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Microbial Interactions in Sediment Communities [and Discussion]

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Microbial interactions in sediment communities

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Mineralization of organic matter in aquatic ecosystems with shallow waters occurs to a large extent in their sediments under anoxic conditions. This is achieved by a community of bacteria, which are the catalysts in a sequence of processes. Of the two possible terminal processes, methanogenesis and sulphate reduction, the first usually dominates in freshwater systems, whereas in estuarine and marine sediments electrons are mainly channelled to sulphate. Interactions between sulphate-reducing and methanogenic bacteria are described.

Sulphate-reducing bacteria also show interactions with fermentative bacteria. After a brief description of properties of sulphate-reducing and fermentative bacteria occurring in sediments, examples are given of interactions between them. This is followed by the presentation of some results obtained from studies on competition for L-lactate between organisms belonging to both groups. It is shown that sulphate-reducing bacteria could successfully compete for L-lactate when this was available in growth-limiting amounts with sufficient sulphate and iron.

Finally, a brief discussion is given of ecological niches of sulphide-oxidizing bacteria thriving in the upper sediment layers.

Introduction

In aquatic systems with shallow waters the major part of the particulate organic material settles on the sediments before it has been broken down to any large extent in the aerobic water phase. And as in these sediments anoxic conditions prevail, anaerobic bacteria form a community that is mainly responsible for the mineralization of organic matter in this environment. Even in the surface layers of sediments into which oxygen may penetrate, anaerobic pockets often occur in which strict anaerobes such as sulphate-reducing bacteria may be highly active (Jørgensen 1977*a*).

The microbes involved in the anaerobic mineralization in sediments are generally much less versatile than the aerobes (Sepers 1981) with respect to the number of organic carbon and energy sources that can be used. An exception to this may be some denitrifying bacteria that are active in sediment surfaces and whose anaerobic metabolism is not greatly different from that exhibited under aerobic conditions. But for apparently stringent reasons, such quantitatively important substrates as glucose cannot be oxidized to completion by microbes that can carry out anaerobic respiration in which sulphate or carbon dioxide function as electron acceptors.

In contrast to aerobic environments, the mineralization in sediments is thus the result of a sequence of processes in which the products of one metabolic group of organisms form the substrates for others. The community thus formed therefore consists of microbes that are highly dependent on each other's activities. And for this reason the participants in anaerobic mineralization processes are of particular interest with respect to the study of microbial interactions.

A community similar to that encountered in sediments occurs in the anaerobic environment created by man for the degradation of organic matter in sewage. Bryant (1976, 1979) describes

this process as being carried out by three groups of bacteria. The first is the group of fermentative bacteria that hydrolyse organic polymers and ferment the monomers. The second group consists of acetogenic bacteria. Although many fermentative bacteria as well as some sulphate-reducing bacteria produce acetate, the term acetogenic was originally given to organisms that convert non-fermentable substrates such as butyrate and propionate to CO_2 or acetate and molecular hydrogen, or both. For thermodynamic reasons these processes are only feasible in the presence of a second bacterium (e.g. a methanogen or sulphate-reducing bacterium) that consumes the hydrogen and thus keeps the concentration of molecular hydrogen very low.

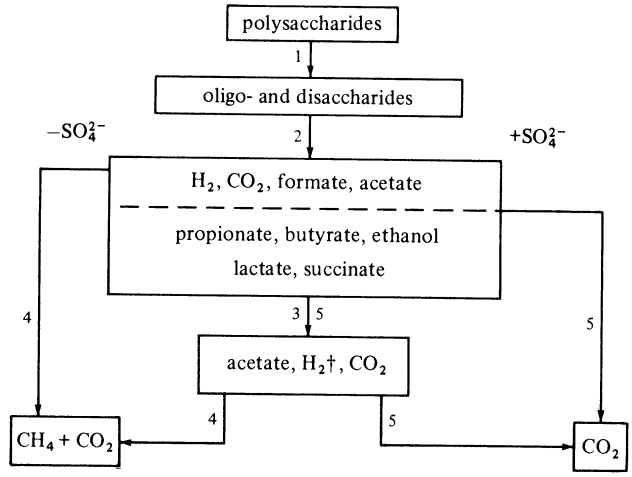

FIGURE 1. Simplified scheme of the sequence of processes involved in the mineralization of polysaccharides in anaerobic sediments. 1, hydrolysis; 2, fermentation; 3, acetogenesis; 4, methanogenesis; 5, sulphate reduction; †, only in acetogenesis.

When no other substrates can be used, such acetogens are condemned to syntrophy. This characteristic led to generic names such as *Syntrophomonas* and *Syntrophobacter* (McInerney *et al.* 1979; Boone & Bryant 1980). Finally, the third group of organisms, the methanogenic bacteria, utilize the end-products of all previous processes, which are acetate, formate, hydrogen and carbon dioxide, in the production of methane. With respect to hydrogen and carbon dioxide they have to compete with organisms such as *Clostridium aceticum* (Wieringa 1940; Braun *et al.* 1981) and *Acetobacterium woodii* (Balch *et al.* 1977), which convert these substrates to acetate. In fact there is also another group of bacteria in sludge that can act as a terminal electron sink. This is the group formed by the sulphate-reducing bacteria, which do compete with the acetogens and methanogens for available substrates. However, because the sulphate concentration is rather low in municipal sewage, the quantitative importance of sulphate reduction in sludge is small. A simplified scheme of the sequence of processes involved in the mineralization of polysaccharides is given in figure 1.

Which of the two terminal processes of anaerobic mineralization, methanogenesis or sulphate reduction, will become dominant depends to a large extent on the availability of sulphate. When this is high, electrons are channelled to sulphate, and methanogenesis is inhibited. A recent example has been given by Zaiss (1981) who studied the sediments of the impounded river Saar (Germany). The sulphate content of its water is relatively high (1.8 mM) compared with other fresh waters, and depending on water discharge and current velocities of the river, the infiltration of sulphate into the sediment varies throughout the year. In periods of increased

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infiltration the rate of sulphate reduction increased and a concomitant decrease of methanogenesis was observed. This was confirmed in laboratory studies. When small amounts of sulphate were added to sediment samples, methane production was inhibited and resumed after sulphate depletion. This latter phenomenon was not observed when larger amounts of sulphate were added, which was presumably due to exhaustion of electron donors. The inhibition of methanogenesis by sulphate could be overcome by the simultaneous addition of hydrogen or acetate (figure 2).

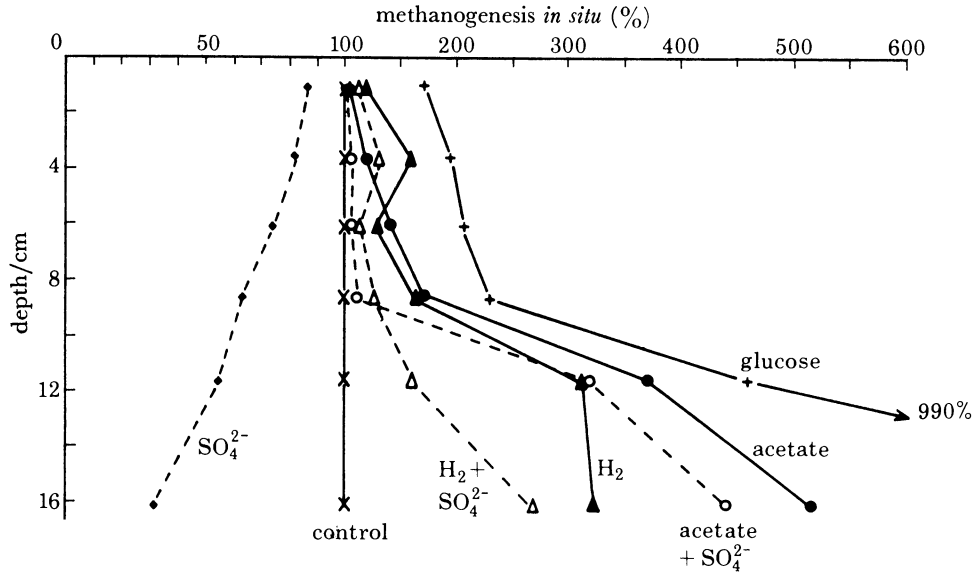

FIGURE 2. Effect of the addition of sulphate (10 μmol g^{-1}), hydrogen (50 μmol g^{-1}), acetate (30 μmol g^{-1}) and glucose (10 μmol g^{-1}) on methanogenesis in a depth profile of river Saar sediment. No substrate was added to control samples. The rates of methanogenesis are expressed as percentages of the control activity. (Zaiss 1981).

The observations of Zaiss (1981) confirm those of Winfrey & Zeikus (1977) who found that methanogenesis in sediments of Lake Mendota (U.S.A.) was inhibited by addition of sulphate and that the inhibition could be reversed by the addition of acetate or hydrogen. Even at very low sulphate concentrations, however, sulphate reduction can be of quantitative importance in the anaerobic mineralization of organic matter when short turnover times of the sediment sulphate pool occur. This was observed by Smith & Klug (1981 *a*) in sediments of a eutrophic lake basin.

In seawater the sulphate concentration (32 mmol/l) is very much higher than in fresh water so that even in estuaries where mixing with fresh water occurs the reduction of sulphate is the dominant terminal process in the anaerobic mineralization of organic matter (Jørgensen 1977 *b*; Sørensen *et al.* 1979; Jørgensen 1980; Mountfort *et al.* 1980). This does not mean, however, that in all anaerobic marine or estuarine environments sulphate reduction always dominates methanogenesis. In the deeper layers of marine sediments which are depleted of sulphate, methanogenesis may become dominant (Martens & Berner 1974, 1977), and when marine sediments are highly enriched with organic matter, methanogenesis may become of quantitative importance. This has been observed by Oremland (1975) in sea grass beds and also by Mountfort & Asher (1981) in heavily polluted intertidal sediments of Waimea Inlet (New Zealand).

The above considerations can be summarized as follows. Acetate, hydrogen and carbon

dioxide are the main end-products of a complex and largely unknown network of processes occurring in sediments of natural waters. Of the two main groups of organisms competing for hydrogen and acetate, the methanogenic and the sulphate-reducing bacteria, the latter group is the more successful under conditions of sulphate sufficiency. The molecular basis of this selective advantage still has to be elucidated.

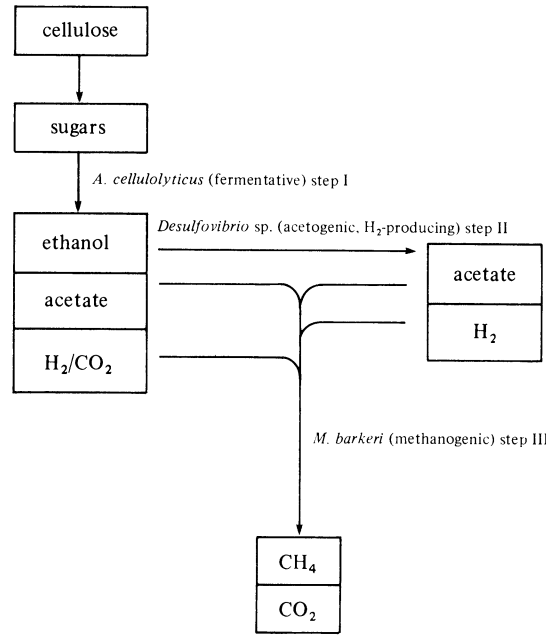

FIGURE 3. Reactions involved in the conversion of cellulose to methane and carbon dioxide by a mixed culture of *Acetivibrio cellulolyticus*, *Desulfovibrio* sp., and *Methanosarcina barkeri* (Laube & Martin 1981).

The picture given above is, however, an oversimplification of the terminal processes occurring in sediments. The energy substrates used by methanogens are not restricted to acetate and hydrogen. Methanol, which is formed in the degradation of pectin (Rode *et al.* 1981), may also be used, as well as methylamines (Hippe *et al.* 1979). In addition, the range of substrates that can be used by the group of sulphate-reducing bacteria is not restricted to acetate and hydrogen at all, as will be shown in the next section. The use of some of these is not even dependent on the presence of sulphate. *Desulfobulbus propionicus* can use lactate with sulphate as electron acceptor but can also ferment lactate (Widdel 1980), and the utilization of ethanol and lactate by *Desulfovibrio* is not always dependent on the presence of sulphate. Growth on these substrates in the absence of sulphate is only possible, however, in the presence of another bacterium that utilizes the hydrogen produced. This was demonstrated by Bryant *et al.* (1977) who applied *Methanobacterium formicicum* as the hydrogen-utilizing bacterium in the presence of carbon dioxide. Thus in the absence of sulphate a synergistic relation may occur between *Desulfovibrio* and a methanogen. Another example of this has been given by Laube & Martin (1981). This pertains to the conversion of cellulose to methane and carbon dioxide by a three-membered culture of *Acetivibrio cellulolyticus*, *Desulfovibrio* sp. and *Methanosarcina barkeri*. The reactions involved are shown in figure 3.

Coexistence of methanogens and sulphate-reducing bacteria may even occur in the presence of sulphate. This may be so when under sulphate limitation lactate and ethanol are oxidized to

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acetate by *Desulfovibrio* and the acetate is subsequently used by a methanogen. Mountfort *et al.* (1980) encountered a field situation that according to their suggestion might be explained by an interrelation of this kind. However, another explanation seems possible, as will be discussed in the section on sulphate-reducing bacteria.

Sulphate-reducing bacteria do not only show interactions with methanogens: they also have to compete with fermentative bacteria for particular energy sources such as lactate. In the next sections a brief discussion will be given of the properties of sulphate-reducing and fermentative bacteria occurring in sediments. This will be followed by the presentation of some results obtained from studies on interactions between organisms belonging to these groups. They were isolated from sediments of the Ems–Dollard estuary, which is situated on the borderline between Germany and the Netherlands. Import of organic matter in the estuary comes from the North Sea, the river Ems and also from a system of canals which in the autumn becomes heavily polluted by waste water of potato starch mills (Van Es 1977). Further details of this estuary have been given by Van Es *et al.* (1980). Finally, a brief discussion will be given of activities of sulphide-oxidizing bacteria in sediment surfaces.

Sulphate-reducing bacteria

In his excellent monograph on sulphate-reducing bacteria, Postgate (1979) describes how after their discovery by Beijerinck (1895) the knowledge of this group of organisms accumulated only slowly. Even in 1949 only few pure cultures were available (Butlin *et al.* 1949) and the organic substrates mainly used as carbon and energy source, malate and lactate, were oxidized to the acetate level. Yet early observations by Hoppe-Seyler (1886) showed a complete oxidation of cellulose to carbon dioxide and methane in anaerobic cultures inoculated with mud, and when calcium sulphate was added, a complete conversion of cellulose carbon to carbon dioxide was observed with a concomitant reduction of sulphate to sulphide. Similar observations were subsequently made in crude enrichment cultures for sulphate-reducing bacteria by Beijerinck (1895), Van Delden (1903) and Rubentschik (1928). It was not until 1930, however, that Baars described the acetate-oxidizing *Desulfovibrio rubentschickii*. Exhaustive attempts to reisolate this species failed (Selwyn & Postgate 1959). In 1976 Pfennig & Biebl reported the isolation of *Desulfuromonas acetoxidans* from cultures of '*Chloropseudomonas ethylica*', which had been shown by Gray *et al.* (1973) to be a syntrophic mixture of the phototrophic *Chlorobium limicola* and a colourless bacterium. However, *Desulfuromonas*, which also occurs in anaerobic sediments, can only oxidize acetate and ethanol with elemental sulphur as the electron acceptor (Pfennig & Biebl 1976). One year later Widdel & Pfennig (1977) described the isolation of an acetate-oxidizing sulphate-reducing bacterium, which was named *Desulfotomaculum acetoxidans*. Its temperature range (20–40 °C) was rather high, however, and as it occurs in animal manure, rumen contents and freshwater habitats contaminated with dung (Widdel & Pfennig 1981*a*), its general importance in the breakdown of acetate in sediments of natural waters seems doubtful. A major breakthrough in the field of sulphate reduction came subsequently through the work of Widdel (1980) in Pfennig's laboratory. In his thesis, Widdel described no less than six new species, belonging to five new genera. Five of these species could oxidize acetate with sulphate as electron acceptor. In addition, Widdel described a new acetate-oxidizing *Desulfovibrio* species, *Desulfovibrio baarsii*. A comparison of substrates used by recently isolated species of sulphate-reducing bacteria with those used by the type species of the genera *Desulfovibrio*

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(*Desulfovibrio desulfuricans*), *Desulfotomaculum* (*Desulfotomaculum nigrificans*) and *Desulfomonas* (*Desulfomonas pigra*) is presented in table 1.

As most of the new genera were isolated from brackish or marine sediments (Widdel 1980; Widdel & Pfennig 1981*b*), a study of the distribution of acetate-oxidizing and propionate-oxidizing sulphate-reducing bacteria was made in the Ems–Dollard estuary (Laanbroek & Pfennig 1981). The propionate-oxidizing *Desulfobulbus* was found in marine as well as in fresh-water sediments, whereas the acetate-oxidizing *Desulfobacter* was only observed in marine

TABLE 1. COMPARISON OF SUBSTRATES USED BY RECENTLY ISOLATED SPECIES OF SULPHATE-REDUCING BACTERIA WITH THOSE USED BY THE TYPE SPECIES OF THE GENERA *DESULFOTOMACULUM* (*DESULFOTOMACULUM NIGRIFICANS*) (CAMPBELL & POSTGATE 1965), *DESULFOVIBRIO* (*DESULFOVIBRIO DESULFURICANS*) (POSTGATE & CAMPBELL 1966) AND *DESULFOMONAS* (*DESULFOMONAS PIGRA*) (MOORE *ET AL.* 1976)

	Desulfobacter postgatei	*Desulfobulbus propionicus*	*Desulfococcus multivorans*	*Desulfomonas pigra*	*Desulfonema limicola*	*Desulfonema magnum*	*Desulfotomaculum acetoxidans*	*Desulfotomaculum nigrificans*	*Desulfosarcina variabilis*	*Desulfovibrio baarsii*	*Desulfovibrio desulfuricans*	*Desulfovibrio sapovorans*
source† ...	1	1	1	2	1	1	1	3	1	1	4	1
complete oxidation of organic substrates	+	−	+	−	+	+	+	−	+	+	−	−
electron donors												
H_2	−	+	−	−	+	−	−	.	+	−	+	−
formate	−	−	+	−	+	+	+	−	+	+	+	−
acetate	+	−	+	−	+	+	+	−	+	+	−	−
propionate	−	+	+	−	+	+	−	−	+	+	−	−
n-butyrate	−	−	+	−	+	+	+	−	+	+	−	+
n-valerate	−	−	+	−	+	+	+	−	+	+	−	+
n-laurinate	−	−	+	−	+	−	−	−	+	+	−	+
n-stearate	−	−	−	−	−	−	−	−	−	+	−	+
lactate	−	+	+	+	+	−	−	+	+	−	+	+
pyruvate	−	+	+	+	+	−	−	+	+	−	+	+
malate	−	−	−	.	−	+	−	.	−	−	+	−
benzoate	−	−	+	.	−	+	−	.	+	−	−	−
glucose	−	−	−	−	−	−	−	+	−	−	−	−
ethanol	−	+	+	.	−	−	+	.	+	−	+	−
propan-1-ol	−	+	+	.	−	−	−	.	+	−	+	−
butan-1-ol	−	+	+	.	−	−	+	.	+	−	+	−

† 1, Widdel (1980); 2, Moore *et al.* (1976); 3, Campbell & Postgate (1965); 4, Postgate & Campbell (1966).

sediments. The sediments of the estuary consist of a semi-aerobic greyish upper layer of several centimetres, situated on black sediment. The upper layer contains many black anaerobic pockets and has similar properties with respect to redox potential and sulphate reduction activities to those found by Jørgensen (1977*a*) in coastal marine sediments in Denmark. In the Ems–Dollard estuary the vertical distribution of *Desulfobulbus* and *Desulfobacter* was similar to that of lactate-oxidizing *Desulfovibrio desulfuricans*, and the highest numbers of all these organisms were found just beneath the transition zone between grey and black sediment (Laanbroek

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& Pfennig 1981). A relatively fast oxidation of acetate and propionate was only found in the presence of excess sulphate. In sediment samples to which no sulphate had been added, the rate of disappearance was much lower and a concomitant production of methane was observed.

Sørensen *et al.* (1981) determined the relative importance of hydrogen and short-chain fatty acids as electron donors for sulphate reduction in marine sediments. They compared the rate of sulphate reduction with that of the accumulation of electron donors in samples to which sodium molybdate had been added, which inhibits sulphate reduction (Peck 1959). Assuming a complete oxidation of propionate and butyrate to carbon dioxide, the electron donors hydrogen, acetate, propionate and butyrate accounted for 5–10, 40–50, 10–20 and 10–20 %, respectively, of the sulphate reduced.

The importance of sulphate reduction in the mineralization of various organic compounds in a freshwater sediment was determined by Smith & Klug (1981 *b*), who measured the change in mineralization rates of ^{14}C-labelled substrates after the addition of an appropriate amount of sodium molybdate. The initial mineralization rate of acetate, propionate and lactate decreased by 14, 52 and 58 %, respectively, after addition of the inhibitor. The initial mineralization rate of glucose was unaffected by molybdate, which was not unexpected since of all known sulphate-reducing species, only *Desulfotomaculum nigrificans* has been reported to utilize glucose (Campbell & Postgate 1965).

The direct participation of sulphate-reducing bacteria in the mineralization of amino acids is still somewhat unclear. Smith & Klug (1981 *b*) reported that at least 85 % of an amino acid mixture was degraded by sulphate reduction in a lake sediment, and that casamino acids could be used for growth of several of their sulphate-reducing isolates. However, enrichment cultures with single amino acids and with mixtures of amino acids inoculated with mud from the Ems–Dollard estuary always resulted in mixtures of fermentative and sulphate-reducing bacteria (T. A. Hansen & A. J. M. Stams, personal communication).

It is also not yet clear whether methane or higher hydrocarbons can be oxidized directly by sulphate-reducing bacteria. Sorokin (1957) could not demonstrate oxidation of methane by these organisms. However, Davis & Yarbrough (1966) observed a slow oxidation of [^{14}C]methane by *Desulfovibrio desulfuricans* during growth on lactate. Reeburgh (1976) and Martens & Berner (1977) suggested that methane produced in deeper layers of marine sediments was oxidized in the upper sulphate-rich layers and they adopted the idea of a slow co-metabolism of methane by sulphate-reducing bacteria to explain the absence of methane in the anoxic upper layers. Mountfort *et al.* (1980) and Devol & Ahmed (1981) found two distinct peaks of sulphate reduction activity in anaerobic sediments. One was located near the surface, the other in a deeper layer where sulphate concentration approached zero. This latter peak occurred in the layer where methane production was observed.

Experiments of Zehnder & Brock (1980) indicated that in sediments sulphate-reducing bacteria occur, which may contribute to the anaerobic oxidation of methane. Addition of ferrous sulphate to anoxic sediments stimulated the anaerobic oxidation of methane, whereas the addition of either sulphate or iron did not enhance the oxidation. Zehnder & Brock (1980) proposed that iron was indispensable as it neutralized the toxic sulphide produced.

Although sulphate is the most common sulphur compound used as electron acceptor, sulphite and thiosulphate are also good acceptors for many bacteria. *Desulfotomaculum acetoxidans* and *Desulfonema magnum* are the only sulphate-reducing bacteria described that cannot use sulphite or thiosulphate (Widdel 1980). Elemental sulphur is only used by some rod-shaped strains of

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Desulfovibrio desulfuricans (Biebl & Pfennig 1977) and by some bacteria that are not sulphate-reducing, such as *Desulfuromonas acetoxidans* (Pfennig & Biebl 1976) and *Campylobacter* species (Wolfe & Pfennig 1977; Laanbroek *et al.* 1978). Smith & Klug (1981*a*) observed a low sulphur reduction rate in freshwater sediments.

Fermentative bacteria

In comparison to the rumen (Bryant 1959; Hungate 1966) little is known about the fermentative bacteria active in sewage digestors and in the sediments of natural waters. Results described by Hobson *et al.* (1974) and Bryant (1976) indicate that in sewage digestors the dominant flora consists of strict anaerobes among which *Bacteroides ruminicola* was very numerous. In addition, clostridia, bifidobacteria and a variety of ill-defined organisms were observed. Among the facultative anaerobes streptococci and members of the Enterobacteriaceae were numerous. Streptococci also occurred in high numbers in piggery waste digestors (Ueki *et al.* 1978), although other Gram-positive cocci have also been isolated from this type of digestor (Spoelstra 1978).

Although occasional isolations of fermentative bacteria from sediments are mentioned throughout the literature, no detailed data about the composition of the flora of these bacteria are available from any sediment ecosystem, and quantitative data with respect to the occurrence of one or more species are almost non-existent. One recent study was carried out by Molongoski & Klug (1976), who encountered large numbers of proteolytic clostridia and saccharolytic streptococci in anoxic sediments of a hypereutrophic lake.

With respect to the Ems–Dollard estuary, early observations of Veldkamp & Elgersma (unpublished, 1964) provided indirect evidence that lactate might be an important intermediate in the sediments. From sediment surfaces around 20 different strains of aerobic spirilla were isolated, most of which grew relatively prolifically on media with lactate as carbon and energy source, indicating that this substrate might be available in their natural environment. A similar observation was made by Hespell (1977), who isolated *Serpens flexibilis* from samples of pond mud. This bacterium grew only aerobically, and lactate was the only major carbon and energy source used.

Laanbroek & Pfennig (1981) studied the fate of lactate added to sediment slurries of the Ems–Dollard estuary and found that this was rapidly degraded (figure 4). Acetate and propionate were formed and the degradation of these products was dependent on the presence of added sulphate. In its absence their degradation took a couple of weeks and a concomitant methane production was observed.

A more detailed study of lactate-utilizing bacteria in the sediment showed that the lactate-fermenting bacteria *Veillonella alcalescens* and an *Acetobacterium* species were present in the mud in approximately equal numbers to lactate-utilizing *Desulfovibrio desulfuricans* only during late summer and autumn when the estuary became heavily polluted with waste water from potato starch mills (Laanbroek *et al.* 1982). In this period 33×10^6 kg organic carbon is released here within 4 months (Van Es 1977). During the rest of the year, however, *Desulfovibrio desulfuricans* was the main lactate utilizer in the estuarine sediments according to results obtained with dilution series made in agar shake tubes with L-lactate as energy source. A few characteristics of the fermentative and sulphate-reducing bacteria are summarized in table 2. These data were generally in good agreement with those found for strains isolated from other environments (Rogosa 1964; Balch *et al.* 1977; Postgate 1979). Details about the interactions between these bacteria will be given in a later section.

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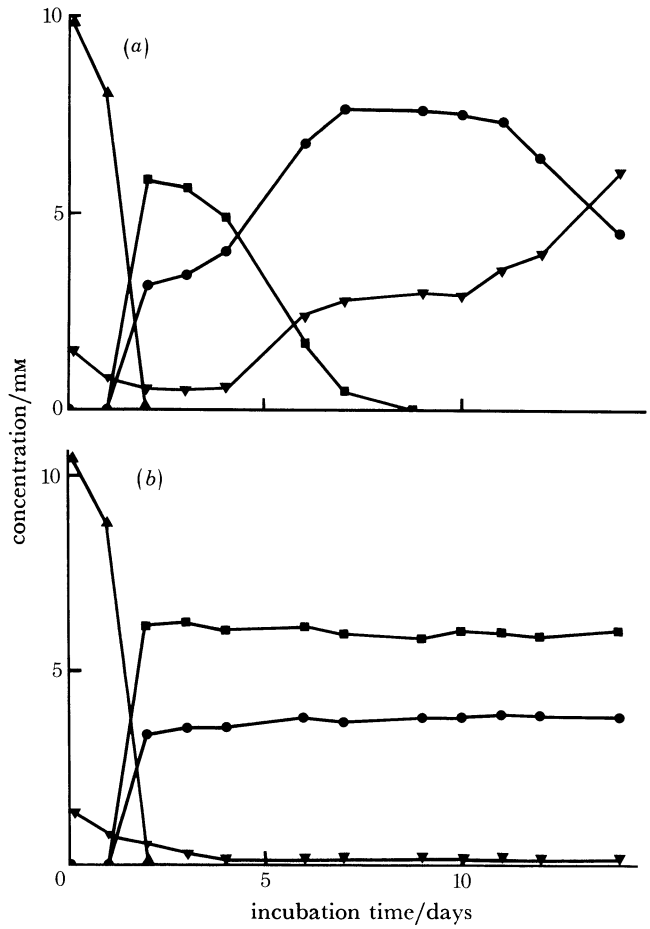

FIGURE 4. Anaerobic mineralization of L-lactate in freshwater sediment slurries: (*a*) with addition of 20 mM sulphate; (*b*) without addition of sulphate. ●, Acetate; ■, propionate; ▲, L-lactate; ▼, free sulphide. Methane production was observed during prolonged degradation of acetate and propionate in the absence of added sulphate (Laanbroek & Pfennig 1981).

TABLE 2. ENERGY SUBSTRATES OF LACTATE-UTILIZING SULPHATE-REDUCING AND FERMENTATIVE BACTERIA ISOLATED FROM ANAEROBIC SEDIMENTS OF THE EMS–DOLLARD ESTUARY (LAANBROEK ET AL. 1982)

	Desulfovibrio desulfuricans H L21	*Veillonella alcalescens* NS L49	*Acetobacterium* sp. NS L40
sulphate reduction . . .	+	−	−
energy source			
H_2	+	−	+
formate	+	−	+
L-lactate	+	+	+
pyruvate	+	+	+
DL-malate	+	+	−
citrate	−	+	−
glucose	−	−	−
ethanol	+	−	−
lactate μ_{max} (30 °C)†	0.16	0.30‡	0.06‡

† Maximal specific growth rate (h^{-1}).
‡ Measured at 1 mM sulphide, halved at 4 mM sulphide.

One of the questions studied subsequently was which bacteria are responsible for the production of lactate in the sediments of the Ems–Dollard estuary. A *Streptococcus* species appeared to be numerous as it occurred in the highest dilutions in a most probable number series with glucose as energy source (Laanbroek 1982). The organism was able to ferment a great number of oligosaccharides and disaccharides and was further characterized by its simple nutrient requirements. In fact, it may well be that lactic acid bacteria of this type are the predecessors of those with highly complicated nutrient requirements that have been known for decades as inhabitants of environments rich in amino acids and vitamins.

In batch culture the *Streptococcus* species showed a homolactic acid fermentation, but in a glucose-limited chemostat when grown at low dilution rates (e.g. 0.02 h^{-1}), acetate, ethanol and formate were the main fermentation products. This behaviour thus was similar to that found in other lactic acid bacteria (De Vries *et al.* 1970; Yamada & Carlsson 1975; Thomas *et al.* 1979). Since additional energy can be generated during the production of acetate (Thauer *et al.* 1977), some lactic acid bacteria appear to use their substrates more economically under energy limitation. Not all streptococci seem, however, to show different fermentation patterns dependent on growth conditions. *Streptococcus bovis*, for instance, was found to have a homolactic acid fermentation also under conditions of energy limitation (Yamada & Carlsson 1975).

Even though the concentration of small and degradable organic molecules seems to be higher in the sediments of natural waters than in the overlying waters, the bacteria in sediments might well often be energy-limited. And therefore these changes of fermentation pattern in streptococci may have consequences with respect to the development of the microbial flora using their fermentation products. In this respect the population density of streptococci fermenting available sugars is of obvious importance, as was shown by Thomas & Turner (1981).

Studies of the occurrence of sulphide-oxidizing colourless sulphur bacteria in the surfaces of the Ems–Dollard sediments indicated that obligately chemolithotrophic bacteria in this area are very numerous (Schröder & Van Es 1980). A rather unexpected feature of this kind of bacteria was recently revealed by Beudeker *et al.* (1981*a*), who showed that *Thiobacillus neapolitanus* does have a mode of energy generation under anaerobic conditions. The reserve material polyglucose that was formed aerobically appeared to be fermented under anaerobic conditions to lactate and ethanol. And as chemolithotrophic sulphide-oxidizing bacteria seem to occur in high numbers on the surface of the sediments of the Ems–Dollard estuary, their lactate and ethanol production may well be of importance for anaerobic processes in the surface layers of the sediment, in particular because alternation of aerobic and anaerobic conditions in microsites may occur frequently.

Interactions between sulphate-reducing and fermentative bacteria

All basic types of bacterial interactions, as formulated by Slater & Bull (1978), may occur between mud-dwelling sulphate-reducing and fermentative bacteria. Commensalism may be observed when the former grow on substrates produced by the latter. A mutualistic relation may occur when growth of fermentative bacteria is favourably influenced by the consumption of their metabolic products. Amensalism may be found when growth of fermentative bacteria is repressed by sulphide produced by sulphate-reducing bacteria. And finally, competition may be encountered when both bacterial types are dependent on a growth-limiting substrate that they have in common.

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The main reason for the necessity of interaction between fermentative and sulphate-reducing bacteria is the inability of most sulphate-reducing bacteria to utilize the monomers of polysaccharides and proteins as carbon and energy source. Their growth depends on the availability of fermentation products, and the utilization of these may increase the growth rate and yield of fermentative bacteria. One example of mutualism between fermentative and sulphate-reducing bacteria is that of interspecies hydrogen transfer in syntrophic mixed cultures. The maintenance of a very low partial hydrogen pressure by sulphate-reducing bacteria allows many hydrogen-producing fermentative bacteria to become more acetogenic (Wolin 1975), and the increase of acetate production is favourable from the point of view of energetics (Thauer *et al.* 1977).

Utilization of fermentation products other than hydrogen may also increase the yield of fermentative bacteria. Otto *et al.* (1980*a*) demonstrated that in *Streptococcus cremoris* the efflux of lactate generated an electrochemical proton gradient that increased the energy yield during growth on lactose by at least 12%. Ten Brink & Konings (1980) subsequently showed that the amount of energy obtained from lactate efflux by *Escherichia coli* was a function of the ratio between the internal and external lactate concentrations. More energy could be generated from lactate efflux when the external concentration was low. In view of these results it was not surprising that *S. cremoris*, when grown on lactose, showed a higher cell yield in the presence of lactate-consuming *Pseudomonas stutzeri* that was allowed to use nitrate as the electron acceptor (Otto *et al.* 1980*b*). Similar mutualistic relations should be possible between fermentative and sulphate-reducing bacteria. The survival value of a high cell yield has been discussed by Yoon *et al.* (1977).

Growth of fermentative bacteria may be repressed by high concentrations of sulphide. The sulphide concentration needed to halve the maximal specific growth rate of *Veillonella alcalescens* and *Acetobacterium* species was 4 mM (Laanbroek *et al.* 1982). This may occur locally in marine sediments in places where sulphide concentrations are known to be high because of the simultaneous occurrence of abundant organic matter and sulphate, as occurs around mussel-banks (Kuenen 1972). A similar situation has been encountered each summer in lagoons around the Mediterranean during the decay of algae (Caumette 1978), but as a rule sulphide production in marine sediments seems to be balanced by the rate of its removal through biological and chemical oxidation as well as by precipitation as ferrous sulphide or the more stable pyrite (Jørgensen 1977*a*, *b*). Detailed information about sulphide toxicity for fermentative bacteria is still lacking, however, if only because of the scant knowledge of occurrence and properties of fermentative bacteria in sediments.

Most sediments seem to be energy-limited with respect to their microbial inhabitants and since lactate is a carbon and energy source for members of the group of sulphate-reducing bacteria as well as for mud-dwelling fermentative bacteria, competition for lactate may well be a common interaction in Nature. Smith & Klug (1981*b*) observed that fermentative bacteria were able to utilize 50% of the lactate added to sediment samples after inhibition of the population of sulphate-reducing bacteria.

Addition of lactate to sediment samples of the Ems–Dollard estuary resulted in almost complete fermentation to acetate and propionate (Laanbroek & Pfennig 1981). This may have been due to iron-limitation of the sulphate-reducing bacteria. This hypothesis is in agreement with the following observation. Estuarine mud inoculated in a lactate-limited chemostat run at a dilution rate of 0·05 h^{-1} gave an initial rapid increase of sulphate-reducing bacteria, which was

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followed by dominancy of fermentative bacteria. After the addition of iron, however, the sulphate reducers again came to the fore (Laanbroek *et al.* 1982). Subsequently pure and mixed culture studies were carried out with bacteria isolated from mud samples.

Mixed culture studies with *Desulfovibrio desulfuricans* and *Veillonella alcalescens* at low sulphide concentrations in an L-lactate-limited chemostat (excess sulphate and iron) resulted in dominancy of *Desulfovibrio*, both at low (0.02 h^{-1}) and high (0.12 h^{-1}) dilution rates. In contrast to pure chemostat cultures of *Veillonella*, hydrogen was never observed in mixed culture with *Desulfovibrio*. When sulphate or iron were limiting, *Veillonella* became dominant. The change in composition of the mixed culture after a sudden increase of the concentration of lactate, when this was limiting growth, depended on the ratio of the population densities. A temporary increase in the number of *Veillonella* cells was only observed when its proportion in the mixed population was higher than 5–10%, which can be explained in part by relatively high sulphide concentrations in mixed culture when the population density of *Veillonella* is very low. A selective advantage of *Veillonella* is of course its ability to use other energy sources than *Desulfovibrio*. This was evident when citrate was introduced into a mixed lactate-limited chemostat culture of both species, which resulted in coexistence (Laanbroek *et al.* 1982).

Competition between *Acetobacterium* species and *Desulfovibrio desulfuricans* in a lactate-limited chemostat, independent of dilution rate, always resulted in dominancy of *Desulfovibrio*, and even sudden increases of the lactate concentration never resulted in a change in the ratio of population densities in favour of *Acetobacterium*. However, the omission of sulphate immediately showed an increase of the *Acetobacterium* population, whereas a lack of iron resulted in the elimination of both populations.

These results show that both *Acetobacterium* and *Veillonella*, which do coexist with *Desulfovibrio* in the sediments of the Ems–Dollard estuary, would never be able to maintain their populations if lactate limitation were a dominant selective factor in their natural environment.

Acetobacterium can also grow at the expense of hydrogen and carbon dioxide (Balch *et al.* 1977). However, *A. woodii* grows much more slowly on these substrates than *Methanobrevibacter arboriphilus* AZ (Winter & Wolfe 1980), and therefore its competitive abilities under such extreme conditions seem to be doubtful. Braun *et al.* (1979), who enumerated bacteria that form acetate from hydrogen and carbon dioxide in sludge and lake sediment, found that their numbers were approximately one-hundredth of those of methanogenic bacteria growing on these substrates. The survival of *Acetobacterium* therefore seems to be highly dependent on its ability to perform mixotrophic growth (Braun & Gottschalk 1981). Generally, mixotrophic growth seems to deserve more attention with respect to the elucidation of ecological niches of anaerobic bacteria.

One other example of mixotrophic growth has been demonstrated in competition experiments with a versatile and a specialistic clostridium (Laanbroek *et al.* 1979). Both types of bacteria could easily be isolated from batch enrichments with glutamate as source of energy, carbon and nitrogen, which had been inoculated with sludge from an anaerobic digestor used for purification of waste water from a potato starch mill. When the sludge was used as an inoculum for a glutamate-limited chemostat, however, the specialistic *C. cochlearium* always became dominant. Coexistence of this clostridium with the more versatile *C. tetanomorphum* could only be obtained in an energy-limited chemostat when both glucose and glutamate were given as energy source. Under these conditions, the versatile *C. tetanomorphum* used not only glucose but simultaneously some of the glutamate; the ratio between the two populations therefore should depend on the ratio in which both substrates are available.

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Sulphide-oxidizing bacteria

The cycles of chemical elements in sediments cannot be considered as separate entities. The sulphur cycle is dependent on the influx of organic carbon oxidized under the simultaneous reduction of sulphate to sulphide.

In the surface layer of sediments, sulphide-oxidizing bacteria are generally quite common. For the study of factors determining the distribution of these as well as for laboratory studies aimed at being ecologically relevant, a knowledge of physicochemical field conditions is indispensable; the recent development of microelectrodes for measuring in the field such parameters as oxygen and sulphide concentrations (Blackburn *et al.* 1975; Revsbech *et al.* 1980) should therefore be considered as a major contribution to sediment ecology.

With respect to sulphide-oxidizing colourless sulphur bacteria exposed to sulphide limitation and simultaneously to an oxygen concentration gradient, a spectrum of bacteria with similar energy metabolisms seems to exist. Timmer-ten Hoor (1977), who made a study of colourless sulphur bacteria occurring in intertidal sediments of the Dutch coast, proposed that increasing oxygen tensions would favour *Thiomicrospira denitrificans* (Timmer-ten Hoor 1975), *Thiomicrospira pelophila* (Kuenen & Veldkamp 1972) and *Thiobacillus thioparus* in this order. Kuenen & Veldkamp (1972) found that the sulphide tolerance of *Tms. pelophila* was considerably higher than that of *T. thioparus* isolated from the same coastal sediment, and Kuenen *et al.* (1977) subsequently showed that this might be interpreted in terms of faster growth at extremely low iron concentrations, which was demonstrated in mixed culture in an iron-limited chemostat.

Jørgensen (1977*c*) reported that *Beggiatoa* species occur abundantly in the surface layers of sediments in a brackish fjord (Limfjorden, Denmark), and, similarly to the situation in the Ems–Dollard estuary with respect to thiobacilli, a difference in optimal growth conditions was observed with respect to oxygen concentration. *B. alba* occurred closer to the surface under more oxidizing conditions than larger *Beggiatoa* species, which preferred a deeper layer. *Beggiatoa* does not occur to any great extent in the intertidal sediments along the Dutch coast (J. G. Kuenen, personal communication), which is probably due to the smaller grain size of its sediment, which may impede vertical migration, reported to be important for its survival (Jørgensen 1977*c*).

The sulphide-oxidizing thiobacilli show a spectrum of characteristics with respect to energy metabolism, and the selective advantages of these were discussed by Gottschal *et al.* (1979) and Beudeker *et al.* (1981*b*). Gottschal & Kuenen (1980) reported the selective enrichment of facultatively chemolithotrophic thiobacilli and related organisms in continuous cultures limited simultaneously by an organic substrate and an inorganic substrate containing reduced sulphur, when inoculated with mud from freshwater sediments. However, similar enrichments inoculated with mud from Dutch coastal sediments always gave rise to mixed cultures of a heterotrophic and a chemolithotrophic bacterial population. It thus seems that mixotrophic thiobacilli are rare in these sediments, although there do not seem to be obvious reasons for their absence. They do occur, however, in other marine environments, as was shown by Adair & Gundersen (1969) and Smith & Finazzo (1981).

Anaerobic sulphide oxidation by photosynthetic bacteria in the upper few millimetres of coastal sediments may well be of quantitative importance (Blackburn *et al.* 1975). In the intertidal sediments along the northern coast of the Netherlands the common occurrence in a thin surface layer of a highly versatile phototrophic bacterium, *Rhodopseudomonas sulfidophila*, was reported by Hansen & Veldkamp (1973). It could grow photoautotrophically with hydrogen, sulphide, thiosulphate and sulphur. Photoheterotrophic growth was possible with

formate, acetate, propionate, butyrate, lactate and several other organic substrates. However, its competition for these substrates with sulphate-reducing bacteria in the anaerobic parts of the sediment surface is probably of little quantitative importance because anaerobically in the light the organic substrates are used by the phototrophs only for assimilatory purposes. The ecological niche of other photosynthetic bacteria encountered in these sediments such as small *Chromatium* species and *Amoebobacter* species (T. H. Hansen, personal communication) has still to be elucidated.

In summary, it can be stated that anaerobic mineralization of organic matter in sediments of natural waters is a highly complex process in which almost certainly many more bacterial species participate than are known at present. As yet, we are not able to depict more than a few details for any particular sediment community with regard to microbial inhabitants and their interactions.

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Discussion

J. R. Postgate, F.R.S. (*Unit of Nitrogen Fixation, University of Sussex, Brighton, U.K.*). One should be careful in stating that a certain organism is tactophilic (surface-loving) or not. Freshly isolated *Desulfovibrio* often shows tactophily but tends to lose it, and grow freely in the fluid, after three or four subcultures. In my experience the tactophilic phenotype can be restored by stress; acclimatization of a freshwater strain to 2.5% NaCl is accompanied by transient tactophily, which is lost after two or three passages. I have also observed the reverse phenomenon: transient tactophily during acclimatization of a marine sulphate reducer to a freshwater medium.

It seems that tactophily or surface adhesion, in this group of bacteria, may well be a response to stress, which would be consistent with the fact that most bacteria, in the natural environment, spend much of their time under stresses of one kind or another. Why tactophily might be helpful to a microbe during stress remains obscure.

H. Veldkamp. We have similar experience with respect to freshly isolated strains of sulphate-reducing bacteria. Initially these may show heavy wall growth in the chemostat and the formation of aggregations of cells is a common phenomenon. These properties are often lost during prolonged cultivation in aqueous media. Probably the constitutive production of extracellular components that enable the cells to stick to solid surfaces has survival value in their natural environment (sediments of natural waters). When cultivated for prolonged periods in

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a liquid medium devoid of particles, mutants may arise that no longer produce extracellular adhesive material. And under these conditions these may be selected for because they do not spend energy and monomers in the production of a useless extracellular polymer. Similar considerations may hold for the often observed loss of motility of bacterial strains (e.g. *Thiomicrospira*) in stirred continuous culture, as under these conditions the synthesis of flagella has become energetically unfavourable.

J. M. Lynch (*Agricultural Research Council Letcombe Laboratory, Wantage, U.K.*). Concerning the choice of surfaces to introduce to liquid cultures to make ecological studies more relevant, we should not be afraid of introducing solids from natural environments. I have successfully introduced soil as a dense slurry into the chemostat. Where the presence of the introduced organic matter is a problem in metabolic studies, this can be removed from the soil by mild oxidation with peroxide. Alternatively, I have recently found that the volcanic ash from Mount St Helens is exactly the same particle size as many of the soils in Washington State but is free from organic matter; this provides an excellent model to study particle–microorganism interactions and can be easily sterilized by irradiation.

H. Veldkamp. In order to study the effect of particles on the growth of bacteria isolated from sediments it seems to be preferable to start with material of known chemical properties. In the marine sediments discussed here, fine clay particles form a dominant fraction; therefore illite may be the material of choice in this particular case.

N. Le Roux (*Warren Spring Laboratory, Stevenage, U.K.*). I have observed sulphate-reducing bacteria associated with a surface film having calcium carbonate and iron sulphide present. Thus the microbial film might not be adhering to the actual surface of the material but could be 'encased' as part of an inorganic film.

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