# Economic Importance of Sulphur Bacteria [and Discussion]

J. R. Postgate and H. Abdollahi

*Phil. Trans. R. Soc. Lond. B* 1982 **298**, 583-600
doi: 10.1098/rstb.1982.0098

*Phil. Trans. R. Soc. Lond.* B **298**, 583–600 (1982)
*Printed in Great Britain*

# Economic importance of sulphur bacteria

By J. R. Postgate, F.R.S.
*A.R.C. Unit of Nitrogen Fixation, University of Sussex, Falmer, Brighton BN1 9RQ, U.K.*

Microbes, through their specialized metabolic processes and ecologies, play a fundamental part in man's economy, and their economic roles are widely recognized as important. The sulphur bacteria have had in past geological eras, and are having today, numerous and diverse effects, often deleterious, scattered throughout the economy. Many of their economic roles are not widely known. Their economic and environmental effects arise, according to the type or combination of species involved, principally from (1) the production of acid, (2) the formation or removal of hydrogen sulphide, and (3) the removal of oxygen. In this paper their various effects on industry and the environment are surveyed.

## 1. Introduction

The fundamental part played by microorganisms in the cycling of the biological elements in the biosphere is now widely recognized. Industry, including agriculture and forestry, depends on exploiting, augmenting or perturbing the biological cycles; hastening the carbon cycle by consuming fossil or renewable fuels; hastening the nitrogen cycle by preparing and using 'chemical' N fertilizer; augmenting other cycles by adding P, K, etc., to soil. The microbes of the carbon and nitrogen cycles have played, and are today playing, a clear and fundamental part in our economy, and it is often those with very specialized metabolisms that are particularly important. One can cite numerous instances of this principle: the critical parts played by cellulolytic bacteria in composting, by methanogens in sewage treatment, by diazotrophs and nitrifying bacteria in agricultural productivity. The sulphur bacteria, in which group I include all those types that play a major part in the biological sulphur cycle, have metabolisms which, from an anthropomorphic viewpoint, seem both strange and highly specialized, as this Discussion Meeting has amply demonstrated. This specialization leads them to occupy ecological niches that are unusual and often not immediately obvious. Such ecological niches are widely, and probably correctly, believed to have been more common and abundant in the early days of this planet's biosphere because these bacteria are ultimately dependent on the essentially anaerobic process of dissimilatory sulphate reduction, and therefore the low or zero concentration of atmospheric $O_2$ before about 2 Ga ago was far more favourable to their activities than is today's 20 % $O_2$. Thus some of their most valuable economic effects are historical, in the deposition and transformation of minerals over geological time. With the emergence of our present atmosphere the sulfuretum has retreated, but twentieth-century man has generated a number of new ecological niches in which some or all of the sulphur bacteria flourish. These provide the contexts in which their economic effects are expressed.

As a preliminary it is necessary to list the general ecological effects of multiplication of the sulphur bacteria. These may be subdivided according to the physiological type of organism that is dominant in a given environment.

## 2. GENERAL ECOLOGICAL EFFECTS OF SULPHUR BACTERIA

### (a) *Sulphate-reducing bacteria*

The special physiology of this group leads to a number of ecological consequences, which have been outlined recently (Postgate 1979) so a detailed presentation will not be made here. Their major effects may be summarized as follows.

1. Formation of hydrogen sulphide. This can be offensive to man, chemically reactive, toxic to aerobic biota, as well as generating a reducing, anoxic environment. Thus they alter environments to favour not only themselves but certain other sulphur bacteria.

2. Removal of sulphate, sulphide-precipitable metals and hydrogen from the ecosystem. These processes, like sulphide formation, alter the character of the associated biota.

3. Generation of alkalinity. Their normal sulphate sources are alkali or alkaline-earth sulphates, the sulphides of which dissociate to form alkali and free $H_2S$.

4. Fixation of dinitrogen. Some strains contribute to this step of the nitrogen cycle and may be quantitatively important in the sea. A recent mention of an authentic nitrate-reducing (ammonia-producing) strain (Liu *et al.* 1980) suggests that they may influence other steps of the nitrogen cycle.

5. Generation of acetate. This, the normal end-product of carbon metabolism by *Desulfovibrio* or *Desulfotomaculum*, can serve as a substrate for syntrophic methane production (see, for example, Laube & Martin 1981; McInerney & Bryant 1981) and thus alter the microflora.

Massive growth of sulphate-reducing bacteria can lead to some or all of the ecological effects summarized above.

### (b) *Sulphide- and sulphur-oxidizing bacteria*

In the anaerobic zone of a sulfuretum the majority of these are phototrophs such as *Chromatium*, *Chlorobium* and *Thiocystis*; some anaerobic thiobacilli (e.g. *T. denitrificans*) may also be present. Their ecological effects are minor: they may alter the colour of waters and generate some sulphur; they then diminish $H_2S$ concentrations and so protect sulphide-sensitive biota to some extent. In aerobic zones, at the periphery of a sulfuretum, aerobic sulphide oxidizers develop, and the thiobacilli, in particular, generate acid ($H_2SO_4$). This decomposes carbonates and, in extreme instances, has a toxic effect on the biota by lowering the environmental pH. The acid may also leach minerals and lead to metal oxide deposition.

## 3. ECONOMIC EFFECTS

The diversity of the environmental and industrial consequences of these ecological effects presents me with a problem in presentation that I shall try to solve by discussing categories of economic activity. Coverage of the sulphate-reducing bacteria will be somewhat superficial because I have surveyed their specific economic activities in a series of reviews and a monograph (Postgate (1960), supplemented by parts of Postgate (1965), LeGall & Postgate (1973) and Postgate (1979)).

### (a) *Pollution of waters*

Canals, harbours, estuaries and stagnant waters near or in population centres are prone to become anaerobic as the detritus of human activity increases their biological oxygen demand. All such waters contain sulphate and, particularly in warm climates or seasons, sulphate reduction ensues, a smell of hydrogen sulphide develops with consequent nuisance to people and damage to metal and paintwork. Examples were presented in the reviews cited above. The condition is self-perpetuating because the reducing properties of $H_2S$ stabilize the anaerobic character of the ecological niche, and its toxic effect on aerobic life, such as fish, augments supplies of nutrient for the sulphate-reducing bacteria. Spectacular examples of pollution leading to mass mortality of fish or the death of birds have been described (see Postgate 1979) and an instance in which humans died from $H_2S$ poisoning is recorded (see LeGall & Postgate 1973). Most instances of pollution are less dramatic, but trips on the canals of Bruges or Venice can be less than agreeable at the end of a warm summer. Such pollution can sometimes be controlled, in closed systems, but generally it arises from an excessive input of organic matter into the water; the cure, if any, is to restrict the input of organic matter to such waters so that they do not become anaerobic. In the 1950s, the lower reaches of the River Thames could be said, without much exaggeration, to be a vast culture of sulphate-reducing bacteria, yet today environmental and effluent controls have improved the water quality impressively.

The effects of sulphate-reducing bacteria *per se* in such situations can be of great economic importance in terms of damage by $H_2S$ to paintwork, corrosion of ships and metal installations (below), tarnishing of domestic instruments and decorations, damage to health, tourism and so on. These matters are well documented; the organisms responsible, long believed to be largely *Desulfovibrio* species (because these are the easiest to isolate) must now be extended to include *Desulfotomaculum*, *Desulfobulbus* and *Desulfococcus* species in fresh water together with *Desulfobacter*, *Desulfonema* and *Desulfosarcina* species, but probably not *Desulfotomaculum*, in marine or brackish waters (Widdel 1980). Postgate (1960) mentioned two other economic consequences of their activities in local aqueous environments: (1) by converting ferric phosphates in sediments to FeS they can release phosphates and augment biomass production; (2) by quantitatively removing sulphate they can render water deficient in sulphate and so decrease biomass production. Some natural waters in East Africa are deficient in sulphate, but non-biological absorption of sulphate by ion exchange, rather than biological sulphate reduction, may be responsible (see Hesse 1956).

In polluted situations the sulphate-reducing bacteria provide an environment favouring the development of other sulphur bacteria, with consequences of varying economic importance. The phenomena of 'bloody seas', '*lagi di sangue*' and other occurrences of red waters can arise because the activities of sulphate-reducing bacteria provide a substrate for the mass growth of the organisms responsible for the colour: red sulphide-oxidizing bacteria such as *Chromatium* and *Thiopedia*. ZoBell (1946) and Butlin & Postgate (1954*a*) cited references to early accounts of such phenomena, and the latter authors described a case in which the mass growth of *Chromatium* turned a polluted stream red. Not all blooms of coloured waters have this origin: in temperate climates green waters contaminated by green algae or cyanobacteria, or brown waters contaminated by brown algae, are most common; waters are occasionally seen that are red or purple because of Athiorhodaceae (coloured non-sulphur bacteria). Halophilic algae usually form the pigments of salt pans. However, when blooms of coloured sulphur bacteria do occur they are spectacular and unmistakable, if only because of the smell of $H_2S$.

When not 'blooming', the red and green sulphur bacteria perform an economically valuable function in confining the pollution of natural waters by $H_2S$. Lyalikova (1957) studied the distribution of *Chromatium* in Lake Belovod in the U.S.S.R. and showed that a zone of these bacteria exists at 13–14 m separating the $H_2S$-bearing water below from the aerobic water above. Considerable $CO_2$ fixation takes place in this zone. Genovese (1963) demonstrated *Chromatium*, *Thiopedia* and *Thiopolycoccus* in the brackish Lake Faro; these organisms formed a zone whose depth was determined by the season and the penetration of light. Reports of green waters due to sulphur bacteria are rare; Bicknell (1949) described a zone of *Chlorobium* at a depth of 25 ± 5 ft (*ca.* 7.6 ± 1.5 m) in the Sodon Lake, Michigan, separating $H_2S$-bearing water from aerobic water. Sulphur springs, some geothermal springs and effluent streams from polluted waters often show a localized growth of coloured photosynthetic bacteria, sometimes as a zoogloeal mass. Jannasch (1957) described and illustrated such outcrops in Wadi Natrun, a soda-bearing lake in Egypt; mosaic records associate this area with the Seven Plagues. The Libyan sulphur-producing lakes described by Butlin & Postgate (1954*b*) had a zoogloeal mud of red and green sulphur bacteria.

Blooms of coloured sulphur bacteria are too rare and capricious to represent a serious economic problem, and a cure is rarely necessary: they usually disappear again after a few weeks. It is a pity that they do not remove all the $H_2S$ of polluted waters when they bloom, for an aesthetically pleasing biological means of controlling such pollution would otherwise be available. Blooms of non-pigmented sulphur bacteria such as *Beggiatoa* and *Thiothrix* occur when $H_2S$ pollution is relatively low and organic pollution high.

The deliberate use of sulphide-oxidizing bacteria for the biological control of $H_2S$ pollution is an attractive idea. It is limited by the relative sensitivity of many of these types to $H_2S$ concentration and illumination levels, as well as the fact that, in Nature, some $H_2S$ often escapes. As a biological control process it might pay reinvestigation, because highly $H_2S$-tolerant strains of sulphur bacteria are now known (see, for example, Kuenen & Veldkamp 1972).

There is one industrial environment in which serious water pollution is caused by the activities of a thiobacillus. Coal mines (both deep and open-cast), gold mines and mines yielding some other minerals normally contain pyritic strata – bands of $FeS_2$ – which, on exposure to air and water, provide a habitat for *Thiobacillus ferro-oxidans* (see, for example, Butlin & Postgate 1954*a*; Tuttle *et al.* 1968; Suzuki 1974; Kleinmann & Crerar 1979). The run-off waters become very acid and rich in $Fe^{3+}$, $SO_4^{2-}$ and hydrogen ions; they can cause corrosion problems to mining machinery and environmental damage in streams or watercourses. Such acid effluents make the water turbid and yellow, kill aquatic fauna and flora and render the water unsatisfactory for industrial use. Industries that generate such waters are therefore obliged to neutralize their effluents and this may be an expensive process. The yellow material ('yellow boy' or, incorrectly, 'sulphur mud') is deposited in the water near the source of pollution, which is thus easily recognized. Braley (1956) concluded that bactericides on the working face of the mine provided no satisfactory solution to the problem; chemical neutralization with lime or chalk can be used successfully. A biological treatment is discussed in § 3*c* and a biotechnological approach is mentioned in § 3*i*.

### (*b*) *Infection of sands and soils*

Marine muds and sands, particularly near estuaries and dense population centres, have a property that is familiar to every child who has made sandcastles. When freshly exposed by the retreating sea such sand has a greyish tint and, when dug into, is found to be black underneath. In a matter of minutes the freshly exposed black sand turns brown. In a similar way, some classes of black mud turn from an intense black to a brownish colour on aeration; in both cases a smell of $H_2S$ is noticeable. The interpretation (Ellis 1932; Bunker 1936) is that black FeS, formed in the sand or soil during anaerobic conditions, becomes autoxidized on exposure to air to brown ferric oxides and sulphates. In the marine intertidal zone the oxidation is reversed as the tide comes in and the process becomes cyclic. Although sulphate-reducing bacteria are the primary generators of sulphide in marine sediments, Gunkel & Oppenheimer (1963) concluded that a considerable contribution could come from the decomposition of organic S by other bacteria. Apart from having a negative effect on tourism, such reversible blackening of sand is of little economic moment, but the phenomenon serves as a warning that metal and stone installations in such environments are threatened by microbiological corrosion (see below).

Soils wholly deficient in sulphate-reducing bacteria are extremely rare but the bacteria are inactive if soils are aerobic. Soils showing pronounced blackening or clays showing 'gleying' are normally anaerobic and often heavily infected. They present a threat of corrosion and can also cause 'nuisance', as in a case of 'blackened and stinking soil' (see Postgate 1960), the smell from which was annoying local residents and causing darkening of paint and metalwork; it was traced to buried builders' refuse and organic debris supporting exuberant activity of sulphate-reducing bacteria.

On the credit side, certain soils infected by sulphate-reducing bacteria have been held to have medicinal properties. 'Ripening' of hot medicinal muds at Piestany-spa, Czechoslovakia, is associated with the activities of thermophilic sulphate-reducing bacteria (Starka 1949).

Rice paddy soils have a particular tendency to become anaerobic and, if sulphate reduction ensues, damage to the rice crop occurs; Postgate (1960) cited Russian, Hungarian and Japanese papers on this topic. Takai & Kamura (1966) mentioned a 33% increase in rice yields in post-war Japan, largely as a result of controlling the *Eh* of paddies. Yet their role in rice cultivation need not be wholly destructive: Jacq & Fortuner (1980) showed that biogenic sulphide could, out of season, be exploited to control nematodes and increase rice yields. Dommergues and his colleagues (Dommergues *et al.* 1969; Jacq & Dommergues 1971) have studied the sudden death of lucerne or maize seeds that can be caused by sulphate reduction in the rhizosphere.

Thiobacilli, though normal inhabitants of soils, do not ordinarily influence its character in any way that is of economic importance. Sulphur dressings augment the growth of thiobacilli and thus the acidity, and have been used to neutralize black alkaline soils and to increase the availability of soil phosphates. Sulphur dressings on vines, potato haulms and so on probably function because thiobacilli then generate and sustain a low pH at the plant surface, so inhibiting the growth of parasites (see Butlin & Postgate 1954*a*). I have found no evidence that phototrophic sulphur bacteria play any economically important role in the soil, although they may be among the anaerobic phototrophic diazotrophs that contribute to the nitrogen status of rice paddies (Habte & Alexander 1980).

### (*c*) *Effluent treatment*

Effluent liquors from the gas industry contain thiosulphates and thiocyanates, which are toxic to fish and sewage organisms. Thus they would constitute a disposal problem, but biological treatment in activated sludge plants or trickling filters is successful. The functional organism is often a thiobacillus and the so-called *Thiobacillus thiocyanoxidans* (see Happold 1957) was isolated from such an effluent. Many thiobacilli can, in fact, metabolize thiocyanate, and most such effluent systems contain thiobacilli. Hutchinson & White (1964), however, showed that non-specific heterotrophic bacteria can also conduct such oxidations; they isolated one such organism able to oxidize thiocyanate but not thiosulphate.

The sulphate waste liquor of the paper industry presents a disposal problem. The ability of sulphate-reducing bacteria to grow in such liquor can be exploited. Bannink & Muller (1952) passed waste liquors from the digestion of straw with sulphite through a column impregnated with sulphate-reducing bacteria and removed sufficient sulphite from the liquor for it to undergo an ordinary methane fermentation (which is ordinarily inhibited by sulphite (Noordam-Goedewagen *et al.* 1949)).

Freke & Tate (1961) discussed the use of sulphate-reducing bacteria for the removal of iron from ferruginous waters, and concluded that the process was feasible. In certain conditions magnetic iron sulphide was formed and the Fe could be removed with a magnet. The conditions for reproducible formation of the magnetic product were not, unfortunately, established. A rotating-disc biological contacter has been evaluated under conditions simulating the treatment of acid mine drainage in which continuous oxidation of $Fe^{2+}$ by thiobacilli was envisaged as preceding precipitation of the $Fe^{3+}$ species by neutralization (Olem & Unz 1980). It is not only the iron but also the acid in mine waters (see above) that presents a serious disposal problem. Tuttle and his colleagues (Tuttle *et al.* 1968, 1969*a*, *b*) described a system in which acid mine water flowed through a porous dam of wood-dust within which a consortium of cellulolytic and sulphate-reducing bacteria reduced sulphate and generated sufficient alkalinity to render the effluent acceptable, as well as removing iron and sulphate; in principle a biological approach is possible to the economic problem presented by acid mine waters.

Microbiological production of reduced sulphur is discussed later in §3*h*. Most of the proposed processes involve exploiting sulphate-reducing bacteria for sulphide formation and presuppose waste materials in aqueous solution or suspension as substrates for bacterial action. Thus they may be regarded equally as processes for the purification of such wastes. Sulphide-fermented sewage sludge has improved settling qualities, which makes it more amenable for disposal. The reason is that, with normal digested sewage sludge, settling or 'dewatering' is interfered with by residual gas production in the sludge, so that the final 'settled' product contains as much as 97% water. Sulphide-fermented sludge does not produce gas and the water content can rapidly be lowered in settling tanks to 92%. If the digested sludge has to be transported any distance for final disposal this improved settling saves moving great quantities of water with it: up to four times as much water may be removed after settlement from sulphide digestion (H.M.S.O. 1958).

McKinney & Conway (1957) discussed sulphate as one of three possible oxidants for anaerobic biological disposal processes, and Pipes (1960) investigated the practicability of a purification process with the use of first hair + starch + filter paper as an 'artificial' sludge, and later waste-activated sludge. Pipes obtained a satisfactory reduction in oxygen demand provided that sulphide was extracted successfully; he envisaged a cyclic process whereby $H_2S$ was extracted, burned and used to regenerate sulphate for the fermentation.

The economic value of sulphate digestion for the purification of wastes thus depends on three factors:

(1) whether the advantage in settling properties outweigh the loss of methane, which is a useful source of power in conventional sewage practice;

(2) whether its applicability to strong or otherwise unacceptable wastes (e.g. distillery slop,

sulphite-waste liquor) is worth the capital investment in remodelling plant and arranging for extraction of $H_2S$;

(3) whether a sufficient market exists for the $H_2S$ obtained as a by-product to render the process practicable.

### (*d*) *Mineral technology*

#### (i) *Formation of mineral deposits*

Soda and other carbonate deposits can arise from the activities of sulphate-reducing bacteria. In principle, this fact arises from the instability of $Na_2S$ or CaS, the most usual reduction products, in the presence of $CO_2$: the end-products are actually $Na_2CO_3$ or $CaCO_3$. Abd-el-Malek & Rizk (1963) described soda formation in these terms for the Wadi el Natrûn in Egypt, and it is reasonable to suppose that the calcite deposits that often surround sulphur springs arise in a similar manner.

The biogenic formation of natural sulphur deposits is a topic that has been periodically discussed over the last few decades, particularly during the 1950s, when a serious world shortage of native sulphur arose. Guides to the literature on this subject may be obtained from Postgate (1979); Trudinger (1976) and Ivanov (1964), and the topic, although it is of great economic importance, will not be discussed in any detail here, for it has been reviewed adequately in those publications. Essentially the position is that 95% of the world's sulphur resources are of biogenic origin, according to their geological location and sulphur isotope ratio, and they represent a fossil reserve that is now being depleted. They are probably the end-products of massive sulfureta that existed in an earlier, less aerobic era of this planet's history. Localities in which biogenic sulphur formation is still taking place still exist and have been studied (e.g. some sulphur-producing lakes in Libya). Sulphate-reducing bacteria are responsible for the reduction of sulphate to sulphide and both photosynthetic sulphide oxidizers and thiobacilli contribute to the oxidation of this to free sulphur. As an economic process this is very important but a historical one.

Sulphide-forming bacteria have been believed to have been involved in the genesis of metal sulphide ores (e.g. those of lead and zinc) since the early decades of this century (see, for example, Siebenthal (1915), and Bastin (1926), reviewed by Bunker (1936)). The topic was discussed by Silverman & Ehrlich (1964) and more recently by Trudinger (1976). Their responsibility for the deposition of copper sulphide ores was mentioned by ZoBell (1946), and Miller (1950) pointed out that sulphate-reducing bacteria might be responsible for the genesis of many types of sulphide ore. Miller grew these bacteria in the presence of carbonates or oxides of Pb, Zn, Sb, Bi, Cd, Co and Ni. Cu as the basic carbonate showed some toxicity. Booth & Mercer (1963) agreed, and concluded that sulphate-reducing bacteria play no significant part in the genesis of copper sulphide ores, but their experiments did not exclude the facts that (*a*) a well established sulfuretum could precipitate a lot of copper without the copper ion reaching an inhibitory concentration (Sukow & Schwartz 1963), and (*b*) telluric waters bearing inhibitory $Cu^{2+}$ concentrations are very rare; a continuous supply of $Cu^{2+}$ at a much lower concentration could yield copper sulphide deposits. Sukow & Schwartz (1963) believed that sulphate-reducing bacteria contributed to the formation of the copper sulphide shales at Zechstein in Germany, and set up sulfureta to examine the effects of added copper or iron salts to them. In both cases the metals became precipitated as sulphides, though the balance of the sulfuretum was temporarily disturbed. Recovery as sulphides of metals from laboratory bacterial leaching solutions by the activity of sulphate-reducing bacteria in continuous culture has given a precipitate enriched 6.9-fold, 38-fold and 1-fold for Cu, Zn and Fe respectively compared with the metal concentrations in the unleached ore (Tomizuka & Yagisawa 1978). Ilyaletdinov *et al.* (1977) reported that chopped reeds enhanced CuS precipitation. Iron sulphide and pyrite deposits are often, though not always, biogenic (see Postgate 1960; Trudinger 1976).

Sulphur isotope distribution experiments suggest that sulphide deposits of Cu, Fe, Pb, Zn, Ag and U may often be of biogenic origin, but Trudinger (1976) advised caution over accepting all such data as evidence for biogenesis; he concludes (Trudinger, this symposium) that the biological contribution to metal sulphide ore genesis is, at best, indirect, except for iron.

Postgate (1960) mentioned the possible role of sulphate-reducing bacteria in the genesis of pyritic fossils, in which the physical form of the original structure is retained as a pyritic replica: presumably the decaying organism provides a 'sink' of $H_2S$ via sulphate reduction, at which FeS accrues and $FeS_2$ is ultimately formed.

### (ii) *Leaching of metal ores*

*Thiobacillus ferro-oxidans* oxidizes pyrites to form a solution containing ferric sulphate and free sulphuric acid. As this becomes diluted (e.g. at the outlet of a mine-draining plant), hydrated ferric oxides are precipitated causing the 'yellow boy' pollution cited earlier. The precipitated oxides form ochre, and exploitable amounts of ochre may be obtained from the leaching of pyrites deposits. Copper and zinc are leached at the same time, and useful amounts of copper have also been obtained by treating the run-off waters of pyrites mounds with scrap iron. Accelerated leaching of pyritic ores by *T. ferro-oxidans* has been demonstrated by many workers, for example by Smirnov (1963) and Duncan *et al.* (1964). Smirnov claimed that they accelerated pyrites oxidation 11-fold to 13-fold in the Kizel coal basin.

Tuovinen & Kelly (1972) reviewed the involvement of *T. ferro-oxidans*; surface-active agents, free sulphur and other thiobacilli, if present, may augment the extent of leaching. Kelly *et al.* (1979) discussed the exploitation of microbial ore treatment in some detail; other thiobacilli and non-sulphur bacteria can be involved and thermophilic thiobacilli capable of accelerated leaching have been obtained. They discussed the mechanism of leaching of pyrites and some copper sulphides with which process direct microbial attack of the ore is likely, but with other sulphide minerals the $Fe^{3+}$ ions formed by $Fe^{2+}$ oxidation may be having a chemical solubilizing effect. They reviewed types of leaching system that can be used in practice and discussed the inhibitory action of metal ions: thiobacilli are relatively insensitive to toxicity by $Cu^{2+}$ or $Zn^{2+}$ ions but sensitive to uranyl ions; in most cases resistant variants can be obtained. Their review will not be rehearsed here, but it is pertinent to mention the following points. Among the minerals leached are sulphides of Cu, Bi, As, Mo, Sb, Ni (see Summers & Silver 1978; Kelly *et al.* 1979) and Sn (Teh *et al.* 1981). Tributsch & Bennett (1981) presented evidence that the initial attack of Cd or Zn sulphides is non-biological, due to acidity, as noted previously by Kelly, Norris & Brierley (1979). Uranite ($UO_2$) is normally associated with pyrite, and the ferric sulphate generated microbiologically converts it to uranyl sulphate, which is soluble, and regenerates ferrous sulphate. The latter can be reoxidized microbiologically, so a cyclic microbiological process for leaching uranium from pyrite is available. This process has excited considerable interest in the last two decades and forms of it are in use on a production scale in North America and in the Bacfox process in South Africa (Livesey-Goldblatt 1977). Leaching of uranium and copper are clear practical examples of the economic use of sulphur bacteria and their exploitation for the recovery of other metals, particularly from low-grade ores, seems promising.

### (*e*) *Corrosion and deterioration*

The anaerobic corrosion of buried ferrous metals, enhanced by the activities of sulphate-reducing bacteria, is one of the best known of the economic activities of this group, though it is worth adding that, in my experience, many working engineers are still unaware of the biological nature of the process. The topic has been discussed by many authorities including Miller & Tiller (1971), Iverson (1972, 1974), Miller & King (1975) and Postgate (1979), and surveyed with recent examples by Crombie *et al.* (1980) so the ground will not be covered yet again. It must be sufficient to remind readers that both the consumption of cathodic hydrogen by *Desulfovibrio* and the generation of sulphide contribute to the process, which tends to occur in anaerobic or intermittently aerobic environments and can be very rapid; I must add that my statement that the mechanism 'is now largely understood and agreed upon' (Postgate 1979) has been challenged by Professor R. L. Starkey (vigorous personal communication). Despite the obvious economic importance of anaerobic corrosion, useful estimates of its economic cost are difficult to make, although it is clearly very high: replacement of small gas or water service mains can be £20 $m^{-1}$ rising to £300–400 $m^{-1}$ for larger mains (Wakerley 1979). The estimate of £20M as the total cost of such corrosion to the U.K. economy is over 25 years old (Vernon 1957) but appears to be the most recent parochial estimate available. Anaerobic corrosion of non-ferrous metals, involving the sulphide generated by sulphate-reducing bacteria, has also been reported.

Less well known is the phenomenon of corrosion of metals, stone and concrete by thiobacilli. Essentially, the process requires a source of sulphur or sulphide, originating by atmospheric deposition, from pyrites or from a sulphide such as $H_2S$ from a nearby source (biological or chemical). The S source supports the autotrophic growth of thiobacilli with concomitant formation of sulphuric acid and it is this acid that is the corrosive agent, sometimes augmented, if the substrate is pyrites, by iron salts. Stone statues, concrete structures such as sewerage pipes and metal machinery are susceptible. Examples were quoted by Butlin & Postgate (1954*a*) and the process was discussed by Krumbein & Pochon (1964) and Purkiss (1971); Iverson (1972) quoted an example of rapid biogenic acid corrosion of copper piping in Maryland soil. The acid mine waters mentioned earlier (generated by *T. ferro-oxidans*) present an instance of this kind when they corrode pumping machinery. Although buildings, stone and concrete structures are undoubtedly susceptible to such biogenic corrosion, one must beware of attributing all such damage to bacteria: atmospheric sulphur oxides can be highly corrosive on their own (see Schaffer 1966).

Deterioration of materials containing native sulphur may occur through the action of thiobacilli. Thaysen *et al.* (1945) attributed damage to fire hoses during World War II to thiobacilli: the sulphur remaining in the rubber from the vulcanizing process provided a substrate for growth and acid production by thiobacilli. The remedy was to dry out the hoses efficiently. Rubber gaskets over stored foods and industrial materials can become infected and so deteriorate; jointing mixtures for sealing pipes together may contain sulphur which thiobacilli attack, causing leakage and corrosion of the pipe metal (Frederick & Starkey 1948). On a more positive theme, Kino *et al.* (1981) described a process using thiobacilli for the biochemical removal of rust.

In the 1950s a form of disintegration attacked pyritized fossils in Britain's Natural History Museum. The 'disease' appeared to spread from specimen case to specimen case and was associated with destructive efflorescence of the fossil, intense local acidity and ferric sulphate formation. A microbiological origin seemed probable, because organisms resembling *T. ferro-oxidans* were obtained by my colleagues from such fossils. This view was accepted by Booth & Sefton (1970), who recommended vapour-phase inhibitors such as chlorocresols as preservatives on the basis of tests with cultures. However, Howie (1979) reported that several decomposing fossils did not carry *T. ferro-oxidans* and pointed out that the local environment on such a fossil would be, for various reasons, inimical to such microbes. The weight of evidence is now against the involvement of sulphur bacteria. The trouble is best cured by good air-conditioning.

### (*f*) *Involvement in oil technology*

Sulphate-reducing bacteria are normal inhabitants of the watery phase of oil-bearing strata and shales (see, for example, Dockins *et al.* 1980) and they are also normally present in the injection waters used in the secondary recovery of oil. They usually inhabit the drilling muds used in exploration for oil. They cause corrosion of machinery and they pollute the product by introducing reduced sulphur into the oil and its associated gas, so their economic effects are of serious concern to the oil industry; the voluminous literature that now exists only partly reflects the substantial research effort that the industry has put into their control. They may also be involved in the biogenesis of oil; a role in the coalescence of oil deposits seems probable and they have been held to attack and modify oil hydrocarbons – a matter that is disputed. Postgate (1979) provided a brief guide to this topic; more extensive texts are the books of Beerstecher (1954) and Davis (1967). A survey of the subject would not be appropriate here. I have seen no quantitative estimates of their economic cost to the oil industry but, in terms simply of corrosion of machinery, plugging of injection systems and contamination of drilling systems and products, the cost must be substantial.

Three related technological areas should be mentioned in which sulphate-reducing bacteria are important.

1. Contamination of stored petroleum or kerosene with sulphide or sulphur may result from the growth of these organisms in the bottom waters of storage tanks and lead to economic loss; in addition, the associated iron sulphide may be pyrophoric and cause explosion (see Postgate 1960). The pollution problem was at one time particularly serious as far as aircraft fuels were concerned, but the situation changed in the 1970s with the change to turbine fuels; microbes still present problems, however (see Park 1975). Borate has been recommended as a protectant (see Crombie *et al.* 1980).
2. Cutting emulsions, used in the tool industry, are oil–water emulsions that cool and lubricate cutting tools. They are prone to contamination by many types of microbe (see Hill 1975) including sulphate reducers (see Postgate 1960).
3. Proposals have been made to exploit the beneficial aspects of sulphate-reducing bacteria in oil release by injecting organic matter into spent oil formations, thus stimulating their activities. Transient success has been claimed; some references were given by Postgate (1979), and the involvement of bacteria in oil recovery has recently been reviewed (Moses & Springham 1982).

The sulphate-reducing bacteria have a profound and varied impact on the oil industry so, as if in compensation, the other sulphur bacteria have little, although, in experiments, enhanced recovery of oil from shales has been noted after dissolution of the shale carbonate through acid production by thiobacilli (Yen 1976). Bunker (1936) and earlier workers suggested that thiobacilli might be exploited for the desulphurization of lower-grade oil (which often contains organic sulphur), but his experiments (and later experiments by his colleagues; personal communication from the late K. R. Butlin) provided no evidence for desulphurization. The autotrophic habit of most thiobacilli does not make them promising candidates for such a function, but some facultative thiobacilli appear to be capable of attacking aromatic substrates (Taylor *et al.* 1969).

### (*g*) *Animal and human nutrition*

Sulphate-reducing bacteria are normal inhabitants, albeit in low numbers, of the rumens of ruminant mammals, although their characterization is not always clear (see, for example, Huisingh *et al.* 1974; Howard & Hungate 1976). They may contribute to the S nutrition of such animals (see Postgate (1960) for references) and of insects (Haines *et al.* 1960). *Desulfotomaculum ruminis* is a rumen inhabitant and a diazotroph (Postgate 1970) but its contribution to the N status of sheep is probably trivial. Sulphur bacteria in general, including the photosynthetic sulphide-oxidizing bacteria, are parasitized by protozoa. Rodina (1963) observed *Thiopedia*, *Beggiatoa* and *Thiothrix* in a lake deposit serving as food for protozoa, which in turn supported fish; the zoogloeal mass in the north African sulphur lakes described by Butlin & Postgate (1954*b*) was clearly being preyed upon by large protozoa that may have supported sulphide-tolerant cyprinodont fish (Smith 1952). They can form the basis of an anaerobic food chain that may support the development of quite complex ecosystems including metazoa (Fenchel & Riedl 1970); a recent example is the ecosystem around the hydrothermal vents of the Galapagos Rift in the Pacific Ocean (63.5–66.0 m deep (see Orcott 1981)), where autotrophic *Thiomicrospira* and related sulphur-oxidizing bacteria apparently provide the primary biomass to support a rich population of invertebrates (see Jannasch & Wirsen 1979; Ruby *et al.* 1981); in this case the substrate, $H_2S$, is of geochemical, not biological origin. The temperature of the hydrothermal water can rise to about 12 °C compared with the ambient 2.1 °C and there is reason to believe that a tube-worm inhabiting the hydrothermal zone lives in symbiotic association with chemotrophic sulphur-oxidizing bacteria (Cavanaugh *et al.* 1981; Southward *et al.* 1981; Hessler 1981). Jannasch (1979) and Jannasch & Wirsen (1979) suggested that comparable eco-systems might be used economically to produce biomass (e.g. edible clams) from waste hydrogen sulphide.

As I mentioned in the introduction, the sulphur bacteria have been held to be of great importance in the early history of life on this planet because, at an early stage, the sulfuretum may well have been the primary source of biomass for living things (see Postgate (1979) and Pfennig & Widdel and Trudinger (this symposium) for references to this discussion).

Sulphate-reducing bacteria are involved in food spoilage. Canned foods are normally heat-sterilized but certain spore-forming organisms have a sufficient heat resistance to cause spoilage. ‘Sulphur stink’ or ‘sulphide spoilage’ (e.g. of canned corn or canned peas), characterized by blackening or greying of the food and a smell of $H_2S$, is due to thermophilic sulphate-reducing bacteria (once known as *Clostridium nigrificans*, now *Desulfotomaculum nigrificans* (see Campbell *et al.* 1956; Campbell & Postgate 1965)). *D. nigrificans* can contaminate infant food preparations (Donelly & Busta 1980) and canned mushrooms (Lin & Lin 1970); its heat tolerance can also cause trouble in the handling of molasses and of sugar during refining, because both of these materials are handled hot. Non-sporulating sulphate-reducing bacteria may also be involved in food spoilage: Levin *et al.* (1959) observed a *Desulfovibrio* species in spoiled olive brines in which the olives had darkened and developed a smell of $H_2S$. The late Professor E. McCoy of the University of Wisconsin described to me an incident in which an American pharmaceutical firm was troubled by blackening and 'putrefaction' of aluminium hydroxide sols stored in drug stores for treatment of digestive disorders. It was traced to *Desulfovibrio* and 'cured' by adding $H_2O_2$ to the preparation.

### (*h*) *Biotechnology*

Biotechnology means many things to many scientists. The manner in which it differs from applied microbiology or economic microbiology is not always clear, so I choose to restrict its meaning to economic processes that, potentially or actually, involve the use of large-scale culture of microbes, be they natural or genetically manipulated. The leaching of mineral ores by *T. ferrooxidans* is, of course, a process of this kind and has been discussed already (§ 3*d* (ii)).

A second example of the biotechnological exploitation of sulphur bacteria is the microbiological production of sulphur. It arose historically from the world sulphur shortage of the 1950s coupled with recognition of the biogenic origin of natural sulphur deposits. It soon became evident that hydrogen sulphide could be as useful to industry as elemental sulphur, but that the determining factor, as far as the economics of the process was concerned, was the cost of the reducing agent. To reduce sulphate, a reasonably cheap raw material, to sulphide in quantities sufficient to contribute significantly to the sulphur requirements of an industrialized country, i.e. to produce in excess of $10^5$ t S per year, required immense amounts of cheap reductant. Sewage was in fact the only feasible substrate. Thus the process had to compete with the economically useful production of methane, which is a by-product of good sewage technology. Despite improved settling qualities of sulphide-fermented sewage sludge mentioned earlier (§ 3*c*), the process did not become competitive (see discussion and refernces in Postgate (1979)). Nevertheless, it continues to attract attention where the marginal cost of effluent treatment is high and sulphur is expensive or where $H_2S$-bearing water is 'free', as in Sokolova's (1961) use of halophilic thiobacilli to precipitate elemental sulphur from oil seam-water. In well insolated areas, where demand is small but local resources are scarce, plans have been made to mimic the North African sulphur lakes described by Butlin & Postgate (1954*b*) by supplementing brackish lakes with organic matter (e.g. farm wastes, sewage). I am not aware of any economically useful exploitation of such a process but, as the economies of many countries evolve towards ones based on small-scale, low-energy technologies, the microbial production of sulphur at the expense of solar energy might well achieve marginal economic viability. Quantitative conversion of sulphate to elemental sulphur has been obtained with sequential cultures of *Desulfovibrio desulfuricans* and *Chromatium vinosum*, but the suggested application to industrial effluents still requires an economically acceptable source of carbon to drive the sulphate reduction (Cork & Cusanovich 1978).

A third biotechnological use of sulphur bacteria arises from the ability of *Thiobacillus ferrooxidans* to solubilize pyrites. Low-grade coal contains pyrites, which spoils its coking properties and causes damage to flues, etc., as the S becomes oxidized during combustion; the S oxides are also an atmospheric pollutant. Microbial 'desulphurization' of coal has been discussed since the 1950s at least (see, for example, Silverman *et al.* 1951), and the process has been used successfully in the laboratory (see Silverman *et al.* 1963). Mixed populations appear to be more effective than pure cultures (Dugan & Apel 1978) but the process is slow and I am not aware of its actual use on an industrial scale; Hoffman *et al.* (1981) described condition for the maximum rate of removal of pyrites. Some trials of desulphurization with the use of *T. ferrooxidans* and thermophilic *Sulfolobus* have indicated that the microbial process could be economically competitive with chemical processes (Detz & Barvinchak 1979).

A fourth area of biotechnological application is in the biological production of $H_2$ as an energy source. Photolysis of water to $H_2$ $(+O_2)$ on a mass-production scale, making use of a chloroplast (or chloroplast-like) system coupled to ferredoxin and hydrogenase, is attractive as a method of storing solar energy. It so happens that *D. vulgaris* possesses one of the most active hydrogenases known (Sadana & Morey 1961; van der Westen *et al.* 1978), and this could well be the enzyme of choice for any viable biotechnological process because it can be extracted in an $O_2$-tolerant form (van der Westen *et al.* 1980). Not all desulfovibrios have such active hydrogenases.

A fifth area in which sulphur bacteria might be biotechnologically exploitable is in biochemical fuel cells. Organisms that generate a redox or pH gradient are particularly suitable for such use; it will be obvious that the sulphate-reducing bacteria lower the redox potential and the thiobacilli lower the pH. The use of sulphate-reducing bacteria in redox biochemical fuel cells has been discussed by Sisler (1961), Sisler & Senftle (1963) and Lewis (1966); Fischer *et al.* (1965*a*, *b*) mentioned biochemical cells based on thiobacilli; Sisler *et al.* (1977) combined both types in a process for the electrobiochemical neutralization of acid mine water.

### (*i*) *Involvement in the paper industry*

Paper manufacture suffers from the depredations of sulphate-reducing bacteria (see reviews by Russell (1961) and Starkey (1961)). Blackening of paper pulp can occur as a result of their activities; Starkey (1961) enlarged on the corrosion problem that they could cause and also the disposal problem that could arise from their presence (see also §3*c*). He quoted the example that the disposal of paper pulp wastes into the Androscoggin River led to sulphide pollution unless about 4½ tonnes of nitrate was added per day at the same time to control the pollution. The disposal problem has been studied by Lawrence and his colleagues (Lawrence 1948, 1950; Lawrence & Fukui 1956; Lawrence & Sakamoto 1959).

Russell (1961) described a situation in which logs for paper pulpwood, stored in seawater (a common practice), became saturated with sea salts and infected with '*D. aestuarii*'. The sulphide formed by these bacteria reacted with the lignin of the wood to form thiolignin. When, in due course, the wood was used for pulp, the thiolignin reacted with the mercurials usually added to inhibit wood-rotting fungi, and thus inactivated them. Thus the overt symptom of attack by *Desulfovibrio* was unexpected rotting of pulp by fungi, and Russell described the microbiological detective work required to trace this symptom to its origin.

### (*j*) *Involvement in the gas industry*

Town gas has been stored over water since the last century. Growth of sulphate-reducing bacteria in gas holder water, with consequent contamination of the gas with $H_2S$, as well as corrosion of the gas holder, has been recognized as a problem for several decades (see Postgate 1960). Transfer to natural gas has entrained a different version of the same problem in the underground storage of gas supplies. Pankhurst (1968*a*, *b*) provided an account of this problem and some of the remedies available.

### (*k*) *Miscellaneous economic activities*

Le Gall & Postgate (1973) mentioned blackening of leather through the action of sulphate-reducing bacteria during tanning; they also cited reference to their isolation from human dental caries and Postgate (1979) described the one recorded case of (seemingly opportunist) *Desulfovibrio* septicaemia in man. Postgate (1960) mentioned the role of sulphate-reducing bacteria in sulphurous spa and spring waters, whose medicinal effect (like the sulphurous muds of Piestany Spa; see §3*b*) is of undoubted economic value. Perhaps the brilliant and variegated displays of sulphur bacteria that bring tourists to geothermal areas such as Yellowstone Park should be included among the useful economic activities of this group?

## 4. CONCLUSION

The nitrogen-fixing bacteria exert their major economic effect in a clearly defined sector of man's economy – which in this instance is agriculture – and their value to the community is very obvious. In principle, it could be expressed in cash. In the methanogenic bacteria, their major economic effects are in sewage treatment and ruminant digestion; it is obvious that sewage treatment is of transcendent social importance, yet it is impossible to assign a cash value to the economic activities of the methanogens because they are essentially non-productive in this context. This is a problem that has dogged economic microbiologists for decades, and which will trouble their successors, the biotechnologists: it is in principle possible, and sometimes fairly easy, to assess the economic value or cost of a microbe that produces or destroys a saleable product; it is rarely possible to assess in financial terms the economic value or cost of microbes involved in recovery or purification processes, deterioration, destruction or recycling. That is why I have included only one such estimate (§3*e*).

The sulphur bacteria present a second problem. It should now be obvious that their economic effects are very diverse, ramifying through many aspects of twentieth-century human society, and that they are sometimes beneficial, sometimes destructive. This diversity of impact and ambivalence of economic consequence has had an unfortunate effect on research on these microbes: studies have been, on the whole, made piecemeal, by small research groups making relatively narrow (if creditable) inroads into specialized aspects of these bacteria. Pleas have been made (e.g. Report of the N.E.R.C. Working Party on Terrestrial Microbiology 1976) for integrated multidisciplinary research into the bacteria of the sulphur cycle, with emphasis on their economic and environmental aspects, but the difficulty of identifying single, clear-cut economic impacts seems regrettably to have contributed to their scientific neglect. Yet sewage disposal, corrosion and such unattractive matters are as important to mankind as silicon chips, medicine and agriculture.

I thank Dr E. M. P. Howie, Dr N. Leroux, Professor H. W. Jannasch, Professor D. P. Kelly, Dr P. Norris and Dr P. A. Trudinger for information that has greatly helped the preparation of this text.

## REFERENCES

Abd-el-Malek, Y. & Rizk, S. G. 1963 Bacterial sulphate reduction and the development of alkalinity. III. Experiments under natural conditions in the Wadi Natrun. *J. appl. Bact.* **26**, 20–26.

Bannink, H. F. & Muller, F. M. 1952 Utilization of waste liquors from the digestion of straw with monosulfite. *Antonie van Leeuwenhoek* **18**, 45–54.

Bastin, E. S. 1926 A hypothesis of bacterial influence in the genesis of certain sulfide ores. *J. Geol.* **34**, 773–792.

Beerstecher, E. 1954 *Petroleum microbiology*. Amsterdam: Elsevier.

Bicknell, A. K. 1949 The occurrence of a green sulphur bacterium in Sodon Lake. *Lloydia* **12**, 183–184.

Booth, G. H. & Mercer, S. J. 1963 Resistance to copper of some oxidizing and reducing bacteria. *Nature, Lond.* **199**, 622.

Booth, G. H. & Sefton, G. V. 1970 Vapour phase inhibition of thiobacilli and ferrobacilli: a potential preservative for pyritic mesuem specimens. *Nature, Lond.* **226**, 185–186.

Braley, S. A. 1956 Acid coal-mine drainage. *Min. Engr* **8**, 314–318.

Bunker, H. J. 1936 *A review of the physiology and biochemistry of the sulphur bacteria*. London: H.M.S.O.

Butlin, K. R. & Postgate, J. R. 1954*a* The economic importance of autotrophic micro-organisms. In *Autotrophic micro-organisms* (*Soc. gen. Microbiol. Symp.* no. 4) (ed. B. A. Fry & J. L. Peel), pp. 271–305.

Butlin, K. R. & Postgate, J. R. 1954*b* The microbiological formation of sulphur in Cyrenaican lakes. In *Biology of deserts* (ed. J. Cloudsley-Thompson), pp. 112–122. London: Institute of Biology.

Campbell, L. L., Frank, H. A. & Hall, E. R. 1956 Studies on thermophilic sulphate reducing bacteria. I. Identification of *Sporovibrio desulfuricans* as *Clostridium nigrificans*. *J. Bact.* **73**, 516–521.

Campbell, L. L. & Postgate, J. R. 1965 Classification of the spore-forming sulfate-reducing bacteria. *Bact. Rev.* **29**, 359–363.

Cavanaugh, C. M., Gardiner, S. L., Jones, M. L., Jannasch, H. W. & Waterbury, J. B. 1981 Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. *Science, Wash.* **213**, 340–342.

Cork, D. J. & Cusanovich, M. A. 1978 Sulfate decomposition: a microbiological process. In *Metallurgical applications of bacterial leaching and related microbiological phenomena* (ed. L. E. Murr, A. E. Torma & J. A. Brierley), pp. 207–221. New York: Academic Press.

Crombie, D. J., Moodie, G. J. & Thomas, J. D. R. 1980 Corrosion of iron by sulphate-reducing bacteria. *Chemy Ind., Lond.*, pp. 500–504.

Davis, J. B. 1967 *Petroleum microbiology*. Amsterdam: Elsevier.

Detz, C. M. & Barvinchak, G. 1979 Microbial desulfurization of coal. *Min. Congr. J.* **65**, 75–82, 86.

Dockins, W. S., Olson, G. J., McFeters, G. A. & Turbak, S. C. 1980 Dissimilatory bacterial sulfate reduction in Montana groundwaters. *Geomicrobiol. J.* **2**, 83–98.

Dommergues, Y., Combremont, R., Beck, G. & Ollat, C. 1969 Note préliminaire concernant la sulfato-réduction rhizospherique dans un sol salin tunisien. *Revue ecol. Biol. Sol* **6**, 115–129.

Donelly, L. S. & Busta, F. F. 1980 Heat resistance of *Desulfotomaculum nigrificans* spores in soyprotein infant formula preparations. *Appl. envir. Microbiol.* **40**, 721–725.

Dugan, P. R. & Apel, W. A. 1978 Microbial desulfurization of coal. In *Metallurgical applications of bacterial leaching and related microbiological phenomena* (ed. L. E. Murr, A. E. Torma & J. A. Brierly), pp. 223–250. New York: Academic Press.

Duncan, D. W., Trussell, P. C. & Walden, C. C. 1964 Leaching of chalcopyrite with *Thiobacillus ferrooxidans*: effect of surfactants and shaking. *Appl. Microbiol.* **12**, 122–126.

Ellis, D. 1932 Dilution of sewage in the sea. *Jl R. tech. Coll. Glasg.* **2**, 698–707.

Fenchel, T. M. & Riedl, R. J. 1970 The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *Mar. Biol.* **7**, 255–268.

Fischer, D. J., Herner, A. E., Landes, A., Batlin, A. & Barger, J. W. 1965*a* Electrochemical observations in microbiological processes. Growth of *Thiobacillus thiooxidans*. I. *Biotech. Bioengng* **7**, 471–490.

Fischer, D. J., Landes, A., Sandford, M. T., Herner, A. E. & Wiegand, C. J. W. 1965*b* Electrochemical observations in microbiological processes. Growth of *Thiobacillus thiooxidans*. II. *Biotech. Bioengng* **7**, 491–506.

Frederick, L. R. & Starkey, R. L. 1948 Bacterial oxidation of sulfur in pipe-sealing mixtures. *J. Am. Wat. Wks Ass.* **40**, 729–736.

Freke, A. M. & Tate, D. 1961 Formation of magnetic iron sulphide by bacterial reduction of iron solutions. *J. biochem. microbiol. Technol. Engng* **3**, 29–39.

Genovese, S. 1963 The distribution of the $H_2S$ in the Lake of Faro (Messina) with particular regard to the presence of 'red water'. In *Marine microbiology* (ed. C. H. Oppenheimer), pp. 194–204. Springfield, Ill.: Thomas.

Gunkel, W. & Oppenheimer, C. H. 1963 Experiments regarding the sulfide formation in sediments of the Texas Gulf coast. In *Marine microbiology* (ed. C. H. Oppenheimer), pp. 674–684. Springfield, Ill.: Thomas.

Habte, M. & Alexander, M. 1980 Nitrogen fixation by photosynthetic bacteria in lowland rice culture. *Appl. envir. Microbiol.* **39**, 342–347.

Haines, T. H., Henry, J. M. & Block, R. J. 1960 The sulfur metabolism of insects. V. The ability of insects to use sulfate in the synthesis of methionine. *Contr. Boyce Thompson Inst. Pl. Res.* **20**, 363–364.

Happold, F. C. 1957 Biological treatment of effluents. *Research, Lond.* **10**, 443–448.

Hesse, P. R. 1956 Sulphate deficiency in Lake Victoria. *Nature, Lond.* **177**, 389–390.

Hessler, R. 1981 Oasis under the sea – where sulphur is the staff of life. *New Scient.* **92**, 741–747.

Hill, E. C. 1975 Biodeterioration of petroleum products. In *Microbial aspects of the deterioration of materials* (*Soc. appl. Biol. tech. Ser.* no. 9) (ed. D. W. Lovelock & R. J. Gilbert), pp. 127–136. London: Academic Press.

Hoffman, M. R., Faust, B. C., Panda, F. A., Koo, H. H. & Tsuchiya, H. M. 1981 Kinetics of the removal of iron pyrite from coal by microbial catalysis. *Appl. envir. Microbiol.* **42**, 259–271.

H.M.S.O. 1958 *Chemistry research* 1957 (*Annual report of the Chemical Research Board*), pp. 60–67.

Howard, B. H. & Hungate, R. E. 1976 *Desulfovibrio* of the sheep rumen. *Appl. envir. Microbiol.* **32**, 598–602.

Howie, F. M. P. 1979 Museum climatology and the conservation of paleontological material. *Spec. Pap. Paleont.* no. 22, pp. 103–125.

Huisingh, J., McNiell, J. J. & Matrone, G. 1974 Sulfate reduction by a Desulfovibrio species isolated from sheep rumen. *Appl. Microbiol.* **28**, 489–497.

Hutchinson, M. & White, D. 1964 The types and distribution of thiobacilli in biological systems treating carbonization effluents. *J. appl. Bact.* **27**, 244–251.

Ilyaletdinov, A. N., Enker, P. B. & Loginova, L. V. 1977 Role of sulfate-reducing bacteria in the precipitation of copper. *Mikrobiologiya* **46**, 113–117 (transl. 92–95).

Ivanov, M. V. 1964 *Microbiological processes in the formation of sulfur deposits.* [Translation.] Washington, D.C.: U.S. Department of Agriculture.

Iverson, W. P. 1972 Biological corrosion. *Adv. Corros. Sci. Technol.* **2**, 1–42.

Iverson, W. P. 1974 Microbial corrosion of iron. In *Microbial iron metabolism* (ed. J. Nielands), pp. 475–513. New York: Academic Press.

Jacq, V. A. & Dommergues, Y. 1971 Sulfato-reduction spermospherique. *Annls Inst. Pasteur, Paris* **121**, 199–208.

Jacq, V. A. & Fortuner, R. 1980 Biological control of rice nematodes using sulphate-reducing bacteria: laboratory and field trials. In *Abstr. 2nd Int. Symp. Microbial Ecology, University of Warwick*, p. 203.

Jannasch, H. W. 1957 Die bakterielle Rotfärbung der Salzseen des Wadi Natrun (Ägypten). *Arch. Hydrobiol.* **53**, 425–433.

Jannasch, H. W. 1979 Chemosynthetic production of biomass: an idea from a recent oceanographic discovery. *Oceanus* **22**, 59–63.

Jannasch, H. W. & Wirsen, C. O. 1979 Chemosynthetic primary production at East Pacific sea floor spreading centers. *BioScience* **29**, 592–598.

Kelly, D. P., Norris, P. R. & Brierley, C. L. 1979 Microbiological methods for the extraction and recovery of metals. In *Microbial technology: current state, future prospects* (*Soc. gen. Microbiol. Symp.* no. 29) (ed. A. T. Bull, D. C. Ellwood & C. Ratledge), pp. 263–308. Cambridge University Press.

Kino, K., Sugimoto, N., Kuroda, K. & Usami, S. 1981 Biochemical rust removal by *Thiobacillus ferrooxidans. Eur. J. appl. Microbiol. Biotechnol.* **13**, 128–132.

Kleinmann, R. L. P. & Crerar, D. A. 1979 *Thiobacillus ferrooxidans* and the formation of acidity in simulated coal mine environments. *Geomicrobiol. J.* **1**, 373–388.

Krumbein, W. E. & Pochon, J. 1964 Ecologie bactérienne des pierres altérées des monuments. *Annls Inst. Pasteur, Paris* **107**, 724–732.

Kuenen, J. G. & Veldkamp, H. 1972 *Thiomicrospira pelophila* gen.n., sp.n., a new obligately chemolithotrophic colourless sulfur bacterium. *Antonie van Leeuwenhoek* **38**, 241–256.

Laube, V. M. & Martin, S. M. 1981 Conversion of cellulose to methane and carbon dioxide by triculture of *Acetovibrio cellulolyticus*, *Desulfovibrio* sp. and *Methanosarcina barkeri*. *Appl. envir. Microbiol.* **42**, 413–420.

Lawrance, W. A. 1948 Determination of the time of passage of pollution in the Androscoggin River and pond. *Sewage Wks J.* **20**, 881–896.

Lawrance, W. A. 1950 The addition of sodium nitrate to the Androscoggin River. *Sewage ind. Wastes* **22**, 820–832.

Lawrance, W. A. & Fukui, H. N. 1956 Microbial oxidation of pure carbohydrate in the presence of calcium lignosulfonate. *Sewage ind. Wastes* **28**, 1484–1494.

Lawrance, W. A. & Sakamoto, W. 1959 The microbial oxidation of cellobiose and butyric and lactic acids in the presence of calcium lignosulfonate. *Tappi* **42**, 93–96.

LeGall, J. & Postgate, J. R. 1973 The physiology of sulphate-reducing bacteria. *Adv. microb. Physiol.* **10**, 81–133.

Levin, R. E., Ng, H., Nagel, C. W. & Vaughn, R. H. 1959 Desulfovibrios associated with hydrogen sulfide formation in olive brines. *Bact. Proc.*, p. 7.

Lewis, K. 1966 Symposium on bioelectrochemistry in microorganisms. IV. Biochemical fuel cells. *Bact. Rec.* **30**, 101–113.

Lin, C. C. & Lin, K. C. 1970 Spoilage bacteria in canned foods. II. Sulfide spoilage bacteria in canned mushrooms and a versatile medium for the enumeration of *Clostridium nigrificans*. *Appl. Microbiol.* **19**, 283–286.

Liu, M. C., DerVertanian, D. V. & Peck, H. D. 1980 On the nature of the oxidation-reduction properties of nitrate reductase from *Desulfovibrio desulfuricans*. *Biochem. biophys. Res. Commun.* **96**, 278–285.

Livesey-Goldblatt, E., Tunley, T. H. & Nagy, I. F. 1977 Pilot-plant bacterial film oxidation (Bacfox process) of recycled acidified uranium plant ferrous sulphate leach solution. In *Conference – Bacterial Leaching 1977* (ed. W. Schwartz), pp. 175–190. Weinheim and New York: Verlag Chemie.

Lyalikova, N. N. 1957 Research on assimilation of free carbon dioxide by purple sulfur bacteria in Lake Belovod. *Mikrobiologiya* **26**, 92–98.

McInerney, M. J. & Bryant, M. P. 1981 Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of $H_2$ on acetate degradation. *Appl. envir. Microbiol.* **41**, 346–354.

McKinney, R. E. & Conway, R. A. 1957 Chemical oxygen in biological waste treatment. *Sewage ind. Wastes* **29**, 1097–1106.

Miller, J. D. A. & King, R. A. 1975 Biodeterioration of metals. In *Microbial aspects of the deterioration of materials* (*Soc. appl. Biol. tech. Ser.* no. 9) (ed. D. W. Lovelock & R. J. Gilbert), pp. 83–103. London: Academic Press.

Miller, J. D. A. & Tiller, A. K. 1971 Microbial corrosion of buried and immersed metal. In *Microbial aspects of metallurgy* (ed. J. D. A. Miller), pp. 61–105. Aylesbury: Medical and Technical Publication Co.

Miller, L. P. 1950 Formation of metal sulfides through the activities of sulfate-reducing bacteria. *Contr. Boyce Thompson Inst. Pl. Res.* **16**, 85–89.

Moses, V. & Springham, D. G. 1982 *Bacteria and the enhancement of oil recovery*. Barking, Essex: Applied Science Publishers.

Noordam-Goedewagen, M. A., Manten, A. & Muller, F. M. 1949 The influence of sulphide on the methane fermentation of sodium and calcium acetates. *Antonie van Leeuwenhoek* **15**, 65–85.

Olem, H. & Unz, R. F. 1980 Rotating-disc biological treatment of acid mine drainage. *J. Wat. Pollut. Control Fed.* **52**, 257–269.

Orcott, J. 1981 Geology of the deep-sea vents. *New Scient.* **92**, 743.

Pankhurst, E. S. 1968*a* Significance of sulphate-reducing bacteria to the gas industry: a review. *J. appl. Bact.* **31**, 179–193.

Pankhurst, E. S. 1968*b* Bacteriological aspects of the storage of gas underground. *J. appl. Bact.* **31**, 211–322.

Park, P. B. 1975 Biodeterioration in aircraft fuel systems. In *Microbial aspects of the deterioration of materials* (*Soc. appl. Biol. tech. Ser.* no. 9) (ed. D. W. Lovelock & R. J. Gilbert), pp. 105–126. London: Academic Press.

Pipes, W. O. 1960 Sludge digestion by sulphate-reducing bacteria. In *Proc. 15th Indust. Waste Conf., Purdue Univ. Engng, Extn. Ser.* no. 106, pp. 308–319.

Postgate, J. R. 1960 The economic activities of sulphate-reducing bacteria. *Prog. ind. Microbiol.* **2**, 48–69.

Postgate, J. R. 1965 Recent advances in the study of the sulfate-reducing bacteria. *Bact. Rev.* **29**, 425–441.

Postgate, J. R. 1970 Nitrogen fixation by sporulating sulphate-reducing bacteria including rumen strains. *J. gen. Microbiol.* **63**, 137–139.

Postgate, J. R. 1979 *The sulphate-reducing bacteria*. Cambridge University Press.

Purkiss, B. E. 1971 Corrosion in industrial situations by mixed microbial floras. In *Microbial aspects of metallurgy* (ed. J. D. A. Miller), pp. 107–128. Aylesbury: Medical and Technical Publishing Co.

Report of the N.E.R.C. Working Party on Terrestrial Microbiology 1976 *Research in terrestrial microbiology*. *N.E.R.C. Pub. Ser.* B, no. 13.

Rodina, A. G. 1963 Sulfur bacteria in the detritus of lakes in the Ladoga district. *Mikrobiologiya* **32**, 675–682. (transl. 575–580.)

Ruby, E. G., Wirsen, C. O. & Jannasch, H. W. 1981 Chemolithotrophic sulfur-oxidizing bacteria from the Galapagos Rift hydrothermal vents. *Appl. envir. Microbiol.* **42**, 317–324.

Russell, P. 1961 Microbiological studies in relation to moist groundwood pulp. *Chemy Ind., Lond.*, pp. 642–649.

Sadana, J. C. & Morey, A. V. 1961 Purification and properties of the hydrogenase of *Desulfovibrio desulfuricans*. *Biochim. biophys. Acta* **50**, 153–163.

Schaffer, R. J. 1966 Some aspects of the decay of stone buildings. *Chemy Ind., Lond.*, pp. 46–51.

Siebenthal, C. E. 1915 Origin of the zinc and lead deposits of the Joplin region. *Bull. U.S. geol. Surv.* no. 606, p. 283.

Silverman, M. P. & Ehrlich, H. L. 1964 Microbial formation and degradation of minerals. *Adv. appl. Microbiol.* **6**, 153–198.

Silverman, M. P., Rogoff, M. H. & Wender, I. 1951 Bacterial oxidation of pyrite materials in coal. *Appl. Microbiol.* **9**, 491–496.

Silverman, M. P., Rogoff, M. H. & Wender, I. 1963 Removal of pyrite sulfur from coal by bacterial cation. *Fuel* **42**, 113–124.

Sisler, F. D. 1961 Electrical energy from biochemical fuel cells. *New Scient.* **12**, 110–111.

Sisler, F. D. & Senftle, F. E. 1963 Possible influence of the Earth's magnetic field on geomicrobiological processes in the hydrosphere. In *Marine microbiology* (ed. C. H. Oppenheimer), pp. 159–171. Springfield, Ill.: Thomas.

Sisler, F. D., Senftle, F. E. & Skinner, J. 1977 Electrobiochemical neutralization of acid mine water. *J. Wat. Pollut. Control Fed.* no. 1, pp. 369–374.

Smirnov, V. V. 1963 Role of the microbiological factor in increasing the corrosive aggressivity of mine waters of the Kizel coal basin. *Mikrobiologiya* **32**, 695–699 (transl. 591–594).

Smith, J. L. B. 1952 Cyprinodont fishes from a sulphur-producing lake in Cyrenaica. *Ann. Mag. nat. Hist.* **5**, 888–892.

Sokolova, G. A. 1961 Microbiological production of sulfur from sulfidic water strata. *Mikrobiologiya* **29**, 888–893 (transl. 638–640).

Southward, A. J., Southward, E. C., Dando, P. R., Rau, G. H., Felbeck, H. & Flugel, H. 1981 Bacterial symbionts and low $^{13}C/^{12}C$ ratios in tissues of Pogonophora indicate unusual nutrition and metabolism. *Nature, Lond.* **293**, 616–620.

Starka, J. 1949 Sulphate-reducing bacteria in the Piestany-spa medicinal mud. *Čas. čsl. Lek.* **62**, 100–103.

Starkey, R. L. 1961 Sulfate-reducing bacteria, their production of sulfide and their economic importance. *Tappi* **44**, 493–496.

Suckow, R. & Schwartz, W. 1963 Redox conditions and precipitation of iron and copper in sulphureta. In *Marine microbiology* (ed. C. H. Oppenheimer), pp. 187–193. Springfield, Ill.: Thomas.

Summers, A. & Silver, S. 1978 Microbial transformations of metals. *A. Rev. Microbiol.* **32**, 637–672.

Suzuki, I. 1974 Mechanisms of inorganic oxidation and energy coupling. *A. Rev. Microbiol.* **28**, 85–101.

Takai, Y. & Kamura, T. 1966 Mechanism of reduction in waterlogged paddy soil. *Folia microbiol.* **11**, 304–313.

Taylor, B. F., Currie, M. & Hoare, D. S. 1969 Aromatic ring cleavage by a *Thiobacillus*. *J. Bact.* **97**, 959–960.

Teh, G. H., Schwartz, W. & Amstutz, G. C. 1981 Geomicrobiological leaching of tin minerals by *Thiobacillus ferrooxidans* and organic agents. *Z. allg. Mikrobiol.* **21**, 157–167.

Thaysen, A. C., Bunker, H. J. & Adams, M. E. 1945 Rubber acid damage in fire hoses. *Nature, Lond.* **155**, 322–325.

Tomizuka, N. & Yagisawa, M. 1978 Optimum conditions for leaching of uranium and oxidation of lead sulfide with *Thiobacillus ferrooxidans* and recovery of metals from bacterial leaching solution with sulfate-reducing bacteria. In *Metallurgical applications of bacterial leaching and related microbiological phenomena* (ed. L. E. Murr, A. E. Torma & J. A. Brierley), pp. 321–344. New York: Academic Press.

Tributsch, H. & Bennett, J. C. 1981 Semiconductor-electrochemical aspects of bacterial leaching. I. Oxidation of metal sulphides with large energy gaps. *J. chem. technol. Biotechnol.* **31**, 565–577.

Trudinger, P. A. 1976 Microbiological processes in relation to ore genesis. In *Handbook of strata-bound and strati-forma ore deposits* (ed. H. K. Wolff), pp. 135–190. Elsevier.

Tuovinen, O. H. & Kelly, D. P. 1972 Biology of *Thiobacillus ferrooxidans* in relation to the microbiological leaching of sulphide ores. *Z. allg. Mikrobiol.* **12**, 311–346.

Tuttle, J. H., Randles, C. I. & Dugan, P. R. 1968 Activity of micro-organisms in acid mine water. I. Influence of acid water on aerobic heterotrophs of a normal stream. *J. Bact.* **95**, 1495–1503.

Tuttle, J. H., Dugan, P. R., MacMillan, C. B. & Randles, C. I. 1969*a* Microbial dissimilatory sulfur cycle in acid mine water. *J. Bact.* **97**, 594–602.

Tuttle, J. H., Dugan, P. R. & Randles, C. I. 1969*b* Microbial sulfate reduction and its potential utility as an acid mine water abatement procedure. *Appl. Microbiol.* **17**, 297–302.

Vernon, W. H. 1957 Metallic corrosion and conservation. In *The conservation of natural resources*, pp. 105–137. London: Institution of Civil Engineers.

Wakerley, D. S. 1979 Microbial corrosion in UK industry: a preliminary survey of the problem. *Chemy Ind., Lond.*, pp. 656–658.

van der Westen, H. M., Mayhew, S. G. & Veeger, C. 1978 Separation of hydrogenase from intact cells of *Desulfovibrio vulgaris*. *FEBS Lett.* **86**, 122–126.

van der Westen, H. M., Mayhew, S. G. & Veeger, C. 1980 Effect of growth conditions on the content and $O_2$ stability of hydrogenase in the anaerobic bacterium *Desulfovibrio vulgaris* (Hildenborough). *FEMS Microbiol. Lett.* **7**, 35–39.

Widdel, F. 1980 Anaeroberabbau von Fettsäuren und Benzoesäure durch neu isolierte Arten sulfat-reduzierender Bakterien. Dissertation: Göttingen.

Yen, T. F. 1976 Microbial oil shale extraction. In *Microbial energy conversion* (ed. H. G. Schlegel & J. Barnea), pp. 309–328. Göttingen: Goltze.

ZoBell, C. E. 1946 *Marine Microbiology*. Waltham, Mass.: Chronica Botanica Co.

## *Discussion*

**H. Abdollahi** (*University College Cardiff, U.K.*). Several workers have experienced difficulties in determining the purity of cultures of sulphate-reducing bacteria. I wonder to what extent the possible presence of undetected contamination might have led to discrepancies over the elemental sulphur reduction and carbon metabolism. I should be glad if Professor Postgate could comment on these problems.

**J. R. Postgate.** Though some early studies were done with what proved later to be impure cultures, they were later checked with purified cultures and it is unlikely in principle that the contaminants would have been sulphur reducers. I think it likely that sulphur reduction is strain-specific and possibly species-specific.