques such as those used by Garlich's *et al.* (1974) might provide information on microbial metabolism within such small particles.

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