Biodegradation **9:** 201–212, 1998.

Bioenergetics of sulphate-reducing bacteria in relation to their environmental impact

W. Allan Hamilton
Molecular & Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland

Accepted 9 June 1998

Key words: consortia, corrosion, energetics, souring, SRB

Abstract

The cellular physiology of the sulphate-reducing bacteria, and of other sulphidogenic species, is determined by the energetic requirements consequent upon their respiratory mode of metabolism with sulphate and other oxyanions of sulphur as terminal electron acceptors. As a further consequence of their, relatively, restricted catabolic activities and their requirement for conditions of anaerobiosis, sulphidogenic bacteria are almost invariably found in nature as component organisms within microbial consortia.

The capacity to generate significant quantities of sulphide influences the overall metabolic activity and species diversity of these consortia, and is the root cause of the environmental impact of the sulphidogenic species: corrosion, pollution and the souring of hydrocarbon reservoirs.

Introduction

Microbiology, the scientific study of microorganisms, can be considered under three main headings; *molecular biology*, *cell physiology* and *population ecology*.

Clearly there is a hierarchical relationship amongst these sub-disciplines, both with regard to the increasing structural complexity they represent, and in respect of the functional dependence a) of ecological relationships on the physiology of the individual organisms, and b) of that physiology on the molecular genetic make up of the particular species. One of the most striking features of microbiology, however, is that the genetics, physiology and ecology of microorganisms relate with one another in the manner of a matrix rather than solely according to this simple linear model. Progress in our overall understanding of the discipline is largely dependent upon problems in one area being illuminated by techniques and insights developed in another. In the case of the sulphate-reducing bacteria (SRB), for example, one can cite the demonstration of acetate turnover in natural ecosystems (Sørensen, Christensen & Jørgensen, 1981; Jørgensen, 1982) as giving confirmation of the widespread occurrence of acetate-utilising SRB (Widdel & Pfennig, 1977; Widdel & Pfennig, 1981), whose very existence had previously been doubted (Postgate, 1979). Also, the current widespread application of molecular methods to microbial ecosystems is revolutionising our understanding, both of present day ecological relationships and of past evolutionary development.

This review will seek to expand this interactive view of microbiology in general, and of the SRB in particular, through the consideration of three separate topics:

- the deterministic influence of cell physiology on population ecology;
- techniques for elucidating the true complexity of microbial consortia;
- some technological consequences of the activities of microbial ecosystems.

Cell physiology of the SRB, and its ecological consequences

Redox mechanisms and bioenergetics

Although the extent of the diversity within the group has only recently been fully recognised (see, for example, Rabus et al., 1996), the SRB have generally been regarded as a collection of organisms, largely unrelated in the convential taxonomic sense, but clearly forming a broad physiological/ecological grouping. This latter property derives directly from their mode of energy-generating metabolism. The SRB are obligately anaerobic, employing a respiratory mechanism with sulphate as terminal electron acceptor and consequently giving rise to sulphide as the major metabolic end product. The anaerobiosis and requirement for sulphate determine the environments in which they are active, while the sulphide produced underlies their environmental and technological impact.

Most of the sulphate-reducing bacteria can also use other oxyanions of sulphur as terminal electron acceptor, eg. sulphite and thiosulphate. The capacity to react with other potential reductants such as nitrate (Widdel, 1988; Moura et al., 1997), or the metal ferric and manganic ions (Lovley, 1995; Lonergan et al., 1996), has also been noted with certain species. Even oxygen has been reported to be able to serve as electron acceptor, although such aerobic respiration does not appear to be coupled to growth (Dilling & Cypionka, 1990; Teske et al., 1996).

Additionally, there exists another, quite separate, mixed group of archaea and bacteria that couple their energy metabolism to the reduction of sulphur (Widdel 1988; Davey et al., 1993; Lonergan et al., 1996). Like the more familiar sulphate-reducing bacteria, these sulphur-reducing organisms are characterised by their sulphide production. As this common property is of such defining practical importance, and since the two groups are often found together in a range of natural environments, it is often appropriate to use the more all-embracing generic terms sulphide-producing bacteria (SPB), or sulphidogens to cover both groups of organisms.

Sulphate, however, is unique in that it is only capable of acting as terminal electron acceptor after its metabolic conversion to adenosine phosphosulphate (APS). The generation of APS involves reaction with ATP, with a net energy cost of two ‘high energy bonds’. There is also a further small energy cost from the cellular uptake of sulphate by co-transport with either H^+ or Na^+ (Cypionka, 1995). Although sulphite, thiosulphate and, possibly, sulphur have similar transport-dependent energy costs associated with their terminal reductant role, they do not require the more energy-demanding activation step by direct reaction with ATP.

What all four sulphur-based electron acceptors share, however, is a greatly reduced energy yield, as compared to that generated from oxygen reduction in aerobic respiratory processes. These lower energy yields are the direct result of the negative values for the redox potentials of the critical reductive reactions:-

$$\begin{array}{ll} APS/AMP + HSO_3^- & E^{o\prime} = -60mV \\ HSO_3^-/HS^- & E^{o\prime} = -116mV \\ S^\circ/HS^- & E^{o\prime} = -270mV \end{array}$$

for comparison:-

$$^1/_2 O_2/H_2O \quad E^{o\prime} = +818mV$$

The potential energy available from the oxidation of any given substrate coupled by respiratory mechanisms to the reduction of a terminal electron acceptor is given by the formula:-

$$\Delta G^{o\prime} = -nF\Delta E^{o\prime}$$

where $\Delta G^{o\prime}$ is the free energy change under standard conditions, n is the number of electrons, F the Faraday constant (96,694kV/J.mole), and $\Delta E^{o\prime}$ the difference in redox potentials between the oxidant and the reductant. Since the redox potentials of biological energy substrates lie in the range -414mV (H_2) to approximately 0mV, it is clear that the energy yield for all anaerobic metabolic sequences employing sulphur or one of its oxyanions must be, by definition, greatly minimised in comparison with utilisation of the same substrates in aerobic metabolism.

The principles governing this energetic analysis are presented in their most accessible form in the review by Thauer et al. (1977), and more detailed discussions of their application to sulphidogenic bacteria are given in two recent review articles (Hamilton, 1998; Hansen, 1994).

Cellular physiology

These energetic limitations very largely determine the character of the cells’ carbon metabolism, and the nature of their ecological interrelationships.

One specific difficulty arises from the apparent impossibility of coupling acetate oxidation via the tricarboxylic acid (TCA) cycle to reduction of any one of the sulphur-based terminal electron acceptors in a manner compatible with the normal energy-generating capacity of that metabolic sequence. The problem arises from the redox potential for the succinate/fumarate couple which, at +33mV under standard conditions, would predict that electron flow to APS, HSO_3^- or S° would be energy-requiring rather than energy-generating. Broadly speaking, there are three solutions to this problem.

A. At least three sulphidogenic organisms have a functional TCA cycle, and acetate can serve as both carbon and energy source. However, *Desulfobacter postgatei* (mesophilic, SO_4^{2-}), *Desulfuromonas acetoxidans* (mesophilic, S°), and *Desulfurella acetivorans* (thermophilic, S°) each carries its own unique set of modifications, including elements of energy-driven reversed electron flow, ensuring a small net energy yield from the operation of the TCA cycle (Thauer 1988; Thauer et al., 1989; Schmitz et al., 1990).

B. Such a situation appears to be the exception rather than the rule, however, and the evidence suggests that all other acetate-oxidising sulphidogenic species utilise the acetyl CoA or carbon monoxide dehydrogenase pathway (Fuchs, 1986; Wood et al., 1986). For example, this pathway, which is universally found amongst the acetogenic bacteria and the methanogenic archaea, has been identified in such sulphidogens as *Desulfotomaculum acetoxidans, Desulfobacterium autotrophicum* and the archaeon *Archeoglobus fulgidus* (Schwörer et al., 1993).

C. The third option is likely to be the most widespread amongst the sulphate- and sulphur-reducing bacteria. Certainly it was for long thought to be the defining characteristic of these organisms that lactate and other low molecular compounds are only partially oxidised, with equimolar amounts of acetate being generated as a metabolic end product. That is to say, neither the TCA cycle nor the acetyl CoA pathway are present, nor is growth possible where acetate is the sole potential energy source. Mechanisms are present, however, for the assimilation of carbon, and growth on hydrogen with acetate plus CO_2 has been shown to be extremely important, both in laboratory studies designed to elucidate the nature of SRB metabolism (Badziong & Thauer, 1978; Peck, 1993), and in energy and carbon flux through anaerobic ecosystems (Jørgensen, 1982).

In recent years our appreciation of the spectrum of carbon substrates capable of metabolic conversion by one or other species of sulphidogenic bacteria has had to be radically revised from this earlier belief that their substrate range was confined to a relatively restricted number of short chain acids and alcohols (see, for example, Hansen, 1993). The balance of evidence would still seem to suggest, however, that in most natural ecosystems the major biodegradative pathways involve other hydrolytic and fermentative species with their metabolic products such as lactate, hydrogen and acetate being then the principal primary substrates for the terminal oxidative steps carried out by the SRB (Jørgensen, 1982; Parkes, 1987).

Mixed species microbial communities

These considerations of energy yield and carbon flux lead us, therefore, to the appreciation that the sulphidogenic bacteria occur in nature, and exert their influence therein, most usually as integral members of mixed species microbial communities, or consortia. In such consortia, the major interactions amongst the constituent species are essentially nutritional in character in that end products of the metabolism of one species can serve as carbon and/or energy nutrients for a second species.

Fermentative species play a central role in such interrelationships since, in order to maintain redox balance in their overall metabolism in the absence of an external electron acceptor, they are obliged to generate substrate concentrations of a range of reduced fermentation products such as, for example, lactate, succinate, butyrate, etc., acetate and, of course, hydrogen. Many of these are subsequently further metabolised by the hydrogen-producing acetogenic bacteria to generate more acetate and hydrogen. These two fermentation/acetogenic products, which can then function as favoured substrates for the terminal oxidant species, sulphidogens and methanogens, thus serve as pivotal metabolites in anaerobic microbial consortia.

Such reaction sequences are often genuinely symbiotic in as much as both species involved derive positive benefit. In this regard, a key reaction is the interspecies hydrogen transfer that links hydrogen-producing acetogens to either the SRB or the methanogens (Thauer & Morris, 1984; Pfennig, 1984). In order to maintain redox balance during the further breakdown of such primary fermentation products as propionate or butyrate, the acetogenic bacteria use protons as electron acceptor with the production of hydrogen. The free energy change associated with these reactions under standard conditions, however, is generally positive such that, in isolation, they cannot supply the energy requirements for microbial growth. Consequently, it is only the removal of this hydrogen by the SRB and/or methanogens that alters the position of equilibrium of the acetogenic fermentation such that it becomes exergonic and thus able to support cellular biosynthesis.

A further significant feature of microbial consortia is that they often form structural entities in the form, for example, of flocs or, where there is attachment to a surface, biofilms. Such biofilms have dimensions in the μm to mm range and demonstrate internal structural and functional heterogeneities (Hamilton, 1987; Costerton et al., 1994). One particularly striking feature of such heterogeneity is the regular development of an aerobic/anaerobic interface within the biofilm (Nielsen et al., 1993) as a consequence of the competition between the diffusion of oxygen into the biofilm and its removal as a result of the activities of aerobic species in the surface layers. Thus, in addition to performing certain of the initial reactions in the biodegradation of the primary nutrients available to the biofilm, these aerobic species are also responsible for creating the conditions of anaerobiosis necessary for the growth of sulphidogenic bacteria within flocs or biofilms, even where these structures are themselves situated within an aerobic bulk phase (Hamilton 1985).

As we shall consider later, aerobic/anaerobic interfaces arising in this manner are of particular significance within biofims associated with microbially induced corrosion of metallic substrata. They are equally important, however, in other microbial ecosystems such as marine sediments and even deep water columns as, for example, in the Black Sea. Whereas in the case of corrosion biofilms the overall dimensions are of the order of 1–3mm and the interface between the two domains is likely to be extremely sharp with an immediate change from oxygen- to sulphate-based respiratory processes, in the larger dimension ecosystems there is evidence of a more complex gradation from fully oxic conditions through a series of progressively more reduced environments, each characterised by a unique terminal electron acceptor (Nealson & Saffarini, 1994). In order of decreasing redox potential values, these are nitrate, manganese, iron, and sulphate, with CO_2 reduction to methane sometimes also being evident at maximal depth even in marine systems. Whereas nitrite and ammonia, sulphide and methane are generally recognised as endproducts of respiratory activity in their respective zones, there is seldom significant build-up of reduced manganese or iron consequent upon the utilisation of Mn (IV) or Fe(III) as terminal electron acceptor. It appears rather that metabolic activity in these regions is associated with the balanced operation of Mn(IV)/Mn(II) and Fe(III)/Fe(II) redox cycles (Canfield et al., 1993; Lovley, 1995). These manganese and iron-based respiratory systems, therefore, operate as electron carriers coupling sulphate respiration ultimately with oxygen, in a manner directly analogous with the operation of hydrogen and electron carriers in cellular bioenergetics. It is only relatively recently that the quantitative importance of manganese and iron respiration in environmental microbial ecosystems has been appreciated, and it remains to be established what might be their role, if any, within the restricted confines of corrosion biofilms (Lewandowski et al., 1997).

In respect of the corrosion and pollution events associated with certain anaerobic microbial ecosystems, the build-up of sulphide as a respiratory end product is almost certainly the single most significant component of the overall processes. Since, however, sulphide is also toxic to all life forms, including the SRB themselves, the maintenance of viability and metabolic activity within such ecosystems depends upon one or more mechanisms of removal or neutralisation of the sulphide. In the cases of corrosion and pollution, this necessary biological function is achieved, at least in part, by the deposition of insoluble metal sulphides as corrosion products or blackened sediments. Particularly in the latter case, the extent of the release of gaseous hydrogen sulphide from such deposits defines the nature and extent of the pollution, at least in terms of its impact on human sensibilities. True removal of sulphide, on the other hand, is dependent upon diffusion, exposure to air, and reoxidation to less reduced forms, most noticeably sulphate or even sulphur. Whereas such redox cycling may arise through abiotic reactions with oxygen or with Mn(IV) (Nealson et al., 1988; Nealson & Myers, 1992), very often it requires the direct involvement of various of the sulphur oxidising bacterial species (Jørgensen, 1982).

From these illustrations of the nature of anaerobic microbial communities, one can make three general statements of widespread significance.

A. Biological sustainability requires active redox cycling of both organic and inorganic elements.
B. Where some perturbation is introduced into such redox cycling, one or more negative consequences follow; eg. cell death, metal corrosion, environmental pollution.

C. Anaerobic microbial consortia display both structural heterogeneity and a high degree of species complexity.

Species diversity of microbial consortia

Whereas there has been a developing appreciation over the last ten or fifteen years that meaningful data on microbial consortia can only be obtained where due recognition is given to the complex nature of these entities, the true extent of that complexity is only now becoming widely recognised. This increase in our knowledge stems very largely from the addition of molecular methods, such as 16S rDNA sequencing, to the armory of techniques used to examine the species diversity of natural consortia and of enrichment cultures derived therefrom. With regard to SRB, the application of molecular techniques to biofilm systems has been relatively limited (Amann et al., 1992), but recent studies of the nature of subsurface microbial communities, and their putative role in the souring of petroleum reservoirs, have brought to light much information both on newly identified species, and on the scale of the species diversity within such natural consortia.

Broadly speaking, the techniques employed to analyse species diversity in subsurface microbial communities should be considered in terms of a number of comparable approaches. This is particularly important in view of the difficulties associated with the sampling of such environments, which serve to compound the inaccuracies already recognised with data derived solely from traditional culturing methods (Teske et al., 1996). Thus in the study of microbial populations associated with oilfield operations, samples may be withdrawn either directly from core samples obtained during drilling, or indirectly from produced water or from backflowing of injection wells. Microbial analyses may employ culturing techniques, or make use of the various molecular approaches now available. Such molecular analyses may be performed either directly on the environmental sample, or on an enrichment culture derived therefrom. Each of these approaches gives information which must be interpreted with due regard to any limitations inherent in the method itself. A true or complete picture of the nature of subsurface, or downhole microbial communities to use the language of the oil industry, can only be arrived at when several such approaches are adopted, and the data derived therefrom are analysed on comparative bases. The final conclusions reached can be greatly strengthened where other physical and chemical data characterising the subsurface environment are included in the final analyses (Parkes et al., 1994). At the present stage of our knowledge of microbial consortia associated with hydrocarbon reservoirs, there is a rapidly expanding body of information pertaining to individual studies, but still a very incomplete picture as to the overall status of such environments.

From an extensive study of produced water samples from fourteen different oil field sites in France, the North Sea and the Gulf of Guinea, Tardy-Jacquenod et al., (1996a) reported the isolation and characterisation by molecular, morphological and physiological parameters of thirty seven strains of SRB. While the majority of the identified strains were members of the genera *Desulfovibrio* and *Desulfotomaculum*, twenty of the isolates were not related to any known species. One example of such a new species is *Desulfovibrio vietnamensis*, isolated from production waters and crude oil storage tanks in Vietnam (Nga et al., 1996). In a more specialised study of enrichment cultures capable of direct anaerobic growth on aromatic hydrocarbons, Rabus et al. (1996) demonstrated the preponderance of complete oxidizers of the family *Desulfobacteriaceae.* These authors also discussed many of the significant problems associated with the extrapolation of microbiological data to considerations of diagenesis, maintenance of hydrocarbon reserves through geological time, biodegradation and souring (build-up of sulphide) subsequent to exploration and production, physical heterogeneities within the formation, and survival and movement of bacteria within such an environment. This last point was examined experimentally by Beeder et al. (1996) who were able to demonstrate the survival and movement through the reservoir to the production well of an antibody-marked benzoate-degrading SRB introduced with the injection water.

In an extension of their seminal work on the application of molecular techniques to the comparative analysis of oil field microbial populations, Voordouw et al., (1996) have most recently identified sixteen *Desulfovibrionaceae* and eight *Desulfobacteriaceae*, along with more limited numbers of fermentative and acetogenic species such as *Clostridium*, *Eubacterium*, and *Synergistes*. They have also shown the presence of potential sulphide oxidizers such as *Thiomicrospira, Arcobacter, Campylobacter* and *Oceanospirillum* spp. It is clear from such work, therefore, that oil field microbial consortia contain both a plethora of SRB species, and an extensive range of companion facultative and anaerobic species linked to the SRB by either their fermentative or acetogenic activity, or by their capacity to oxidise sulphide.

Since hydrocarbon reservoirs are generally high temperature environments, many microbiological analyses have been directed toward the identification and characterisation of thermophilic organisms. One of the earliest of such studies was carried out by the Bergen group who demonstrated the presence of spore-forming *Desulfotomaculum* in produced waters from North Sea oil operations (Rosnes et al. 1991). Subsequently, Stetter et al. (1993) found evidence in produced waters from both North Sea and Alaskan fields for hyperthermophilic sulphidogens capable of growth at temperatures in excess of 85 °C. Genera identified were archaeal sulphate-(*Archaeoglobus)* and sulphur-reducers (*Thermococcus* and *Pyrococcus*), and bacterial sulphur-reducers (*Thermotoga*). Since such organisms have also been isolated from submarine hot vents, these authors suggested that they may have been introduced to the reservoirs during the seawater injection employed to stimulate the secondary oil production phase. Currently however, the balance of evidence supports the contention that at least a significant proportion of the downhole microbial populations are indigenous to the formation rather than introduced during drilling and production (see, for example, Nilsen et al., 1996; Grassia et al., 1996).

A number of recent reports have further extended our knowledge of subsurface microbial communities, some focussing of the spread of organisms present while others identify particular isolates. For example, fermentative organisms of the genera *Thermoanaerobacter, Thermoanaerobacterium* and *Thermococcus,* and of the order Thermotogales have been characterised in glucose-based enrichment cultures from production waters from thirty six high temperature reservoirs (Grassia et al., 1996). The authors also draw attention to the capability of *Thermoanaerobacter* and *Thermoanaerobacterium* to reduce thiosulphate to sulphide or sulphur, respectively. In a more detailed study, Davey et al., (1993) proposed the recognition of two new genera within the order Thermotogales, *Petrotoga* and *Geotoga.* These sheathed bacteria have a fermentative mode of metabolism, but are also capable of reducing elemental sulphur and thiosulphate to sulphide (Ravot et al., 1995).

Examples of other sulphate-reducing thermophilic bacteria that have been identified are *Desulfacinum infernum* (Rees et al., 1995) and *Thermodesulforhabdus norvegicus* (Beeder et al., 1995). Unlike previous thermophilic isolates other than *Desulfotomaculum thermoacetoxidans* (Min & Zinder, 1990) and *Desulfotomaculum kuznetsovii* (Nazina et al., 1988), these two organisms are acetate-oxidizers. This is potentially an important finding as evidence has been put forward suggesting that acetate may be a major energy and carbon substrate for the growth downhole of souring sulphidogenic species (Herbert, 1987; Cochrane et al., 1988; Burger et al., 1992). On the other hand, a completely contradictory view is put forward by Mueller & Nielsen (1996) who conclude from their studies of populations enriched from North Sea and Alaskan fields that sulphidogenic souring is more likely to be the result of incomplete oxidative activity of SRB, with a concomitant increase in acetate concentration in formation waters.

Another interesting feature of the two isolates *Desulfacinum infernum* and *Thermodesulforhabdus norvegicus* is that they are closely related, both to each other, and to the hydrogen-producing acetogen *Syntrophobacter wolinii,* which last organism has been shown to be capable of propionate oxidation to acetate in monoculture, using sulphate as electron acceptor (Wallrabenstein et al., 1994).

These various studies, therefore, leave us in no doubt that subsurface anaerobic microbial communities such as are likely to be indigenous to hydrocarbon reservoirs show a high degree of species diversity, embracing:- fermentative and acetogenic catabolic activities; sulphidogenic capacity from the reduction of sulphate, thiosulphate and/or elemental sulphur; mechanisms for the trapping and/or reoxidation of reduced sulphur compounds. Furthermore, these populations are comprised of both mesophilic and thermophilic organisms, with the requisite mechanisms for their survival in such an environment.

Molecular techniques for the analyses of complex microbial communities

Two major difficulties associated with standard microbiological analyses of complex communities incorporating SRB are the bias introduced with the initial selection by enrichment culture, and the technical problems underlying the unequivocal establishment of pure monocultures of the organisms under study. In addressing these problems, the Bergen group have been particularly successful in their development of antibody-based techniques for the isolation and identification of SRB strains from oil field samples (Christensen et al., 1992; Nilsen et al., 1996).

In our own studies of the putative role of SRB-containing microbial consortia in the souring of hydrocarbon reservoirs, we have been fortunate to have direct access to drill cores from a number of oil fields, mostly in the North Sea. The drilling lubricants (known as muds) have also been available to us, and the comparative analyses of muds and inner core sections have allowed conclusions to be drawn as to which organisms may be introduced during the drilling operations, and which are more likely to be indigenous to the formation. We have also compared data from standard microbiological cultural methods with findings based on partial sequence analysis of 16S rDNA fractions. These molecular methods have been applied directly to core and mud samples, and to enrichment cultures derived therefrom.

In our intial study based on the analysis of enrichment cultures (Leu et al., 1998), we found evidence of thermophilic *Desulfotomaculum* spp. in cores and muds, and a mesophilic *Desulfomicrobium* sp. apparently only present in a core sample. Additionally, clones were obtained whose sequences were most closely related to thermophilic *Thermoanaerobacter* and *Clostridium* spp.

More recent work, which is entirely based on 16S rDNA sequence analysis and is still on-going (C. Devine, C. McGovern-Traa & I.S.C. Spark *unpublished results*), has allowed us to draw several general conclusions.

Even so-called blank muds prior to their first use and introduction downhole, are rich sources of potential microbial contamination of geological formations. From one such blank mud we have identified five *Clostridia* spp., and one species each of the genera *Spirochaeta, Cytophaga, Acinetobacter* and *Haloanaerobium.*

Analysis of a lactate-based SRB enrichment culture from the same blank mud showed some minimal degree of overlap (*Spirochaeta, Cytophaga* and one *Clostridium* spp.), but otherwise gave a quite different picture, with three non-identical *Clostridium* spp., and one species each of the genera *Geotoga, Microscilla, Desulfotomaculum* and *Porphyromonas.*

By comparison, sequence data derived directly from a core sample, although still subject to further analysis, indicates an altogether different population, with several sequences apparently bearing little or no relation to previously identified organisms. Sequences so far retrieved from the core belong to the genera *Sphingomonas, Azoarcus, Ralstonia* (previously *Alcaligenes*) and *Acinetobacter.* It is worthwhile, at this point, to remind ourselves of the point made by Nilsen et al. (1996) that there is no reason to expect that water samples taken from subsurface environments will necessarily reflect the true nature of the sessile downhole populations growing as biofilms within the pore structure of the geological strata.

It is clear from these findings, and from the data from other groups as discussed above, that downhole microbial populations are extremely complex and demonstrate very considerable species diversity. Equally, it remains true that the inherent difficulty of sampling such environments determine that findings must be interpreted with caution. While the advent of the new molecular techniques have greatly increased our experimental power, they are still at the developmental stage in terms of their direct application to environmental samples, and particularly so where quantitative analyses of mixed populations are sought.

Technological consequences of sulphidogenic activity

This paper will confine its consideration of practical systems to one or two selected examples. Reviews of other technological applications such as, for instance, anaerobic treatment of waste water are to be found elsewhere in the literature (Colleran et al., 1995).

Microbially influenced corrosion

The exact nature of the role of microorganisms in general, and of the SRB in particular, in microbially influenced corrosion (MIC) remains only partially understood. Some aspects are clearly established, however.

MIC is fundamentally an electrochemical phenomenon, with metal dissolution at an anodic site being coupled to electron uptake by an acceptor at a cathodic site. The rate and extent of these processes are critically affected by the chemical and physical nature of the precipitated iron sulphide corrosion products, and by access of oxygen to the system (Hamilton & Lee 1995).

The fact that so-called anaerobic pitting corrosion caused by SRB is often more pronounced under environmental situations that allow for some degree of access of oxygen, has been recognised for many years at the anecdotal level. This facet was confirmed and its nature further explored in a series of field studies and laboratory simulations (Hamilton, 1991; Moosavi et al., 1991; McKenzie & Hamilton, 1992). It was established that the extent of corrosion weight loss could not be correlated with either the numbers or the activity of SRB within the biofilm adherent to test coupons. There was a correlation, however, with the presence of oxygen and this, in turn, was associated with conversion of sulphide corrosion products to non-acid volatile forms (most likely pyrite and elemental sulphur), with a surface overlay of iron oxides and hydroxide.

These findings led to an extensive laboratory study with a defined mixed population biofilm containing *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Desulfovibrio desulfuricans,* and controlled conditions of oxygenation (Lee et al., 1993a & b, 1995; Nielsen et al., 1993). The critical role of oxygen was confirmed, most remarkably in an experiment in which periods of intermittent exposure to alternating oxic and anoxic conditions were followed by exposure for one month to saturating concentrations of oxygen in the absence of carbon and energy nutrients. During the period of oxic/anoxic cycling, high corrosion rates of up to 4mm.y^{-1} were measured, and the 2mm biofilm was shown to have developed a steep oxygen gradient with an aerobic/anaerobic interface at approximately 1mm. The biofilm as a whole demonstrated a high oxygen consumption rate, with a comparable rate of sulfate reduction being noted in the anaerobic base biofilm region. During the prolonged aerobic exposure in the absence of added substrate, these high rates of corrosion, oxygen consumption, and sulfate reduction were maintained. Also the content of iron sulphide corrosion products remained essentially unaltered.

On the basis of these findings, the authors proposed the model which is illustrated in Figure 1. The hypothetical scheme shows the electrochemical corrosion cell, with protons acting as the primary electron acceptor in accordance with the classical cathodic depolarisation theory (von Wolzogen Kuhr & van der Vlugt, 1934). H_2 formed from this reaction is oxidized by the SRB with the production of sulphide, the majority of which reacts with the Fe^{2+} to form a mixture of iron sulphide corrosion products. In a series of abiotic reactions, the reduced HS^- and Fe^{2+} ions then react across the aerobic/anaerobic interface with oxygen to give sulphur and iron redox cycling and the formation of a surface deposit of ferrihydroxide corrosion product. That is to say, the electrochemical corrosion reaction is correctly portrayed as an oxidation of metallic iron, with electron transfer across the metal surface and protons acting as the primary electron acceptor. Thereafter, in a linked series of biotic and abiotic reactions, sulphate acts as a secondary electron acceptor, with oxygen being the terminal electron acceptor whose presence and function in that role ultimately determines the rate and extent of the initial corrosion reaction.

It should be recognised, however, that this scheme may be, at least in part, an oversimplification. For example, Fe^{2+} and HS^- are recognised energy donors for microbial oxidation, and genera such as *Gallionella* have been implicated in certain corrosion processes (Tatnall, 1991). Also, it is now becoming clear that many sulphidogenic bacteria are capable of reducing other terminal electron acceptors such as S°, Fe^{3+} and even O_2 (Lovley & Phillips, 1994; Lovley & Woodward, 1994; Lovley 1995; Dannenberg et al., 1992). Whether any of these reactions play a role, direct or indirect, in SRB anaerobic corrosion remains to be determined.

The above scheme proposes that anaerobic MIC is entirely the end result of microbial sulphidogenesis, and beyond that there is no suggestion of any degree of species-specificity. Two recent studies of particularly severe corrosion failures, however, have identified two new closely related SRB species, *Desufovibrio gabonensis* (Tardy-Jacquenod et al., 1996b) and *Desulfovibrio indonensis* (Feio et al., 1998) whose presence have been implicated in the corrosion processes. Further studies with the Indonesian strain have produced evidence of an exopolymer fraction with a very high binding affinity for iron and the potential to accelerate metal loss from the substratum (I.B. Beech unpublished results).

Marine and estuarine pollution

The marked toxicity of sulphide to all forms of life, including the SRB themselves, make the reoxidation of any microbially-produced sulphide essential for the long-term biological stability of anaerobic ecosystems. Where such a redox balance is not maintained, the consequences are variously identified as pollution of a natural environment, souring of a hydrocarbon reservoir, or corrosion of a metal vessel or pipeline.

Apart from specific instances of the introduction of some noxious chemical, pollution occurring in many estuarine or marine environments most commonly arises from an increased carbon loading. This may take the form of domestic sewage or industrial

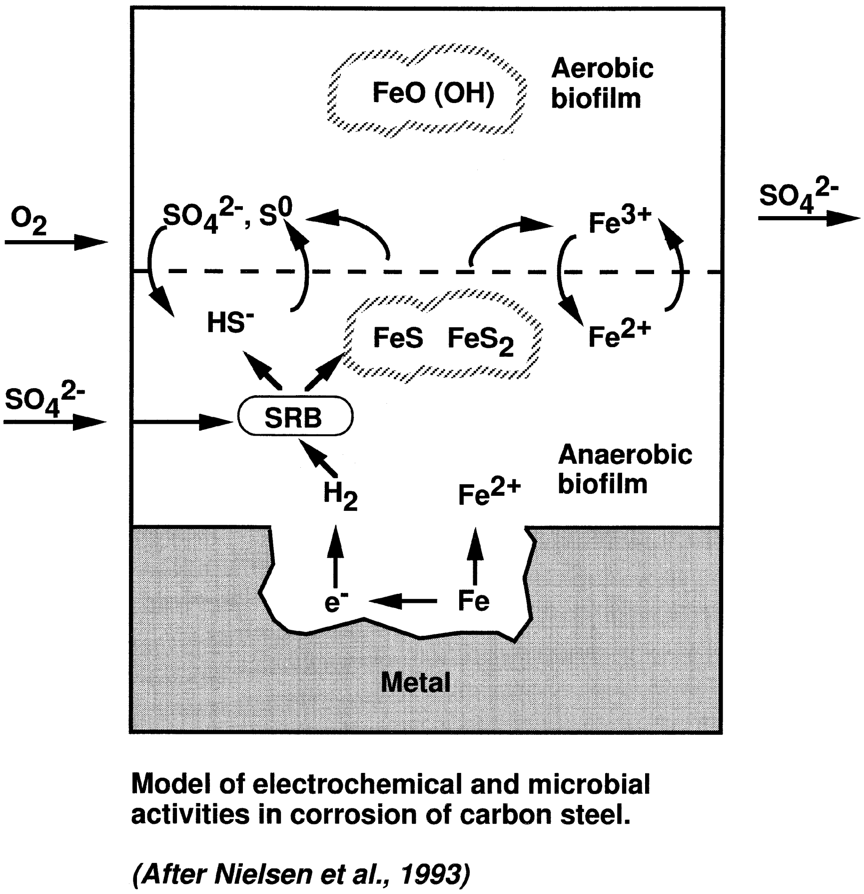

Figure 1. A hypothetical model for the role of oxygen in the sulphate-reducing bacterial corrosion of carbon steel. (Reproduced, with permission, from *Biofouling* (1993) 7: 267-284).

effluent, decay of an algal bloom, or deposition of drill cuttings from an oil production platform (Sanders & Tibbets 1987). In each case, the localised stimulation of microbial degradative activity leads to greatly increased oxygen uptake and a consequent redox imbalance within the system, which manifests itself through the development of anaerobiosis and an excess of sulphide production. It is this sulphide and the associated anoxia that constitute the true nature of the pollution; as evidenced by blackened metal sulphide-rich sediments, release of noxious H_2S gas, and decreased species diversity.

Hydrocarbon reservoir souring

As is clearly evident from the discussions above of subsurface microbial communities, studies of the potential for SRB and other sulphidogens to cause souring of underground petroleum reservoirs are actively being pursued in many laboratories at the present time. There is now little argument that bacterial activity is the sole, or at least the principal cause of this commercially very serious problem. What is very much less clear are the biological and environmental parameters controlling the rate and extent of the process. For example, certain formations appear to have the high sulphide content characteristic of a soured reservoir at the earliest stages of their production. On the other hand, it is generally considered that souring is a problem arising subsequent to the initiation of sea water flooding which is employed to maintain production pressure within the reservoir. Even within this latter scenario, opinions differ as to what might be the causative factor(s):- Lowering of the formation temperature, from possibly as high as 120 °C, to allow growth of mesophiles, or even of thermophiles? Introduction of sulphate, normally largely absent from formation brines? Introduction of one or more of the necessary organisms for the complex metabolic transformations and nutritional dependencies that underly sulphide production? Introduction of nutrients? Modification of physicochemical conditions within the reservoir such that organisms previously dormant re-establish full metabolic activity?

In seeking the answers to these very practical questions, we are at the same time gaining much new fundamental knowledge as to the nature and extent of microbial diversity in what has turned out to be a new corner of the biosphere (Parkes et al., 1994).

Removal of heavy metals

Undoubtedly the main technological consequences of microbial sulphidogenic activity are of a negative character in respect of corrosion, pollution and souring. Nonetheless, to conclude on a more positive note, at least one reaction has been harnessed in an advantageous biotechnological process.

Metal sulphides are universally characterised by their exceedingly low solubility in aqueous systems. Sulphidogenic activity therefore offers the potential for the removal of toxic heavy metals during downstream processing from a range of industrial plants, and in bioremediation in general. Such processes are now in operation (Barnes et al., 1994).

References

Amann RI, Stromley J, Devereux R, Key R & Stahl DA (1992) Molecular and microscopic identification of sulfate-reducing bacteria in multispecies biofilms. Appl. Environ. Microbiol. 58: 614–623

Badziong W & Thauer RK (1978) Growth yields and growth rates of *Desulfovibrio vulgaris* (Marburg) growing on hydrogen and sulfate and hydrogen and thiosulfate as sole energy sources. Arch. Microbiol 117: 209–214

Barnes LJ, Scheeren PJM & Buisman CJN (1994) Microbial removal of heavy metals and sulphate from contaminated groundwater In: Means JL & Hinchee RE (Eds) Emerging Technology for Bioremediation of Metals (pp 38–49). Lewis Publ., Boca Raton

Beeder J, Torsvik T & Lien T (1995) *Thermodesulforhabdus norvegicus* gen. nov., sp. nov., a novel thermophilic sulfate-reducing bacterium from oil field water. Arch. Microbiol. 164: 331–336

Beeder J, Nilsen RK, Thorstenson T & Torsvik T (1996) Penetration of sulfate reducers through a porous North Sea oil reservoir. Appl. Environ. Microbiol. 62: 3551–3553

Burger ED, Addington DV & Crews AB (1992) Reservoir souring: bacterial growth and transport. In: Proceedings of the Fourth International IGT Symposium on Gas, Oil and Environmental Biotechnology

Canfield DE, Thamdrup B & Hansen JW (1993). The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction. Geochim. Cosmochim. Acta 57: 3867–3883

Christensen B, Torsvik T & Lien T (1992) Immunomagnetically captured thermophilic sulfate-reducing bacteria from North Sea oil field waters. Appl. Environ. Microbiol. 58: 1244–1248

Cochrane WJ, Jones PS, Sanders PF, Holt DM & Mosely MJ (1988) Studies on the thermophilic sulfate-reducing bacteria from a souring North Sea field. SPE Production Eng. 1988: 301–316

Colleran E, Finnegan S & Lens P (1995) Anaerobic treatment of sulfate-containing waste streams. A. van Leeuwenhoek Int. J. Gen. Mol. Microbiol. 67: 29–46

Costerton JW, Lewandowski Z, DeBeer D, Caldwell D, Korber D & James G (1994) Minireview: biofilms the customized microniche. J. Bacteriol 176: 2137–2142

Cypionka H (1995) Solute transport and cell energetics. In: Barton LL (Ed) Sulfate-Reducing Bacteria (pp 151–184). Plenum Press, New York

Dannenberg S, Kroder M, Dilling W & Cypionka H (1992) Oxidation of H_2, organic compounds and inorganic sulfur compouds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. Arch. Microbiol. 158: 93–99

Davey ME, Wood WA, Key R, Nakamura K & Stahl DA (1993) Isolation of three species of *Geotoga* and *Petrotoga*: two new genera, representing a new lineage in the bacterial line of descent distantly related to the '*Thermotogales*'. System. Appl. Microbiol. 16: 191–200

Dilling W & Cypionka H (1990) Aerobic respiration in the sulfate-reducing bacteria. FEMS Microbiol. Lett. 71:123–128

Feio MJ, Beech IB, Carepo M, Lopes JM, Cheung CWS, Franco R, Guezenec J, Smith JR, Mitchell JI, Moura JJG & Lino AR (1998) Isolation and characterisation of a novel sulphate-reducing bacterium of the *Desulfovibrio* genus. Anaerobe. 4: 117–130

Fuchs G (1986) CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol. Rev. 39:181–213

Grassia GS, McLean KM, Glenat P, Bauld J & Sheehy AJ (1996) A systematic survey for thermophilic fermentative bacteria and archaea in high temperature petroleum reservoirs. FEMS Microbiol. Ecol. 21: 47–58

Hamilton WA (1985) Sulphate–reducing bacteria and anaerobic corrosion. Ann. Rev. Microbiol. 39: 195–217

— (1987) Biofilms: microbial interactions and metabolic activities. Symp. Soc. Gen. Microbiol. 41: 361–385

— (1991) Sulphate-reducing bacteria and their role in biocorrosion. In: Flemming H-C & Geesey GG (Eds) Biofouling and Biocorrosion in Industrial Water Systems (pp 187–193). Springer-Verlag, Berlin

— (1998) Sulfate-reducing bacteria: physiology determines their environmental impact. Geomicrobiol. J. 15: 19–28

Hamilton WA & Lee W (1995) Biocorrosion. In: Barton LL (Ed) Sulfate-Reducing Bacteria (pp 243–264). Plenum Press, New York

Hansen TA (1993) Carbon metabolism of sulfate-reducing bacteria. In: Odom JM & Singleton R (Eds) The Sulfate-Reducing Bacteria: Contemporary Perspectives (pp 21–40). Springer-Verlag, New York

Hansen TA (1994) Metabolism of sulfate-reducing prokaryotes. A. van Leeuwenhoek Int. J. Gen & Mol. Microbiol. 66: 165–185

Herbert BN (1987) Reservoir souring. In: Hill EC, Shennan JL & Watkinson RJ (Eds) Microbial Problems in the Offshore Oil Industry (pp 63–71). Instit. Petroleum, London

Jørgensen BB (1982) Ecology of the bacteria of the sulfur cycle with special reference to anoxic-oxic interface environments. Phil. Trans. Roy. Soc. Ser B 298: 543–561

Lee W, Lewandowski Z, Okabe S, Characklis WG & Avci R (1993a) Corrosion of mild steel underneath aerobic biofilms containing sulfate-reducing bacteria. Part I: at low dissolved oxygen concentration. Biofouling 7: 197–216

Lee W, Lewandowski Z, Morrison M, Characklis WG, Avci R & Nielsen PH (1993b) Corrosion of mild steel underneath aerobic biofilms containing sulfate-reducing bacteria. Part II: at high bulk oxygen concentration. Biofouling 7: 217–239

Lee W, Lewandowski Z, Nielsen PH & HamiltonWA (1995) Role of sulfate-reducing bacteria in corrosion of mild steel: a review. Biofouling 8: 165–194

Leu J-Y, McGovern-Traa CP, Porter AJR, Harris WJ & Hamilton WA (1998) Identification and phylogenetic analysis by 16 S rDNA gene cloning and sequencing of thermophilic sulfate-reducing bacteria in oil field samples. Anaerobe 4: 165–174

Lewandowski Z, Dickinson W & Lee W (1997) Electrochemical interactions of biofilms with metal surfaces. Wat. Sci. Tech.36: 295–302

Lonergan DJ, Jenter HL, Coates JD, Phillips EJP, Schmidt TM & Lovley DR (1996). Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J. Bacteriol. 178: 2402–2408

Lovley DR (1995) Microbial reduction of iron, manganese, and other metals. Adv. Agronomy 54: 175–231

Lovley DR & Phillips EJP (1994) Novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. Appl. Environ. Microbiol. 60: 2394–2399

Lovley DR & Woodward JC (1994) Stimulated anoxic biodegradation of aromatic hydrocarbons using Fe(III) ligands. Nature 370: 128–131

McKenzie J & Hamilton WA (1992) The assay of *in situ* activities of sulphate-reducing bacteria in a laboratory marine corrosion model. Internat. Biodeterior. Biodegrad. 29: 285–297

Min H & Zinder SH (1990) Isolation and characterization of a thermophilic sulfate-reducing bacterium *Desulfotomaculum thermoacetoxidans* sp. nov. Arch Microbiol. 153: 399–404

Moosavi AN, Pirrie RS & Hamilton WA (1991) Effect of sulphate-reducing bacteria activity on performance of sacrificial anodes. In: Dowling NJ, Mittleman MW & Danko JC (Eds) Microbially Influenced Corrosion and Biodeterioration (pp 3.13–3.27). National Association of Corrosion Engineers, Washington, DC

Moura I, Bursakov S, Costa C & Moura JJG (1997) Nitrate and nitrite utilization in sulfate-reducing bacteria. Anaerobe 3: 279–290

Mueller RF & Nielsen PH (1996) Characterization of thermophilic consortia from two souring oil reservoirs. Appl. Environ. Microbiol. 62: 3083–3087

Nazina TN, Ivanov AE, Kanchaveli LP & Rozanova EP (1988) A new spore-forming thermophilic methylotrophic sulfate-reducing bacterium *Desulfotomaculum kuznetsovii* sp nov. Microbiology 57: 659–663

Nealson KH, Tebo BM & Rosson RA (1988) Occurrence and mechanisms of microbial oxidation of manganese. Adv. Appl. Microbiol. 33: 279–318

Nealson KH & Myers CR (1992) Microbial reduction of manganese and iron: new approaches to carbon cycling. Appl. Environ. Microbiol. 58: 439–443

Nealson KH & Saffarini D (1994) Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. Ann. Rev. Microbiol. 48: 311–343

Nga DP, Ha DTC, Hien LT & Stan-Lotter H (1996) *Desulfovibrio vietnamensis* sp. nov., a halophilic sulfate-reducing bacterium from Vietnamese oil fields. Anaerobe 2: 385–392

Nielsen PH, Lee W, Lewandowski Z, Morrison M & Characklis WG (1993) Corrosion of mild steel in an alternating oxic and anoxic biofilm system. Biofouling 7: 267–284

Nilsen RK, Beeder J, Thorstenson T & Torsvik T (1996) Distribution of thermophilic marine sulfate reducers in North Sea oil field waters and oil reservoirs. Appl. Environ. Microbiol 62: 1793–1798

Parkes RJ (1987) Analysis of microbial communities within sediments using biomarkers. Symp. Soc. Gen. Microbiol. 41: 147–177

Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, Fry JC, Weightman AJ & Harvey SM (1994) A deep bacterial biosphere in Pacific Ocean sediments. Nature 371: 410–413

Peck HD (1993) Bioenergetic strategies of the sulfate-reducing bacteria. In: Odom JM & Singleton R (Eds) The Sulfate-Reducing Bacteria; Contemporary Perspectives (pp 41–76). Springer-Verlag, New York

Pfennig N (1984) Microbial behaviour in natural environments. Symp. Soc. Gen. Microbiol. 36: 23–50

Postgate JR (1979) The Sulphate-Reducing Bacteria. Cambridge University Press, Cambridge

Rabus R, Fukui M, Wilkes H & Widdel F (1996) Degradative capacities and 16S rRNA-targeted whole-cell hybridization of sulfate-reducing bacteria in an anaerobic enrichment culture utilizing alkylbenzenes from crude oil. Appl. Environ. Microbiol. 62: 3605–3613

Ravot G, Ollivier B, Magot M, Patel BKC, Crolet J-L,Fardeau M-L & Garcia J-L (1995) Thiosulfate reduction, an important physiological feature shared by members of the Order *Thermotogales*. Appl. Environ. Microbiol. 61: 2053–2055

Rees GN, Grassia GS, Sheehy AJ, Dwivedi PP & Patel BKC (1995) *Desulfacinum infernum* gen. nov., sp. nov., a thermophilic sulfate-reducing bacterium from a petroleum reservoir. Int. J. Syst. Bacteriol. 45: 85–89

Rosnes JT, Torsvik T & Lien T (1991) Spore-forming thermophilic sulfate-reducing bacteria isolated from North Sea oil field waters. Appl. Environ. Microbiol. 57: 2302–2307

Sanders PF & Tibbetts PJ (1987) Effects of discarded drill muds on microbial populations. Phil. Trans. Roy. Soc. Ser B 316: 567–585

Schmitz RA, Bonch-Osmolovskaya EA & Thauer RK (1990) Different mechanisms of acetate activation in *Desulfurella acetivorans* and *Desulfuromonas acetoxidans*. Arch. Microbiol. 154: 274–279

Schwörer B, Breitung J, Klein AR, Stetter KO & Thauer RK (1993) Formylmethanofuran:tetrahydromethanopterin formyltransferase and N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase from the sulfate-reducing *Archaeoglobus fulgidis*: similarities with the enzymes from methanogenic Archaea. Arch. Microbiol. 159: 225–232

Sørensen J, Christensen D & Jørgensen BB (1981) Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. Appl. Environ. Microbiol. 42: 5–11

Stetter KO, Huber R, Blochl E, Kurr M, Eden RD, Fielder M, Cash H & Vance I (1993) Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. Nature 365: 743–745

Tardy-Jacquenod C, Caumette P, Matheron R, Lanau C, Arnauld O & Magot M (1996a) Characterization of sulfate-reducing bacteria isolated from oil-field waters. Can. J. Microbiol. 42: 259–266

Tardy-Jacquenod C, Magot M, Laigret F, Kaghad M, Patel BKC, Guezennec J, Matheron R & Caumette P (1996b) *Desulfovibrio gabonensis* sp. nov., a new moderately halophilic sulfate-reducing bacterium isolated from an oil pipeline. Int. J. Sys. Bacteriol. 46: 710–715

Tatnall R (1991) Case histories: biocorrosion. In: Flemming H-C & Geesey GG (Eds) Biofouling and Biocorrosion in Industrial Water Systems (pp 165–185). Springer-Verlag, Berlin

Teske A, Wawer C, Muyzer G & Ramsing NB (1996) Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. Appl. Environ. Microbiol. 62: 1405–1415

Thauer RK (1988) Citric-acid cycle, 50 years on: modifications and an alternative pathway in anaerobic bacteria. Eur. J. Biochem. 176: 497–508

Thauer RK & Morris JG (1984) Metabolism of chemotrophic anaerobes: old views and new aspects. Symp. Soc. Gen. Microbiol. 36: 123–168

Thauer RK, Jungermann K & Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41: 100–180

Thauer RK, Möller-Zinkhan D & Spormann AM (1989) Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. Ann. Rev. Microbiol. 43: 43–67

von Wolzogen Kuhr CAH & van der Vlugt LS (1934) The graphitization of cast iron as an electrobiochemical process in anaerobic soils. Water 18: 147–165

Voordouw G, Niviere V, Ferris FG, Fedorak PM & Westlake DWS (1990) Distribution of hydrogenase genes in *Desulfovibrio* spp. and their use in identification of species from the oil field environment. Appl. Environ. Microbiol. 56: 3748–3754

Voordouw G, Armstrong SM, Reimer MF, Fouts B, Telang AJ, Shen Y & Gevertz D (1996) Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate–reducing, fermentative, and sulfide-oxidizing bacteria. Appl. Environ. Microbiol. 62: 1623–1629

Wallrabenstein C, Hauschild E & Schink B (1994) Pure culture and cytological properties of *Syntophobacter wolinii.* FEMS Microbiol. Lett. 123: 249–254

Widdel F (1988) Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In: Zehnder AJB (Ed) Biology of Anaerobic Microorganisms (pp 469–585). John Wiley, New York

Widdel F & Pfennig N (1977) A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium *Desulfotomaculum (emend.) acetoxidans.* Arch. Microbiol. 112: 119–122

— (1981) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. 1. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. Arch. Microbiol. 129: 395–400

Wood HG, Ragsdale SW & Pezacka E (1986) The acetyl-CoA pathway: a newly discovered pathway of autotrophic growth. FEMS Microbiol. Rev. 39: 345–362