PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Bacteria of the Sulphur Cycle [and Discussion]

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The bacteria of the sulphur cycle

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This paper concentrates on the bacteria involved in the reductions and oxidations of inorganic sulphur compounds under anaerobic conditions. The genera of the dissimilatory sulphate-reducing bacteria known today are discussed with respect to their different capacities to decompose and oxidize various products of fermentative degradations of organic matter. The utilization of molecular hydrogen and formate by sulphate reducers shifts fermentations towards the energetically more favourable formation of acetate. Since acetate amounts to about two-thirds of the degradation products of organic matter, the complete anaerobic oxidation of acetate by several genera of the sulphate-reducing bacteria is an important function for terminal oxidation in sulphate-sufficient environments. The results of pure culture studies agree well with ecological investigations of several authors who showed the significance of sulphate reduction for the complete oxidation of organic matter in anaerobic marine habitats.

In the dissimilatory sulphur-reducing bacteria of the genus *Desulfuromonas* the oxidation of acetate is linked to the reduction of elemental sulphur. Major characteristics of the anaerobic, sulphide-oxidizing phototrophic green and purple sulphur bacteria as well as of some facultative anoxygenic cyanobacteria are given. By the formation of elemental sulphur and sulphate, these bacteria establish sulphur cycles with the sulphide-forming bacteria. In view of the morphological diversity of the sulphate-reducing bacteria the question of possible evolutionary relations to phototrophic sulphur bacteria is raised.

1. Introduction

When we think of the sulphur cycle, we have in mind the different reductions and oxidations of inorganic sulphur compounds performed by living organisms. Depending on the organisms and the conditions, these transformations may be assimilatory or dissimilatory metabolic functions. Under aerobic conditions the reduction of sulphate is assimilatory (e.g. in green plants and most microorganisms), whereas the oxidation of reduced sulphur compounds (e.g. sulphide minerals) is dissimilatory in many bacteria (e.g. the colourless sulphur bacteria (Kuenen 1975)) and equivalent to the oxidation of organic compounds for energy-conserving reactions. In water and soil, there is in addition a great variety of chemoorganotrophic bacteria, actinomycetes and fungi that oxidize different sulphur compounds to sulphate (Tuttle 1980; Killham *et al.* 1981). Growth of these microorganisms may or may not be stimulated by the oxidation of the sulphur compounds.

Under anaerobic conditions, both oxidized and reduced sulphur compounds are substrates for metabolic processes of bacteria only. The oxidized sulphur compounds, including elemental sulphur, represent counterparts of oxygen as electron acceptors in the terminal oxidation of organic substances and hydrogen; this is true for the strictly anaerobic, dissimilatory sulphate- and sulphur-reducing bacteria, which form hydrogen sulphide as product. The reduced sulphur compounds also including elemental sulphur, occupy the position of electron donors instead of

water, for autotrophic carbon dioxide assimilation; this is true for the phototrophic bacteria with anoxygenic photosynthesis. Sulphate is formed by these bacteria as the ultimate oxidation product without the participation of molecular oxygen. This is an essential difference in comparison with the nitrogen cycle, which cannot proceed without the participation of oxygen, since the first oxidation step of ammonia is an oxygen-dependent reaction. This paper will concentrate on the bacteria of the sulphur cycle under anaerobic conditions.

2. Dissimilatory sulphate-reducing bacteria

In our mostly aerobic biosphere the development of anaerobic environments depends essentially on two conditions: (1) continued supply and degradation of organic matter, and (2) limitation of the access of air by physical means, e.g. prevention of mixing in sediments, or by density gradients in water. In the anaerobic decomposition of organic matter two basically

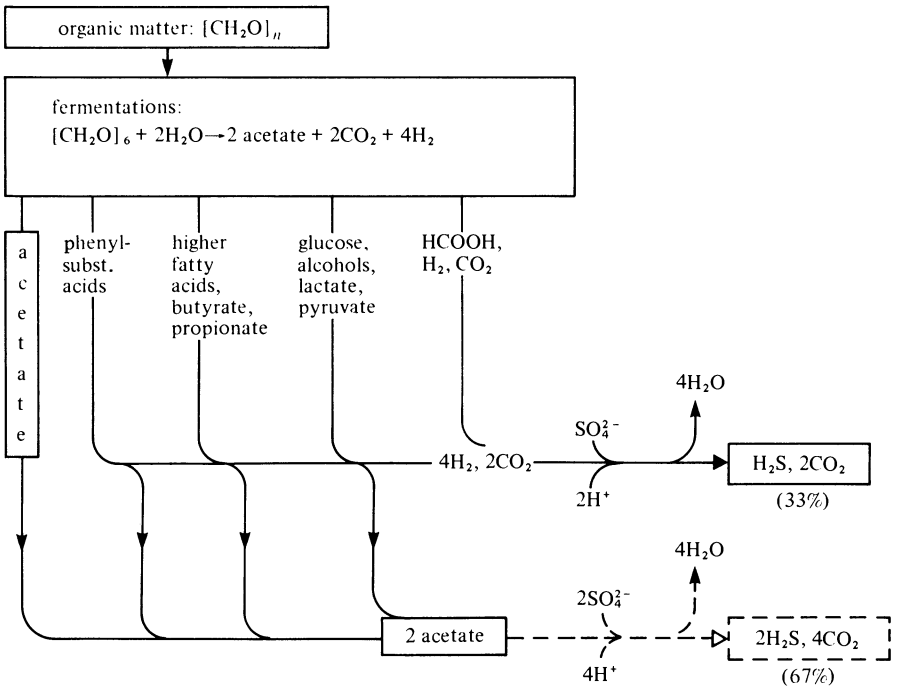

Figure 1. Scheme of the anaerobic degradation of organic matter by fermentations and different pathways of subsequent oxidation linked to sulphate reduction.

different major steps have to be differentiated: the initial degradation of polymers with subsequent fermentation of the monomers and then the oxidative degradation of the reduced fermentation products with external electron acceptors, the most important of which under anaerobic conditions are sulphate and carbon dioxide. The overall ratio of carbon, hydrogen and oxygen in complex organic matter is similar to that of carbohydrates. Thauer (1976) pointed out that a maximum of 4 mol molecular hydrogen and 2 mol carbon dioxide can theoretically be formed per mole of hexose by fermentations; the remaining part has to appear in the form of 2 mol acetate. This is true irrespective of the kind of reduced compound that might be formed in intermediary fermentation steps. As a result, not more than 33 % of

organic matter can be degraded completely if an anaerobic decomposition of acetate is impossible (Fenchel & Jørgensen 1977). These considerations show that the terminal oxidation of organic matter ultimately depends on two metabolic capacities representing extremes: (1) the utilization of hydrogen with high affinity, and (2) the anaerobic oxidation of acetate. These two capacities may be distributed among different species of bacteria or may be present in a single species. All the organic compounds that are more reduced than acetate can be placed between the extremes hydrogen and acetate and may therefore still be subject either to incomplete oxidation yielding reducing equivalents and acetate, or to complete oxidation.

If we arrange the electron-donating degradation products other than acetate in order of decreasing complexity, we may have phenyl-substituted acids, higher fatty acids or sugars at one end and hydrogen at the other. Sulphate-reducing bacteria thriving at the hydrogen end may have a very limited capacity to degrade organic carbon compounds, but they will scavenge hydrogen with high affinity and use it as electron donor or carry out incomplete oxidations of ethanol or lactate with acetate as end-product. Most species of the ubiquitous well known genus *Desulfovibrio* belong to this group (Postgate 1979). They can be envisaged as living hydrogen acceptors and electron transport systems to sulphate. In sulphate-limited environments these species have their counterparts in those methanogenic bacteria that likewise do not participate in the degradation of organic substances, but rather utilize hydrogen very efficiently, forming methane from carbon dioxide. *Desulfovibrio* species have long been known to use hydrogen for the reduction of sulphate. It was shown by Sorokin (1966) that carbon dioxide and acetate serve as carbon sources when hydrogen is provided as electron donor for sulphate reduction. Later on, Badziong & Thauer (1978) calculated ATP formation in *Desulfovibrio vulgaris* from quantitative growth experiments with hydrogen plus sulphate or thiosulphate as sole energy sources, and bicarbonate and acetate as carbon sources. More recently, Brandis & Thauer (1981) confirmed for *Desulfovibrio vulgaris* (Hildenborough), *D. desulfuricans* (Essex 6) and *D. gigas* (type strain) the capacity to grow with hydrogen and sulphate as sole energy source. No comparable experiments exist so far for the species of the genus *Desulfotomaculum* or for *Desulfomonas pigra* (Moore *et al.* 1976). All these bacteria were often considered to be predominantly fermentative and dependent on substrates like lactate, pyruvate or ethanol and yeast extract for growth. The demonstration of growth of *Desulfovibrio* species on hydrogen and sulphate as sole energy sources allows us to consider dissimilatory sulphate reduction as an energy-conserving process biologically analogous to dissimilatory nitrate reduction or respiration with oxygen. Consequently, the question arises whether sulphate-reducing bacteria utilize other electron donor substrates in addition to hydrogen, the oxidation of which may or may not be coupled to substrate-linked phosphorylation.

It was recently shown (Widdel 1980; Pfennig & Widdel 1981) that there are hitherto unknown sulphate-reducing bacteria that incompletely oxidize lower and higher fatty acids. Propionate is degraded to acetate and carbon dioxide by bacteria that were selectively enriched from freshwater and marine habitats. Since no other fatty acids are oxidized by these oval or lemon-shaped bacteria, they were called *Desulfobulbus propionicus*. With higher fatty acids, e.g. palmitate, vibrioid sulphate reducers were isolated that degraded all straight-chain fatty acids with a chain length of 4–18 carbon atoms. All eight strains of this *Desulfovibrio sapovorans* type degraded fatty acids with an even number of carbon atoms to acetate, while 1 mol of propionate was formed in addition to acetate from 1 mol of fatty acids with an uneven number of carbon atoms. This result indicated that the fatty acids were degraded by β-oxidation. Comparison of the growth

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

yields (Widdel 1980) obtained from the incomplete and complete oxidation of palmitate with sulphate indicate that substrate-level phosphorylation in the course of β-oxidation may indeed contribute to energy conservation. More detailed studies are required to confirm these calculations.

All incomplete oxidations ultimately result in the formation of acetate, which will account for two-thirds of the organic matter degraded. Therefore, the significance of dissimilatory sulphate reduction for the terminal oxidation of organic matter in sulphate-sufficient environments will depend on whether or not acetate can be oxidized in the course of sulphate reduction. The formation of sulphide in cultures with acetate as substrate was first reported by Rubentschik (1928), Baars (1930) and later by Selwyn & Postgate (1959). Detailed studies with pure cultures, however, were lacking. The isolation of the sporing, acetate-oxidizing, sulphate-reducing bacterium *Desulfotomaculum acetoxidans* in pure culture (Widdel & Pfennig 1977, 1981 *b*) first showed that the sulphate-reducing bacteria had solved in principle the problem of conserving sufficient energy for growth, most probably by electron transport of reducing equivalents not only from hydrogen but also from acetate to sulphate. The composition of the culture medium necessary for the new sulphate-reducing bacteria differed in several respects from the one that has been used so far (Widdel & Pfennig 1981 *a*, *b*; Pfennig *et al.* 1981). When it became clear that *Desulfotomaculum acetoxidans* is primarily an intestinal bacterium (optimum growth temperature 36 °C) that can be isolated regularly only from manure or manure-contaminated mud samples, isolations with acetate were carried out from anaerobic marine or brackish water habitats. Several strains of short rod-shaped, non-sporing bacteria, *Desulfobacter postgatei* (Widdel & Pfennig 1981 *a*), turned out to be highly specialized for acetate as substrate. In view of the significance of acetate as a fermentation product it is not surprising that still other types of sulphate-reducing bacteria were isolated that oxidized acetate (Pfennig & Widdel 1981). Two of the new species, the filamentous gliding *Desulfonema limicola* and *Desulfovibrio baarsii*, are specialized on lower and higher fatty acids, which are oxidized completely to carbon dioxide. The remaining three new species, *Desulfococcus multivorans*, *Desulfosarcina variabilis* and *Desulfonema magnum*, are catabolically the most diverse sulphate reducers known today. They combine the ability to oxidize acetate, lower and higher fatty acids with the capacity to degrade and oxidize benzoate and a number of phenyl-substituted acids. However, *Desulfotomaculum nigrificans* is still the only species of all sulphate reducers that is able to ferment sugars (Campbell & Postgate 1965). Four of the new species are potentially chemoautotrophic, because they are able to grow with formate, two of them also with hydrogen and carbon dioxide as sole electron donor and carbon source (Widdel 1980).

Taking into account all the species capable of oxidizing acetate by sulphate reduction, the sulphate-reducing bacteria can be considered to function as terminal oxidizers of organic matter in sulphate-sufficient environments and thus to fill the position occupied by the methanogenic bacteria under conditions of sulphate limitation.

This assumption is strongly supported by the results of quantitative experiments with sediments from natural habitats. Using anaerobic marine sediments, Jørgensen & Fenchel (1974) and Jørgensen (1977) showed convincingly that more than half of the added organic matter was completely degraded to carbon dioxide in the course of sulphate reduction. In experiments with anaerobic sediment from Lake Mendota, Winfrey & Zeikus (1977) demonstrated that the carbon and electron flow was altered from methanogenesis to the formation of carbon dioxide and sulphide when sulphate was added to the samples. Jørgensen *et al.* (1979) were able to increase the rate of sulphate reduction 3–4 times in water samples from the hypolimnion (4.5 m

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

depth) of a tropical salt lake, when 0.1 mM acetate was added. Lactate stimulated the reduction rate only 1.8-fold in one experiment. The degradation of added acetate with concomitant formation of sulphide was observed in brackish water sediments by Laanbroek & Pfennig (1981). By using the same sediments the presence of acetate-oxidizing sulphate-reducing bacteria of the *Desulfobacter postgatei* type was shown by the agar shake culture method. In sulphate-rich environments the anaerobic oxidation of organic matter occurs by sulphate reduction. Only when sulphate becomes exhausted in deep layers or microniches of such habitats, the terminal degradation is taken over by the methanogenic bacteria (Abram & Nedwell 1978; Mountford *et al.* 1980; Mountford & Asher 1981; Sørensen *et al.* 1981). Even under these conditions hydrogenase-containing sulphate-reducing bacteria may maintain metabolic activity by cleaving certain reduced fermentation products to acetate and hydrogen, thus providing substrates for methanogenic bacteria (Bryant *et al.* 1977; McInerney & Bryant 1981).

Our knowledge of thermophilic sulphate-reducing bacteria is very limited. Only two species have so far been studied in pure culture: *Desulfotomaculum nigrificans* (Campbell & Postgate 1965), whose optimum growth temperature is 55 °C, and *Desulfovibrio thermophilus* (Rozanova & Khudyakova 1974) isolated from a very deep petroleum deposit and exhibiting an optimum growth temperature around 65 °C. It is to be expected that the sulphate-reducing bacteria are represented by several more types in anaerobic habitats with temperatures between 50 and 80 °C.

3. Dissimilatory sulphur-reducing bacteria

In anaerobic sulphide-containing marine and freshwater sediments and in the wake of the sulphate-reducing bacteria – though quantitatively of minor importance – thrive the sulphur-reducing bacteria of the *Desulfuromonas acetoxidans* type (Pfennig & Biebl 1976). They arouse particular interest by the fact that they carry out the anaerobic oxidation of acetate, with elemental sulphur and polysulphide sulphur as electron acceptors being reduced to hydrogen sulphide. Compounds with disulphide bonds such as cystine or oxidized glutathione can also be used, whereas none of the oxidized sulphur compounds is reduced. Instead of sulphur, many strains can use the non-selective electron acceptors L-malate or fumarate, which are quantitatively reduced to succinate.

Although sulphur-reducing bacteria were regularly isolated from anaerobic marine environments, they appear to be less frequent in freshwater mud. Characteristically, the marine forms are laterally to subpolarly flagellated while the freshwater isolates are ovoid to elongated ovoid, and polarly flagellated. For the latter cell type, which is unable to use any electron donor other than acetate, the new species *Desulfuromonas acetoxidans* was proposed (Pfennig & Widdel 1981).

Dissimilatory reduction of elemental sulphur is not restricted to *Desulfuromonas*, which does not reduce other electron acceptors. Saprophytic *Campylobacter* strains, *Vibrio succinogenes* (Wolfe & Pfennig 1977; Laanbroek *et al.* 1978) and a number of rod-shaped *Desulfovibrio* strains resembling strain Norway 4 (Biebl & Pfennig 1977) were capable of growth with elemental sulphur when other electron acceptors were omitted. Common to all sulphur-reducing eubacteria is the presence of low-potential *c*-type cytochromes; of these the three-haem cytochrome *c*-551.5 (c_7) has been studied most extensively (Probst *et al.* 1977). It was recently discovered that dissimilatory sulphur reduction also occurs among the archaebacteria (Zillig *et al.* 1981). The new extremely thermoacidophilic anaerobic bacteria, Thermoproteales, thrive in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Icelandic solfataras. The long rod-shaped *Thermoproteus tenax* grew best at pH 5 and 88 °C (1.7 h generation time). In sulphide-reduced mineral media, elemental sulphur or malate served as electron acceptor, and formate, methanol, ethanol, glucose or starch was used as electron donor and carbon source. Further cell types were observed but have not yet been described in detail.

4. Phototrophic bacteria participating in the sulphur cycle

The bulk of hydrogen sulphide formed by dissimilatory sulphate reduction is most probably oxidized to sulphate by the respiratory activity of various aerobic sulphur-oxidizing bacteria and by the direct reaction with oxygen resulting in several intermediary oxidation products, e.g. sulphur and thiosulphate (Kuenen 1975). Under special conditions, however, when light has access to the anaerobic, sulphide-containing water or the sediment surface, anaerobic phototrophic bacteria may develop that oxidize sulphide and sulphur to sulphate with the concomitant reduction of carbon dioxide to cell substance. Depending on the conditions established by the opposing gradients of light intensities from above and sulphide concentrations from below, different kinds of bacteria may develop. The general theme has therefore a number of variations that are interesting in a discussion of the sulphur cycle.

(*a*) The phototrophic green sulphur bacteria, Chlorobiaceae, possess with their chlorosomes the most efficient light-harvesting system, which allows them to grow at lower light intensities than any other photographs (Biebl & Pfennig 1978). In most natural environments with vertical zonation of different phototrophic organisms, the green sulphur bacteria are therefore found in the lowermost layer at the end of the light gradient directly adjacent to the sulphide production zone (Jørgensen & Fenchel 1974). Their other characteristics are in agreement with this habitat: highest tolerance for sulphide, obligately anaerobic metabolism and lack of motility (Pfennig 1978). The first oxidation product of sulphide, elemental sulphur, appears outside the cells and can therefore either be oxidized further to sulphate or reduced to sulphide by sulphur-reducing bacteria. The syntrophic relations between sulphur- or sulphate-reducing bacteria and green sulphur bacteria were studied quantitatively in combined pure cultures (Biebl & Pfennig 1978).

A good example of the bacterial sulphur cycle in the water column of a lake (depth 22 m) was recently provided by Parkin & Brock (1981). During summer, a dense layer of gas-vacuolated green sulphur bacteria was present at a depth of 2.2 m in Knaak Lake, Wisconsin, a small and wind-protected brown-water lake 22 m deep. The upper limit of the green layer (2.0 m) was given by the beginning of oxygen-containing water; the lower limit (2.4 m) was caused by light limitation as a consequence of self-shading of the bacteria. At 2.2 m depth the sulphide concentration was zero during the day, because in the light all the sulphide formed was instantaneously reoxidized by the green bacteria. During the night, the sulphide concentration increased to a maximum of 0.18 mg l^{-1}, indicating that sulphate reduction was actually taking place within the layer of the green bacteria. With water samples taken from a depth of 2.2 m, Parkin & Brock were able to show that sulphate did not limit sulphate reduction, although the sulphate concentration was very low. The rate of sulphate reduction could be increased, however, by a factor of 2.5 when acetate was added to the samples to a concentration of 0.1 mM. Sulphate reduction in the layer at 2.2 m was therefore obviously limited by the electron donor substrate, and sulphate-reducing bacteria must have been present capable of using acetate as an energy source.

(*b*) The phototrophic purple sulphur bacteria, Chromatiaceae, comprise many species, the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

cells of which are motile by polar flagella (e.g. the species of the genera *Chromatium*, *Thiocystis* and *Lamprocystis*). Elemental sulphur formed by the oxidation of sulphide is stored as sulphur globules inside the cells; only the final oxidation product, sulphate, is released into the medium. By chemotactic and phototactic responses, the cells attain optimal positions in the gradients of sulphide and light. In Nature, the largest areas for the development of purple sulphur bacteria are provided in shallow and stagnant seawater pools and lagoons along protected beaches; in such environments, which are usually rich in decaying algae and seagrasses, pink to purple red blooms of purple sulphur bacteria often appear during summer (sulfureta (Fenchel 1969)).

The capacity of purple sulphur bacteria for chemoautotrophic and mixotrophic growth in the dark was first reported by Bogorov (1974) and Kondratieva *et al.* (1976). Later studies (Kämpf & Pfennig 1980) revealed that all species of the genera *Chromatium*, *Thiocystis*, *Amoebobacter* and *Thiocapsa* that were able to utilize thiosulphate have the capacity for chemoautotrophic growth under conditions of decreased oxygen partial pressure (5 % oxygen and 1 % carbon dioxide in nitrogen). Although the doubling time was 5–10 times longer than under anaerobic conditions in the light, the dry mass yields were comparable with those of *Thiobacillus* species.

(*c*) It was a great surprise when Cohen *et al.* (1975) and later Garlick *et al.* (1977) first reported on the capacity of certain species of the cyanobacteria to carry out facultatively anoxygenic photosynthesis with sulphide. In *Oscillatoria limnetica* 0.1–1 mM H_2S inhibited the function of photosystem II and, within about 2 h, induced anoxygenic photosynthesis with hydrogen sulphide as electron donor for carbon dioxide assimilation. Hydrogen sulphide was oxidized only to elemental sulphur, which precipitated as small globules around the filaments. Jørgensen *et al.* (1979) later showed that the cyanobacteria (*O. limnetica*, *Microcoleus* spp.) growing at the chemocline of a tropical salt lake performed anoxygenic photosynthesis in the early morning but shifted to oxygenic photosynthesis when sulphide was consumed to concentrations below 5 μM. During the night, elemental sulphur and polysulphide disappeared again owing to the use of elemental sulphur as electron acceptor in the respiratory activity of the cyanobacteria under anaerobic conditions in the dark. In addition, chemoorganotrophic bacteria of the *Desulfuromonas* type were probably also involved in the reduction of the elemental sulphur formed.

These examples show that, under anaerobic conditions, the phototrophic sulphur bacteria established closed microbial sulphur cycles with chemotrophic sulphide-forming bacteria between sulphide and both elemental sulphur and sulphate.

Although in our present aerobic biosphere the biotopes for anaerobic processes are very restricted, they may have had a much greater significance at times in the Earth's history when the atmosphere was still oxygen-free. Broda (1975) pointed out that no sulphate existed on the primeval Earth, and it might well have been formed first by the metabolism of the phototrophic sulphur bacteria from sulphides. This would imply that the sulphate-reducing bacteria could not have evolved before the phototrophic sulphur bacteria. Once sulphate was present, it represented a potential electron acceptor for chemotrophic bacteria that could use it for energy-conserving processes in the dark.

The evolutionary views expressed by Broda (1975) that different groups of aerobic chemotrophic Gram-negative bacteria are closely related to certain genera of the phototrophic non-sulphur bacteria (divergence hypothesis) were fully confirmed by similarity determinations of the 16*S* RNA of these bacteria (Stackebrandt & Woese 1979). In view of the morphologically very different new species and genera of the sulphate-reducing bacteria it is tempting to

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

speculate, in agreement with views of Peck (1974), that closer genetic relationships might be found between these bacteria and certain phototrophic sulphur bacteria than among the sulphate-reducing bacteria themselves. And although the phototrophic sulphur bacteria may have predated the sulphate-reducing bacteria, they, via the sulphur cycle, became more and more dependent on the metabolic activities of the latter in the course of evolution.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Discussion

J. R. Postgate, F.R.S. (*U.N.F., University of Sussex, U.K.*). Has the autotrophy of *Desulfonema limicola* and *Desulfosarcina* been checked with ^{14}C? The history of this subject tells us that growth tests on their own can be misleading.

N. Pfennig. The strains were not tested with $^{14}CO_2$, although this test would be desirable. However, both species were grown over at least four passages with H_2 and CO_2 in defined mineral media without yeast extract or any other organic carbon source. In comparison, *Desulfovibrio* and *Desulfobulbus* species that require acetate as additional carbon source ceased to grow after the second transfer.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES