PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Microbes and Microbial Processes in Sediments [and Discussion]

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Microbes and microbial processes in sediments

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Buried sediments are generally characterized by the absence of oxygen and so microbial processes will be dominated by the activity of anaerobic bacteria. Carbon turnover in the presence of electron acceptors therefore involves interaction of the carbon, nitrogen and sulphur cycles. In the absence of electron acceptors other methods of energy conservation become significant. Interspecies H_2-transfer permits the use of otherwise energetically unfavourable reactions. Conservation of the energy in polyphosphate bonds and reduction of Fe(III) and Mn(IV) to produce more energetically favourable end-products are other mechanisms available to benthic bacteria. The reduction of CO_2 to acetate may require more serious consideration as a hydrogen sink in certain sediments. The list of substrates known to be susceptible to attack by anaerobic bacteria has grown rapidly in recent years and estimates are now available for the turnover of refractory components such as lignin. Finally, bacteria are considered as producers of biomass, particularly of specific cell components that may be used as biomarkers to identify zones of activity. The key features of such biomarkers are identified.

1. Introduction

The microbial population and the processes mediated by microbes in aquatic sediments are subject to several controlling factors. The decomposition and modification of organic matter is affected by (*a*) the nature and source of the organic matter, for example, whether it is freshly sedimented algal material or much processed soil organic matter transported from the catchment, (*b*) temperature, (*c*) the presence of electron acceptors, (*d*) bioturbation (sediment mixing by animals) and finally (*e*) the presence of particular decomposer microbes in the sediment. The geochemist's view of sediment may be confined to the search for a particular mineral and that of the microbiologist to an interest in a particular microbe, but between these extremes lies an area of interest to both disciplines that provides exciting opportunities for collaborative research. This paper does not attempt to provide a comprehensive review of sediment microbiology but attempts rather to identify those areas of common interest and to discuss them in relation to the microbial populations present and the processes in which they are involved. More detailed accounts of sediment microbiology and the anaerobic processes mediated by bacteria are given by Nedwell (1984), Nedwell & Brown (1982), Sleat & Robinson (1985) and Thauer & Morris (1984). Similarly, the processes by which bacteria exchange information (gene transfer), particularly concerning the decomposition of refractory components and xenobiotics, cannot be covered adequately in this contribution. For details of these processes the reader is referred to Reanney *et al.* (1983).

The processes and end-products of organic matter decomposition are to a large degree controlled by the availability of electron acceptors. The first section of this paper will therefore consider the importance of various respiratory processes in carbon turnover and how these differ in marine and freshwater sediments. Mechanisms of organic carbon metabolism in the absence

of electron acceptors are then discussed with particular attention paid to alternative methods of energy conservation and disposal of hydrogen. The apparent inability of microbes to decompose certain classes of compounds in the absence of oxygen is briefly considered and finally it is argued that bacteria should be thought of not only as decomposers effecting the mineralization of organic carbon, but also as producers of certain key organic compounds. Such compounds are currently used as biomarkers to indicate the presence, or activity, of particular bacteria within sediments. The acceptance of biomarkers as indicators of biomass requires careful validation and, by definition, precludes their use as historical markers. Quantitative descriptions of microbial populations require even more rigorous assessment and some of the underlying assumptions of the methods used are discussed.

2. Decomposition processes in the presence of electron acceptors

General thermodynamic considerations suggest that potential electron acceptors might be used in the order O_2; Mn(IV); NO_3^-; Fe(III); SO_4^{2-}; CO_3^{2-} and would thus be expected to be involved in spatially arranged electron-transport mediated processes as illustrated in figure 1. Although they represent broad and often inaccurate generalizations, such diagrams provide a useful back-cloth against which sediment processes may be viewed.

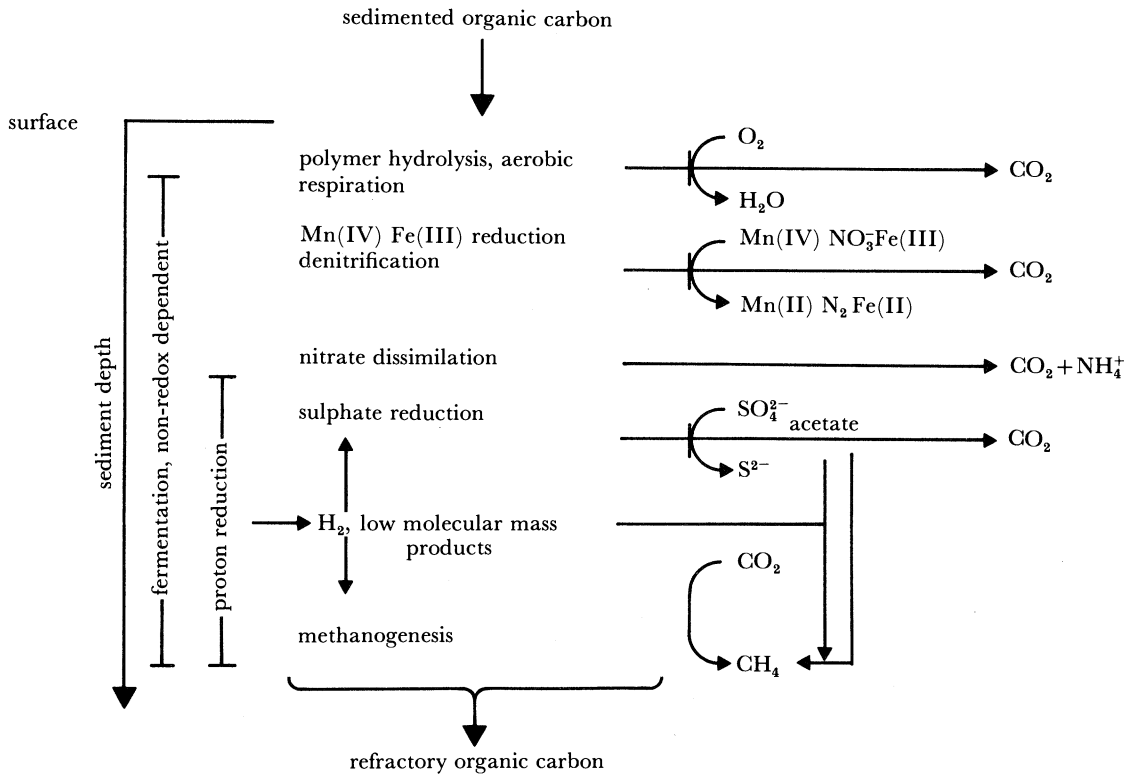

Figure 1. Depth distribution and interactions of decomposition processes in buried sediments.

Microbial attack on polymers, a process that is most active in surface sediments, is usually the step that limits the rate of decomposition of particulate material entering sediment (Cappenberg *et al.* 1982), largely because the process is hydrolytic and expensive in terms of

energy. The amount of material that remains after microbial attack, often referred to as the refractory component, will depend on the nature of the primary producer and the degree of processing that has occurred in the catchment or the oceanic water column. Thus higher plant material rich in lignin may show considerable resistance to attack. The intervening steps, i.e. those of conversion of organic molecules to CO_2 or CH_4, will depend to a large degree on the availability of electron acceptors. The relative importance of these is seen most clearly when marine and freshwater sediments are compared. Jørgensen (1980) estimated that, of the organic carbon oxidized in the marine Limfjord, oxygen was the electron acceptor for 46%, nitrate for 3% and sulphate for 51% (methanogenesis was negligible at this site). By contrast our estimates (Jones & Simon 1980; Jones *et al.* 1984*b*) of decomposition in a soft-water lake show that, of the carbon mineralized, 45% was by aerobic respiration, 20% by denitrification, 25% by methanogenesis, 2% by sulphate reduction, 1% by iron reduction. The remainder is assumed to have been involved in fermentation processes that do not involve electron acceptors. Clearly the concentrations of electron acceptors, particularly sulphate (28 mmol l^{-1} in sea water and 0.15 mmol l^{-1} in English Lake District waters) has a marked effect on terminal respiratory processes. However, the broad generalization that decomposition is controlled in this way requires qualification on several fronts. In the first place, buried sediments differ enormously in their characteristics. Whereas some may be aerobic to considerable depths, microelectrode studies have shown that others may become anoxic within a few millimetres of the sediment water interface (Revsbech *et al.* 1980). The degree of aeration (and the redox cycle of elements) will also be affected by the activity of burrowing animals (bioturbation). Because microbial activity is affected by and in turn affects the redox state of the sediment (Billen 1978), some consideration might be given to how these processes are mediated and whether certain widespread assumptions about bacterial activity within the sediments are in need of modification. Diagenetic models based on gradients of electron acceptors within sediments are not considered here. For further details of their application in relation to the sulphur and nitrogen cycles respectively see Berner (this symposium) and Vanderborght *et al.* (1977).

Given the variability in sediment characteristics and in the chemistry of the overlying water, an even greater variability in the availability of the electron acceptors to the bacteria within the sediment might be expected. An oxidizing redox potential does not indicate that sufficient oxygen is present for aerobic respiration (Revsbech *et al.* 1980) but neither does the presence of oxygen preclude the growth and activity of anaerobic bacteria. Sulphate-reducing bacteria not only exhibit oxygen tolerance (Hardy & Hamilton 1981) but are known to be active within the anaerobic microniches (large particles and faecal pellets) of aerobic sediments (Jørgensen 1977). Similarly nitrate reduction may occur within the larger particles (greater than 250 μm) of oxygenated shallow-water lake sediments (Jones 1979) and methanogenesis in open ocean water (Oremland 1979). Under such circumstances, anaerobic bacteria may play a significant role in carbon turnover although the final products will be those of aerobic respiration.

The presence of an electron acceptor and appropriate bacteria is, however, no guarantee that a particular process can proceed. Westrich & Berner (1984) have shown that sulphate reduction in the anoxic sediments of Long Island Sound is limited by the electron donor (i.e. the quantity of available organic matter). Likewise it is inadvisable to assume that a particular pathway of dissimilation will be the dominant one, or that it will be mediated by the expected group of organisms. It has been shown, for example, that a significant quantity of added ^{15}N-nitrate is reduced to ammonia rather than to nitrogen gas (Sørensen 1978). Although this

reaction is performed by both facultative (Macfarlane & Herbert 1982) and obligate (Caskey & Tiedje 1980) anaerobes, results at this laboratory and elsewhere (Barton *et al.* 1983) have shown that nitrate and nitrite may also be reduced by sulphate-reducing bacteria. The conversion of nitrate to ammonia appears to be restricted to zones of low redox potential (−200 mV) and can be significant in well mixed, reduced environments with a steady input of nitrate, such as digested sludge (Kaspar *et al.* 1981). Its role in stratified sediments is, however, less clear. While it is possible to demonstrate that ^{15}N nitrate injected into deeper sediments is converted to ammonia (Sørensen 1978), it is another matter to show that nitrate actually reaches such depths under natural conditions. The most likely candidates for such a process are estuarine sediments where tidal influx may ensure nitrate movement into reducing zones and under such circumstances up to 15% of the nitrate may be reduced to ammonia (Buresh & Patrick 1981). It is precisely such sediments that readily yield organisms capable of this particular reductive process (Macfarlane & Herbert 1982).

Two other aspects of the activity of sulphate-reducing bacteria in sediments deserve further mention, but because they have been extensively covered in recent reviews they are only considered briefly in this paper. The first is to emphasize how much our understanding of the metabolic capabilities of these organisms has been broadened by the work of Drs F. Widdel and N. Pfennig (see, for example, Pfennig & Widdel 1982). Not only has the substrate range been extended to include such compounds as stearate, palmitate and benzoate, but the terminal oxidation of acetate has been shown to be a widespread phenomenon (see, for example, Widdel & Pfennig 1981). These results tend to support observations on the sulphate-mediated oxidation of fatty acids in sediments (Sørensen *et al.* 1981; Banat & Nedwell 1983). In addition, the use of hydrogen and sulphate as energy sources is more widespread than previously thought (Brandis & Thauer 1981). Second, the terminal process of decomposition in anaerobic, sulphate-rich (i.e. marine) and sulphate-poor (i.e. freshwater) sediments has been seen as one where the sulphate-reducing bacteria out-compete methanogens for hydrogen in the former, but act as net hydrogen donors in the latter. Laboratory experiments have demonstrated the kinetic mechanism for the success of sulphate reducers in competition for both hydrogen and acetate (Kristjansson & Schönheit 1983; Schönheit *et al.* 1982) and that this can occur even at the low sulphate concentrations encountered in lake sediments (Lovley & Klug 1983*a*). It only remains to emphasize that these interactions may fluctuate rapidly, at least in freshwater sediments, and that phases of dominance by hydrogen-utilizing methanogens (Jones *et al.* 1982*a*) may be followed by periods when acetate-utilizing *Desulfotomaculum* species appear to be the most active component of the population (see, for example, Jones & Simon 1984).

3. Decomposition processes in the absence of electron acceptors

In the absence of oxygen, organic matter may be further modified by non-redox dependent fermentors and proton-reducing bacteria. It is the syntrophic association of the latter and of hydrogen-consuming bacteria (sulphate reducers and methanogens), which has received most attention recently. The process, known as inter-species hydrogen-transfer, is discussed in detail by Mah (1982) and Nedwell (1984) and therefore is not considered at length in this paper. Briefly, the catabolism of certain low molecular mass compounds may yield acetate and hydrogen, for example,

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2;$$

($\Delta G^{\circ\prime}$ for the reaction as written is 76.1 kJ, see Thauer *et al.* 1977). The hydrogen is produced because certain bacteria are able to recycle reduced coenzymes by a hydrogenase-mediated release of hydrogen,

$$NADH_2 \rightarrow NAD + H_2.$$

This reaction will only occur at extremely low partial pressures of hydrogen; for example, for thermodynamically favourable metabolism of propionate and butyrate, hydrogen partial pressures of approximately 1.01 and 0.101 Pa respectively are required. It is the hydrogen-scavenging activity of sulphate reducers and methanogens that maintains such low partial pressures. Among the methanogenic syntrophic associations that have been reported since the review by Mah (1982) is one that reports the catabolism of benzoate (Mountfort *et al.* 1984).

In addition to the interactions described above, the metabolic end-products of some anaerobic bacteria may be modified by certain less well defined processes. Although Fe(II) production may dominate redox events in many freshwater sediments, the reductive process may also be involved to a small degree in organic carbon turnover. Lithotrophic bacteria may conserve energy by Fe(III) reduction but the process is so slow that it is unlikely to be of significance in the natural environment. Conversely, many fermentative bacteria reduce Fe(III) but appear to gain no energetic advantage from the process (Jones *et al.* 1983). Such organisms are capable of reducing metals other than Fe(III), including Mn(IV), As(V), U(VI) and Se(VI) (Jones *et al.* 1984*a*). Certain chemo-organotrophic anaerobes, however, have been shown to conserve energy by Fe(III) reduction and this process appears to be coupled in some way to the production of more energetically favourable end-products (table 1*a*), i.e. a reduction in the quantity of alcohol produced with a concomitant increase in the more oxidized volatile fatty acids. Nitrate has been observed to have a similar effect on the fermentation end-products of *Bacteroides multiacidus* (Yamamoto *et al.* 1982). The presence of Fe(III) in anoxic sediments, on the other hand, appears to allow a more complete oxidation of fatty acids by a resident bacterial population that is clearly different from the fermenters described above. Under such circumstances the fermentation of sugars is less likely to be of

TABLE 1. (*a*) THE EFFECTS OF Fe(III) ON THE GROWTH YIELD AND FERMENTATION PRODUCTS OF A FERMENTATIVE VIBRIO SPECIES IN A COMPLEX AND DEFINED MEDIUM

percentage change in the presence of Fe(III)

		final concentration		
medium	molar growth yield	ethanol	acetate	formate, lactate, succinate[a]
complex	+63	−63	+64	+52
defined	+52	−25	+12	+12

(*b*) THE EFFECT OF Fe(III) ON THE ACCUMULATION OF VOLATILE FATTY ACIDS IN FRESHWATER SEDIMENTS

percentage decrease in the presence of Fe(III)

head space gas	C_2	n-C_3	i-C_4	n-C_4
hydrogen	50	94	96	98
nitrogen	66	66	50	25

(The data are taken from Jones *et al.* 1984*b*.)

[a] Under the h.p.l.c. operating conditions used these acids were not separated.

importance and terminal metabolism of fatty acids will assume greater significance. Addition of Fe(III) to the anoxic sediments of Blelham Tarn in late summer decreased the quantity of volatile fatty acids that accumulated (table 1*b*), particularly under an atmosphere of H_2. Added fatty acids also disappeared more rapidly in the presence of Fe(III) (Jones *et al.* 1984*b*). The exact mechanism whereby bacteria might use Fe(III) instead of a syntrophic partner as a hydrogen sink is not known, but it is of interest to note that a recent paper implicates Fe(III) in the bacterial sub-oxic diagenesis of organic matter in banded iron formations (Walker 1984).

Other methods of energy conservation may also be used by sediment bacteria to permit organic carbon turnover in the absence of electron acceptors. Certain sulphate reducers, members of the genus *Desulfotomaculum*, are able to conserve the energy of the pyrophosphate bond (Peck & LeGall 1982) and this mechanism has also been observed in fermentative anaerobes (Cruden *et al.* 1983; Varma *et al.* 1983). The energy of short- and long-chain polyphosphates may be conserved by a similar mechanism (Varma & Peck 1983) and, because such polymers are found as storage materials in many of the algal and cyanobacterial primary producers, it is tempting to speculate that polyphosphates may play a part in the decomposition processes of sediments. Jones & Simon (1984) were able to show that the addition of pyrophosphate to freshwater sediments not only stimulated sulphate uptake, but also increased the use of volatile fatty acids although not to the degree observed when sulphate and nitrate was added (table 2).

TABLE 2. THE EFFECT OF PYROPHOSPHATE, SULPHATE AND NITRATE ON THE ACCUMULATION OF VOLATILE FATTY ACIDS IN FRESHWATER SEDIMENTS

	percentage decrease caused in the accumulation of		
addition	acetate	propionate	formate, lactate, succinate[a]
pyrophosphate	47	40	40
sulphate	79	100	70
nitrate	41	73	79

(The data are taken from Jones & Simon (1984).)

[a] Under the h.p.l.c. operating conditions used these acids were not separated.

It is worth mentioning here that synthetic processes may also occur in the absence of oxygen. In relation to the above, one of the most relevant is the autotrophic conversion of carbon dioxide to acetate;

$$2CO_2 + 4H_2 \rightarrow CH_3COO^- + H^+ + 2H_2O;$$

($\Delta G^{\circ\prime} = -95$ Kj mol^{-1}, see Thauer *et al.* 1977). This reaction may be performed by bacteria such as *Acetobacterium woodii* (Balch *et al.* 1977) and *Clostridium aceticum* (Braun *et al.* 1981) and the enzymes involved have now been determined (Eden & Fuchs 1983). Although Braun *et al.* (1979) reported viable counts of acetogenic bacteria to be *ca.* 1% of those of methanogens in sludge and lake sediments, Lovley & Klug (1983*b*) estimated that acetogens accounted for *ca.* 5% of H_2 consumption in the muds of Wintergreen Lake. These measurements were, however, made by using sediment slurries incubated under an artificial atmosphere. Given the effect of partial pressure of hydrogen on the processes described above, the role of acetogenesis in the sediments of Blelham Tarn was investigated at this laboratory. When small sediment cores were used, taking care to avoid disturbance and to alter the soluble gas state, hydrogen consumption by acetogens could be as high as 50% of that by the methanogens (table 3).

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Table 3. Hydrogen consumption by methanogenic and acetogenic bacteria in the sediments of Blelham Tarn, English Lake District

	hydrogen consumed/(μmol g^{-1} d^{-1})		
	a	*b*	*b*/*a*
sediment depth/cm	$CO_2 \rightarrow CH_4$	$CO_2 \rightarrow CH_3COOH$	%
0–1	2.4	1.2	52
1–2	3.6	0.3	8
2–3	3.1	0.3	9
3–4	2.2	0.5	23

(Jones & Simon, unpublished results.)

Clearly these organisms may play a significant role in the interaction of anaerobic bacteria in some sediments.

Section 2 was concerned with electron acceptors and their reduction. Two products of such reductions, sulphides and ammonia, are not unique to electron transport mediated reactions and may be the result of putrefaction and ammonifaction respectively, i.e. the decomposition of organic molecules containing $-SH$ and $-NH_2$ groups. Both processes may be significant in the sediments of eutrophic lakes (Jones *et al.* 1982*c*; Jones *et al.* 1982*b*) although inadequate analytical methods and chemical binding of both groups prevent quantitative determination of their importance.

4. Limitations on decomposition in sediments

Our understanding of the capabilities of anaerobic bacteria (in particular) has broadened so rapidly in the past decade that any list of their molecular limitations must, of necessity, have contracted. The bacteriology of the anaerobic metabolism of aromatics is reviewed by Sleat & Robinson (1985) and few additional comments are offered here. Some of the compounds recently found to be degraded anaerobically are shown in table 4. Like that of polymer

Table 4. Some substrates used by anaerobic bacteria

substrate	organism or source	reference
syringic acid, gallic acid and derivatives	mixed community in freshwater sediment	Kaiser & Hanselmann (1982)
nicotinic acid	*Desulfococcus niacini*	Imhoff-Stuckle & Pfennig (1983)
benzoate	mixed community in freshwater sediment	Sleat & Robinson (1983)
trihydroxybenzenes	*Pelobacter acidigallici*	Schink & Pfennig (1982*a*)
succinate	*Propionigenium modestum*	Schink & Pfennig (1982*b*)

degradation in sediment, the initial step in the metabolism of aromatics, ring reduction, is endergonic and the following reactions may only proceed at low partial pressures of H_2 (see preceding section). Until recently, lignin was considered to be a refractory component in the absence of oxygen, but work by Benner *et al.* (1984) has shown that lignin and lignified plant tissue is biodegradable in anaerobic sediments. These authors prepared [^{14}C]lignin lignocelluloses from herbaceous plants and hardwoods. Not surprisingly the former (17%

conversion to $^{14}CO_2$ and $^{14}CH_4$ in 294 days) was degraded more rapidly than the latter (1.5% in 246 days). Similar, very slow, rates of conversion to CO_2 were observed when Ward & Brock (1978) studied hydrocarbon (hexadecane) metabolism by anaerobic lake sediments. These rates were not stimulated by the addition of sulphate and nitrate. Methane has long been considered to be resistant to anaerobic attack. Concentration gradients of methane in marine sediments suggested, however, that it was being consumed within the sediment (Bernard 1979) and ^{14}C-labelled methane added to marine and freshwater sediments has been shown to be converted to $^{14}CO_2$ (Reeburgh 1980; Iversen & Blackburn 1981; Panganiban *et al.* 1979; Zehnder & Brock 1980). Although sulphate-reducing bacteria have been implicated in this process, no isolates have been obtained and the mechanism remains to be determined.

The molecular configuration of the substrate confers only one limitation on rates of decomposition; the remainder of this section is devoted briefly to another, namely the effect of temperature on microbial processes. Although many benthic bacteria may grow rapidly at temperatures as low as 0 °C (Innis & Mayfield 1978) other processes, such as methanogenesis, are severely temperature limited in deep-water sediments (Zeikus & Winfrey 1976). In very general terms the reported ranges of temperature optima for denitrification, sulphate reduction and methanogenesis are 20–25, 30–40 and 35–45 °C respectively. Only the first of these is likely to be encountered in natural sediments and probably only in those associated with shallow water (thermal vents and hot springs excluded). The anaerobic production of acetate in freshwater sediment slurries has an even higher temperature optimum (figure 2), averaging 50 °C. Attempts to obtain pure cultures of anaerobes that function in the laboratory at the same temperature as that encountered in the sediment have often failed (see, for example, Jones

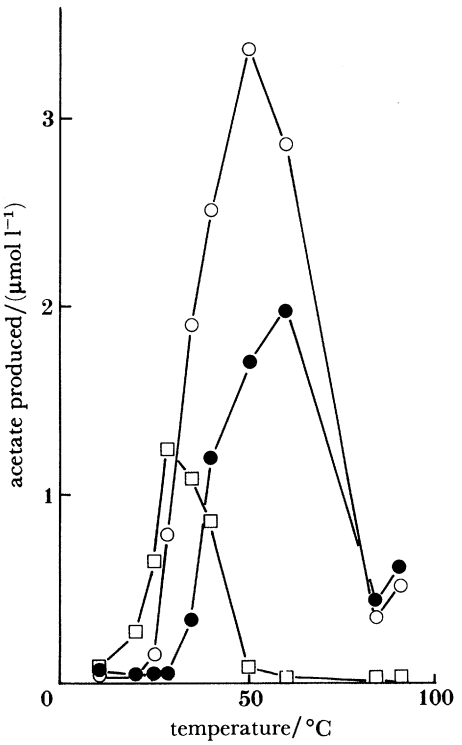

FIGURE 2. The effect of temperature on the production of acetate and methane by sediment slurries. Acetate production in the presence of H_2–CO_2 (○) and N_2–CO_2 (●) and methane production in the presence of H_2–CO_2 (□) are shown.

et al. 1982*a*). Why should bacteria exhibit such high temperature optima and yet function with apparent efficiency at much lower temperatures in the field? Microbiologists might question not only the origin of these bacteria, but also whether the results are due to an inability to reproduce field conditions adequately in the laboratory, or to isolate those organisms that are active in the sediments.

5. Microbes as contributors to sedimented organic matter

The preceding sections of this paper have considered microbes as decomposers, i.e. organisms responsible for the alteration of incoming organic matter and the conversion of susceptible fractions to carbon dioxide and methane. However, they must also be considered as producers, containing components within their biomass such as lipids, which might be used as biomarkers to identify zones where particular organisms are active. This approach to microbial ecology has received considerable impetus from the work of D. C. White and his group and details of the procedures and their applications are provided in a recent review (White 1983). Lipid phosphate has been proposed as a general indicator of microbial biomass (White *et al.* 1979), though variations occur with the nutritional status of the microbial groups concerned. The components of the microbial community can be determined in greater detail depending on the lipid fractions analysed. Short branched-chain C_{15}-saturated acids, *cis*-vaccenic acid and C_{17}- and C_{19}-cyclopropane fatty acids indicate various bacterial groups, polyenoic fatty acids of more than 20 carbon atoms indicate eukaryotes, phosphosphingolipids certain fermenters, while diphytanyl glycerol diether and dibiphytanyl diglycerol tetraether phospholipids are characteristic of halophilic bacteria and methanogens (Tornabene & Langworthy 1979). Communities of aerobic and facultative bacteria grown in the laboratory have been distinguished on the basis of cyclopropyl fatty acids in the former and 7-octadecenoic and lauric acid in the latter (Parkes & Taylor 1983). The same authors (Taylor & Parkes 1983) observed that the fatty acid composition of sulphate-reducing bacteria depended on the substrate used as a carbon source. *Desulfobacter* grown on acetate and *Desulfobulbus* grown on hydrogen and carbon dioxide contained C_{even} fatty acids whereas the latter grown on propionate contained predominantly C_{odd} fatty acids. *Desulfovibrio* contained branched-chain iso- and anteiso-fatty acids. The authors concluded that a single biomarker could not be used to cover all sulphate-reducing bacteria, and that the dominance of C_{even} fatty acids in sediments may indicate the importance of acetate as a substrate for these organisms

These observations raise some interesting questions about the use of biomarkers (also referred to as signatures or fingerprints) in microbial ecology and it is worthwhile considering what key features they should exhibit. If such analytical techniques are to provide quantitative information about a microbial community, rather than merely to indicate dominance or otherwise of a particular group, for example, dominance of fungi or bacteria (which might be ascertained more easily with a microscope), then the biomarker should be chosen with care. It should be unique to the organism (or group) concerned, or at least predominate within that group. It should be a true indicator of biomass; in other words, it should not persist after the death of the microbe. Its turnover time should, therefore, be short in relation to the generation time of the organism. The biomarker should also possess conservative properties, in that its relation to biomass should be constant and not depend on the nutritional status of the organism (i.e. the degree of starvation or satiation or the substrates used). Many of the compounds

discussed above are of relatively high molecular mass, or possess structures that may preclude rapid turnover, particularly in the absence of oxygen. Even phospholipids, now extensively used by microbiologists as biomarkers, may possess $T^{\frac{1}{2}}$ (the time taken to metabolize half of the biomarker in the organisms after death) of as long as 16 days (White *et al.* 1979). Total lipid phosphate has also been shown to decrease by 65% during starvation (Oliver & Stringer 1984) therefore biomass estimates based on such analyses will be subject to considerable error. These same compounds are also considered to be of geochemical significance (Edmunds *et al.* 1982). In addition, the results cited above (Taylor & Parkes 1983) indicate that in certain groups of bacteria the fatty acid biomarkers produced depend on the chain-initiator substrates used. Certain of these substrates chosen as biomarkers (for example, iso- and anteiso-branched acids) are also assumed, by geochemists, to be the residues of the microbial population (Cranwell 1982).

Clearly the same compound, or class of compounds, cannot serve both as biomarker and as historical or geochemical marker. It is the task of the microbiologist to provide a more complete understanding of biomarkers, particularly lipids (which are also used increasingly for taxonomic purposes). The stability of each biomarker under changing environmental conditions must be determined, as must the degree to which it is metabolized following death of the microbe. Perhaps, then, the geochemist will be provided with a more realistic assessment of the role of microbes in the deeper sediments. In the meantime, measurements such as turnover of labelled substrates, incorporation of labelled acetate and phosphate into lipids, may provide the only indicators of metabolic activity at depth. Such experiments are difficult to perform *in situ*.

In addition to the reservations expressed above, it is worth emphasizing that many of the bacteria associated with sediments have yet to be isolated and their role as producers of such biomarkers to be determined. Among these are the filamentous bacteria, which can, on occasions, account for up to 50% of the bacterial biomass. They are notoriously difficult to isolate and, if recent small successes are typical (Maiden & Jones 1984), occupy highly specialized niches in the sediment. Other organisms that may be of greater interest to geochemists (such as *Metallogenium*, *Achromatium*) remain to be isolated.

6. Concluding remarks

Although the microbiologist may be able to make broad predictions about the processes likely to dominate in a sediment, given some characteristics of that sediment and its overlying water, this may not be extended to a detailed description of the microbial community. This paper has attempted to consider those factors that might provide microbiologists and geochemists with a better understanding of such communities; these may be summarized as follows.

Electron acceptors may play a significant role in the control of metabolism, but the acceptor itself may not be the factor that limits microbial activity, nor does the presence of one acceptor preclude the use of another at any given depth in the sediment. In addition, the known range of electron donors, particularly for sulphate-reducing bacteria, is widening rapidly, as is our understanding of the ability of these organisms to compete effectively at low sulphate concentrations. In the absence of electron acceptors, thermodynamically unfavourable reactions may proceed via inter-species hydrogen transfer and other, less well defined reactions, where inorganic species may act as the hydrogen sink. The role of organisms that

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conserve the energy of pyrophosphate and polyphosphate bonds and those that produce acetate from carbon dioxide require further investigation in a greater variety of aquatic sediments. Just as the list of substrates for sulphate reducers has lengthened, so has that of apparently refractory components that are now known to be degraded anaerobically, and that now includes lignin and certain hydrocarbons. The organisms or consortia responsible have yet to be isolated, and this is also true of anaerobic methane oxidation.

Microbes must also be considered as producers of organic matter, but the choice of key compounds as estimators of biomass (biomarkers) is constrained by their specificity, biodegradability and inherent conservative properties. Such compounds cannot serve the dual role of biomarker and historical marker, although some do at present. It is the task of the microbiologist to determine the suitability of these compounds for their role. Finally, it is important to emphasize that many microbes remain to be isolated and their role in sediments is, as yet, not understood. As our understanding of sediments and their microbial populations grows, then so does the opportunity for collaboration between microbiologists and geochemists.

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Discussion

R. A. Berner (*Department of Geology and Geophysics, Yale University, U.S.A.*). Much of the iron in sediments is in the ferric form, for example, as $Fe(OH)_3$ and is insoluble. How do the microbes take this iron into the cell?

J. G. Jones. We do not know the answer to this question. In some cases we know that physical contact between the organism and the iron is important. The iron may well be transported

across the cell membrane in chelated form. In other cases it is clear that iron reduction can occur without contact, presumably by some reaction with a chemical produced by the organism.

R. A. Berner. How much of the organic matter in the sediments is destroyed by the reduction of iron and manganese?

J. G. Jones. The lakes we have examined are not rich in manganese. Though the iron reduction in the lakes is very important to their chemistry, this reaction removes only about 1 % of the carbon in the sediments. Similar estimates have been obtained from studies of Dutch lakes and also from pure culture studies.

P. S. Meadows (*Zoology Department, Glasgow University*). We have recently tried to relate aerobic and anaerobic bacterial biomass (colony-forming units) and microbial activity ([^{14}C]glucose breakdown) with *Eh* profiles in deep-sea sediments from the North Eastern Atlantic.

Both aerobic and anaerobic bacterial biomass and microbial activity fall to zero within 10–20 cm of the sediment–water interface. However, there is often no reduction in *Eh* at all, the sediment remaining fully oxidized.

I wonder whether Dr Jones has any comments on how this system may relate to freshwater sediments, particularly in view of the very different *Eh* régimes?

J. G. Jones. The constant value of *Eh* is presumably caused by the penetration of electron acceptors into the upper parts of the sediment, but it is difficult to say more without a knowledge of the chemistry of the overlying waters. There are considerable problems in interpreting the results of viable counts and microbial activity measurements when the environment of the organisms has been changed. Unless the sediment remains undisturbed and the partial pressure of hydrogen in the experiments is the same as that in the sediments, the results are not likely to be very useful. The respiration rate determined from [^{14}C]glucose suffers from similar difficulties, because glucose is not likely to be the natural substrate of the organisms.

P. S. Meadows. Unfortunately it is not straightforward to perform controlled experiments on deep-sea research vessels. Indeed, any sort of radioactive experiments are difficult.

J. G. Jones. I still believe it is important to perform the experiments under the correct conditions, and that the results will not make sense until this is done. I do, however, sympathize with your difficulties.

B. Durand (*Institut Français du Pétrole, Rueil Malmaison, France*). As a petroleum geologist I am interested in the conditions that must be satisfied before large volumes of methane can be generated biologically.

J. G. Jones. One needs a large and steady input of organic material that can be fermented to produce acetate. A steady input of hydrogen and carbon dioxide will also provide a substrate for the other methanogens. I would also expect that the temperature at which the reactions would proceed most rapidly would be about 40 °C, but in the light of recent discoveries this temperature may be too low.

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G. Eglinton (*Organic Geochemistry Unit, School of Chemistry, University of Bristol*). To what depths does Dr Jones believe that organisms remain active in consolidating marine sediments when water circulation is absent?

J. G. Jones. Most of my work has been concerned with biological processes within centimetres of the water–sediment interface. I see few problems in the organisms remainging active at considerable depths. For instance there is now evidence that lignin is biodegradable, and that about 1 % of the material is consumed in 150 days. So I think that some slow biological activity can continue, provided liquid water is available.

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