

The use of natural bacterial populations for the treatment of sulphur-containing wastewater

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Abstract

Pollution by inorganic and organic sulphur compounds is increasing and, because of the many environmental hazards associated with these compounds (e.g. toxicity, acidification of rain and freshwater, increase of COD, the greenhouse effect), must be taken seriously. There is a wide variety of sulphur oxidizing bacteria available in nature, and these can be used for the effective control of such pollution. The best way to break the sulphur cycle is to stop it at sulphur which, being insoluble, can be easily recovered (e.g. $\text{SO}_4^{2-} \rightarrow \text{S}^{2-} \rightarrow \text{S}^0$). (Eco)physiological knowledge about the sulphur oxidizing bacteria has proved very useful in the prediction of the performance of sulphur oxidizing communities in actual wastewater treatment systems. Appropriate reactor design, based on this type of study, is essential if such bacterial communities are to function efficiently, especially when toxic sulphides must be treated. This paper reviews the natural and anthropogenic sources of sulphur pollution, its consequences and possible solutions.

Introduction

Human activities are disturbing the sulphur cycle on a local, or even global, scale. There are two major causes: the formation of SO_2 and other sulphur compounds by the burning of fossil fuels, and the boosting of the sulphur cycle by various forms of eutrophication. This boosting can result in local pollution as a result of extensive sulphate reduction.

The biological sulphur cycle (Fig. 1) is normally kept in balance by a number of processes that make the cycle, as a whole, very resilient. Thus if high concentrations of hydrogen sulphide accumulate, it may be oxidized (biologically or chemically) to elemental sulphur, and thus removed from the general cycle. That this is sometimes the case on a geological scale is demonstrated by the huge sulphur de-

posits in some geological formations. Similarly, under suitable conditions, over-production of hydrogen sulphide can result in the formation of metal sulphide masses, generally to be found as the various pyritic ores. The oxidized form of sulphur, sulphate, also occurs on the geological scale, as calcium sulphate deposits.

In spite of this impressive resilience and storage capacity, the cycles can be observed to be out of balance, both globally and in localized areas. Of course, this may be due to natural causes, such as a volcanic explosion or 'natural' eutrophication. However, the greatest threat to the balance of the sulphur cycle is human activity, particularly SO_2 generation which is causing acid rain (see below). When it is asked what biology, and especially microbiology, can do to abate these problems, it must always be emphasized that prevention is better

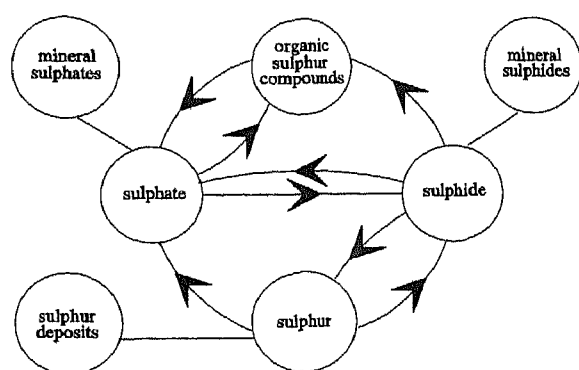


Fig. 1. The biological sulphur cycle.

than a cure. Microbiological contributions to the solution of the sulphur pollution problem can be made along three lines:

1. Expand our understanding of the sulphur cycle, and the ways in which human activity is unbalancing it.
2. On the basis of this information, develop prevention methods.
3. Should prevention not be possible, or fail, provide methods of remediation, especially for localized problems.

This review will catalogue the main natural and anthropogenic causes of sulphur pollution, and discuss existing and conventional techniques for treating reduced and partially oxidized sulphur-containing wastes. Although most of these processes are 'end of the pipe' solutions, some emphasis will be placed on recycling processes which can be integrated into a total process design.

General pollution problems

Sources

Natural sources of reduced and (partially) oxidized sulphur compounds

The most spectacular sources of sulphur and sulphides are undoubtedly geothermal, whether by free dispersal into the atmosphere as a volcano erupts, by diffusion from geothermal springs (e.g. Yellowstone Park), or within the hydrothermal areas of the sea. It is, of course, difficult to quantify

these contributions to the global sulphur cycle, estimates of SO_2 emission from volcanoes, for example, vary by an order of magnitude (from 1.5 to 34 Tg S as SO_2 year⁻¹; Lein 1983). The amount emitted will depend on the type of volcano involved. Andesitic volcanoes emit high concentrations of water vapour and lower amounts of SO_2 . The more common basaltic volcanoes release greater concentrations, with the basaltic volcanoes of the Hawaiian Islands producing the most (Lein 1983). Fumeroles activity, while less spectacular than full scale eruptions, also contributes a certain amount. Interested readers should consult Lein (1983) for an extensive review of the data. Sulphur compounds are also physicochemically released from sedimentary rocks and mineral deposits (e.g. pyrites) by weathering and from the sea by aerosol formation.

Biological activity also contributes significantly to the global sulphur cycle. Thus, the activity of sulphate reducing bacteria releases sulphate from aquatic sediments and biofilms, stratified seas and lakes and, of course, bogs and marshes as hydrogen sulphide. If, however, the area is undisturbed, this H_2S rarely reaches the atmosphere because of the formation of mats of sulphur oxidizing organisms at the sulphide: oxygen interface. The behaviour of the community of phototrophs and chemotrophs (see below) in such a system was described by Jørgensen (1982). As can be seen from Fig. 2, the position of the various species within the mat was determined by the amount of light available for photosynthesis, as well as the position of the sulphide: oxygen boundary. The importance of the organic sulphides being produced within algal blooms in the oceans is only now beginning to be appreciated, and measured (see, for example, de Zwart & Kuenen 1992).

Anthropogenic sources of reduced and (partially) oxidized sulphur compounds

Probably one of the greatest anthropogenic contributors to sulphur pollution is the burning of fossil fuels. Sulphur is present in coal in the form of pyrites crystals, sulphate and, distributed within the matrix of the coal, as organic compounds. Sulphides are also present in natural gas and oil deposits. The actual amounts in fossil fuels vary with the

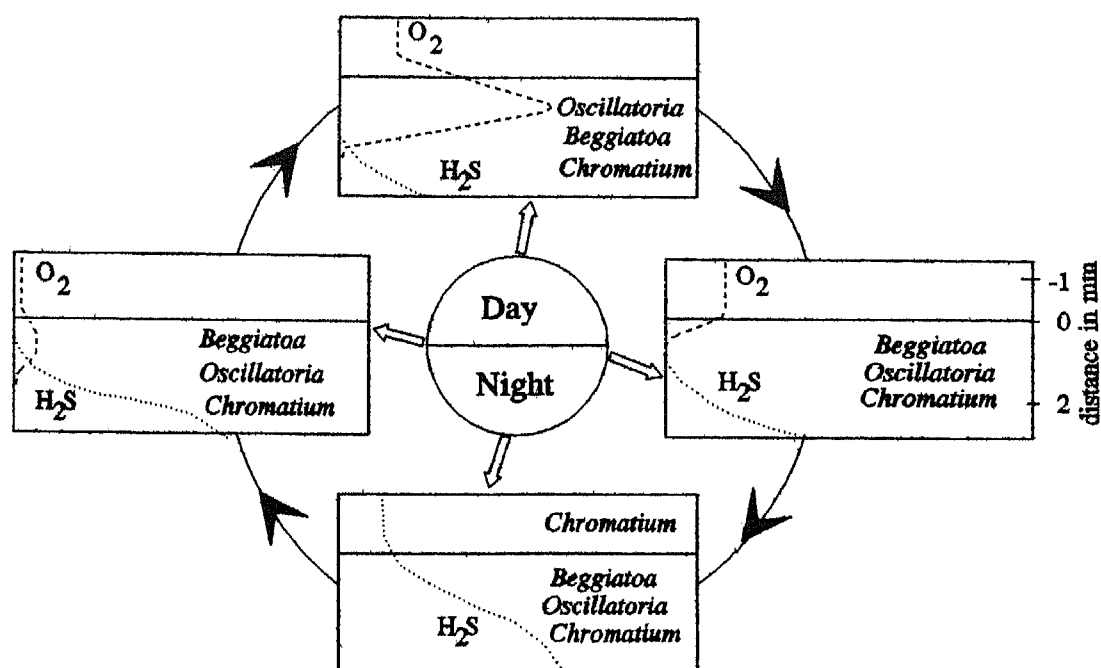


Fig. 2. Diurnal cycle of oxygen and sulphide gradients and microbial distribution in a marine sulfuretum (adapted from Jørgensen 1982).

source. Thus most natural gas sources are fairly low in sulphur content (around 0.05% S by weight), but sources containing 80% H_2S are known (Ryaboshapko 1983). The sulphur content of oil is known to vary from 0.3–0.5% in African oils to 5% in Venezuelan oil. The sulphur content of coal can vary from less than 1% to as much as 8%. Another major source of SO_2 is the mining and smelting of sulphidic metal ores in which sulphides can be as much as 45% of the dry material. Table 1 summarizes the estimates of SO_2 emissions, and Table 2 the sulphide emissions contributed by different anthropogenic activities and collated by Ryaboshapko (1983).

Anthropogenic activity has also boosted biological production of reduced sulphur compounds. Everyone is familiar with the smells associated with the local sewage works, and this is extended by sulphides produced during methanogenesis from organic wastes, as well as sulphate reduction within rubbish dumps. Locally, H_2S and the methylated sulphides (CH_3SH , $(CH_3)_2S$ and $(CH_3)_2S_2$; MAC values 10, 0.5, 20 and < 20 ppm, respectively) may

Table 1. Estimates of SO_2 emission ($TgS\ year^{-1}$) from different processes in various countries (before the political boundaries were changed). Adapted from Ryaboshapko (1983). * = included in Industrial total.

Process type	USA	Great Britain	West Germany	USSR
Energy	10.65	1.53	1.53	7.32
Petroleum processing	1.65	0.2	0.15	0.3
Iron production	4.05	0.25	0.25	1.3
Industrial	0.90	0.61	0.55	3.4
Others	2.1	0.3	*	1.4

Table 2. Estimates of sulphide emissions due to industrial activity (adapted from Ryaboshapko (1983)).

Process	Sulphide	Emission (as $TgS\ year^{-1}$)
Paper making	DMS, H_2S , mercaptans	0.1–0.65
coke production	H_2S	0.07–0.7
smelting of iron	H_2S	0.1–0.5
cement production	HH_2S	1.2

be produced in industrial effluent, notably from paper mills and the nylon industry.

The activities of metal pyrite-oxidizing bacteria were previously limited by the accessibility of the minerals. Mining has exposed enormous areas of minerals to microbial activity, with the resultant acidification and sulphate pollution of ground water and soils in the surrounding area.

Problems associated with sulphur pollution

Health

The only two sulphur compounds mentioned in the World Health organization (WHO) guidelines for drinking water quality (WHO 1984a) are sulphate and hydrogen sulphide. Their recommended levels are 400 mg SO_4^{2-} per litre and 'not detectable by consumer', respectively. In the volume on health criteria (WHO 1984b), dimethyl sulphide is also discussed, but no recommended level is given. At very low concentrations, it is, of course, an important constituent of some beer flavours.

The accepted sulphate level is primarily determined from its taste threshold which is in the range between 200 and 500 mg SO_4^{2-} per litre, depending on the associated cation. However, sulphate can also act as a laxative, especially when combined with Mg^{2+} . Normal adults are affected at levels around 1000 mg per litre, but children and new users can be affected by less. Hydrogen sulphide is very toxic. It inhibits various enzyme systems (WHO 1984b), and irritates the epithelium of the mucous membranes. If inhaled, it paralyses the respiratory centre. Instant death occurs at a concentration of 2800 mg per m^3 , levels of 1400–2100 mg per m^3 will result in death after 30 minutes. Indeed, sudden deaths have occurred when workers cleaning sewers have inhaled lethally high concentrations of H_2S from the sewage. At lower concentrations, H_2S causes depression (0.12 mg per m^3), conjunctivitis and visual problems ((1.5–43 mg per m^3), and psychic changes, dizziness and vomiting, among other problems (70–700 mg per m^3). Perhaps one of its greatest dangers is that hydrogen sulphide also affects the sense of smell, and concentrations above 225 mg per m^3 can thus not be de-

tected in the usual manner. Swallowing alkali sulphides can cause nausea, vomiting and pain. 10–15 g NaS is fatal. Hydrogen sulphide has been detected in drinking water from stagnant sources. However, its taste and odour thresholds are much lower (0.05–0.2 mg per l) than its toxic levels, making poisoning from ingestion unlikely.

Organic sulphides can also be extremely hazardous. At very low concentrations, DMS is an important component of foods and beverages, contributing to the flavour of, for example, beer and milk. It also contributes to the 'smell of the sea'. At higher concentrations, but still in the ppb range, DMS has a very offensive odour. At even higher concentrations, the toxic effects are similar to those described above for H_2S .

The atmosphere

Atmospheric sulphur compounds are dominated by the organic sulphides (especially DMS), SO_2 , and (localized) H_2S . An indicator of anthropogenic sulphur pollution in the atmosphere can be found by comparing the levels of sulphate in ice cores taken from the Arctic and Antarctic. Because the southern hemisphere is thinly populated and industrialized (compared to the north), it can be assumed that most, if not all, sulphate in the Antarctic ice is due to natural causes, and indeed a correlation between volcanic eruptions and sulphate levels has been observed (Legrand & Delmas 1987; Stauffer & Neftel 1987). The steady increase in sulphate (from below 40 ng/g ice in 1900 to above 100 ng/g ice in 1980) in the Arctic does not correlate with volcanic activity, and were thought to be almost entirely due to human activities. The sulphate (as H_2SO_4) comes from the oxidation of SO_2 with H_2O_2 , OH and O_3 in clouds, and is one of the primary causes of acid rain (Isakson 1987). SO_2 is one of the few gaseous pollutants not associated with the Greenhouse Effect (it absorbs at roughly the same wavelength as ozone), but another sulphur compound, COS, does contribute to the 'dirty window effect' as it absorbs at in the same region (although to a much lesser extent) as the CFCs (Ramanathan 1987). The organic sulphides, especially dimethyl sulphide (DMS), are also radiatively significant (de Zwart & Kuenen 1992) through

the formation of the more oxidized dimethyl sulphonate. The occurrence of dimethyl sulphide, itself, corresponds with various types of algal blooms. Indeed, recent investigations indicate that some of the sulphate in the Antarctic ice may originate from dimethyl sulphonate precipitation.

Aquatic

One of the most obvious effects of reduced sulphur compounds in bodies of water is anoxia. In most situations, layers of sulphur oxidizing bacteria practically separate the sulphide and oxygen gradients. However, in extreme situations (e.g. with very high sulphide concentrations or after turnover of a normally stratified lake), chemical oxidation of sulphide can proceed rapidly enough to consume all of the available oxygen. Even an increase in the sulphate content of freshwater may be sufficient to boost the sulphur cycle, promoting sulphate reduction and, again, anoxia. Of course, it is essential that sufficient (organic) electron donors are available in addition to sulphate. In such cases, sulphate reducing bacteria may out-compete the slower-growing bacteria that ferment lower fatty acids and the H_2 metabolizing methanogens, because of the higher affinity for reduced substrates of the sulphate reducing bacteria (Nanninga 1987).

Many sulphidic water bodies are highly acidic, largely because of the sulphuric acid produced by the oxidation of the sulphur compounds. Even in the hot, acid springs of volcanic areas, the sulphur cycle continues, with sulphur oxidizers such as *Sulfolobus* and sulphur reducers such as *Acidianus* active at 80°C and above.

Terrestrial

Most of the terrestrial problems associated with sulphur-linked pollution are, again, linked to acidity. Damage to forests caused by acid rain has been much in the news recently, as has the erosion of famous buildings such as the Parthenon in Athens. In the vicinity of mines or exposed mineral deposits, this takes on another dimension since the acidity combined with bacterial activity can result in heavy metals forming soluble salts, and leaching into the ground water (this facility is actually employed in leaching process for metal recovery).

Bacteria with resistance to relatively high concentrations of mercury, for example, have been isolated from such streams, and the isolation from an acid mine stream of a strain able to reduce chromate as an electron acceptor has been described. Sulphate reducing and sulphur oxidizing bacteria (the latter producing H_2SO_4) have also been associated with the corrosion of pipes, tanks and other metallic structures. The sulphate reducers are thought to cause depolarization of the metal, resulting in electrochemical corrosion of structures such as oil platforms (Kuenen & Bos 1989). The sulphur oxidizing bacteria produce sulphuric acid which may also stimulate electrochemical corrosion. This is admirably illustrated by the example of a fairly new ship loaded with wet, pyrite-containing coal from a waste pond, and then travelled from the USA to South America. The journey was rough, so that the contents of the holds were well mixed. Not long after this, corrosion was noted in the holds. Not only was this greatest in the holds nearest the engines, where it was warmer, but there was also a greenish liquid appearing. The captain tasted this, and found it to be acid. It was eventually concluded that the production of sulphuric acid by the pyrite oxidizing bacteria had created the electrochemical conditions for the corrosion of the stainless steel hull of the ship, also promoting chemical corrosion (P. Bos, pers. comm.). This type of damage is not limited to metallic structures. For example, considerable damage has been observed in concrete sewers. This is due to the fact that the sewer pipes contain anaerobic sewage with active sulphate reduction, but an aerobic head space. H_2S leaves the sewage and may be precipitated as elemental sulphur on the aerobic, wet surfaces above the water level. There it is further oxidized to sulphuric acid, which dissolves the calcium carbonate in the concrete. Indeed, a strain of *Thiobacillus thiooxidans* was formerly known as *T. concretivorans* (Kuenen et al. 1992).

The current state of the art

The colourless sulphur oxidizing bacteria

It is clear that many of the problems associated with sulphur pollution cannot be solved by microbiological means. At the same time, it is also obvious that microbial activity will exacerbate, rather than diminish sulphur-related pollution problems. Fortunately, there are many ways in which microbial activity can be beneficially exploited. Microbiological methods of dealing with problems associated with reduced sulphur compounds depend on bacteria able to oxidize these compounds. The group of bacteria able to oxidize reduced sulphur compounds is extremely heterogeneous, and the most obvious sub-division is that between the phototrophs and the non-phototrophic (generally called colourless) groups. An overview of the phototrophs is given in Table 3. There are a number of reasons why the phototrophic sulphur oxidizing bacteria would not be the first choice for biocatalysts in biotechnological processes. The most obvious problems are associated with reactor design. For example, the phototrophs require light, and many of them retain molecular sulphur within the cell, giving separation problems. An African lake,

Table 3. Physiological types found among the phototrophic sulphur oxidizing bacteria (adapted from Kuenen et al. 1985). obl. = obligate; fac. = facultative.

Family	Metabolic definition	Electron donor
Chlorobiaceae	obl. phototroph, fac. (photo)autotroph, external S ⁰ produced, obl. anaerobe	S ²⁻ , S ₂ O ₃ ²⁻ , S ⁰ , H ₂ , organic acids
Chloroflexaceae	fac. phototroph, fac. photoautotroph, external S ⁰ produced, thermophilic	S ²⁻ , organic acids
Chromatiaceae	phototroph, fac. photoautotroph, internal S ⁰ produced, fac. aerobic	S ²⁻ , S ₂ O ₃ ²⁻ , H ₂ , organic acids
Rhodospirillaceae	fac. phototroph, fac. photoautotroph, external S ⁰ produced	S ²⁻ , S ₂ O ₃ ²⁻ , organic acids

with a very large surface to volume ratio, was observed to have *Chlorobium* species oxidizing the available sulphide to sulphur at a very slow rate (Butlin & Postgate 1954). Moreover, the sulphide oxidation is strictly coupled to growth in these organisms, with the generated electrons being required for CO₂ reduction (i.e. fixation). Thus during S²⁻ oxidation, *Chlorobium* cultures (which excrete their sulphur) and *Chromatium* cultures (which accumulate it intracellularly) can be expected to generate approximately 1–2 grams S⁰ per gram of cells produced. In *Thiobacillus* species, the oxidation of sulphur compounds is not obligately coupled to growth as the main electron acceptor is oxygen (or nitrate) rather than CO₂. In fact, these chemolithotrophs can oxidize sulphur compounds (e.g. extracellular elemental sulphur) without any appreciable growth when nutrients other than their energy source are limiting (Kuenen et al. 1985). They can therefore produce at least 20 grams S⁰ per gram of cells. Since biotechnological processes tend to deal with much more concentrated sulphide solutions, and require higher specific rates of oxidation and low sludge production, the colourless sulphur bacteria from the genus *Thiobacillus* are clearly more appropriate for most applications.

The 'colourless sulphur bacteria' is a general term of physiological, but little taxonomical significance (Kuenen 1989; Kelly & Harrison 1989). All of the physiological types outlined in Table 4 can be found within this group. With such a great diversity, there has been some debate as to the taxonomic

Table 4. Definition of the physiological types of bacteria able to oxidize reduced sulphur compounds. Synonyms: ^A obligate autotrophs. ^B facultative autotrophs, mixotrophs.

Physiological type	Carbon source		Energy source	
	Inorganic	Organic	Inorganic	Organic
Obligate chemo-lithoautotroph ^A	+	–	+	–
Facultative chemo-lithoautotroph ^B	+	+	+	+
Chemolithoheterotroph	–	+	+	+
Heterotroph	–	+	–	+

validity of the group. Indeed, evidence from 5S and 16S RNA analysis (Lane et al. 1990) has indicated that some species (e.g. the members of the genus *Thiomicrospira*) are more closely related to *Escherichia coli* than to other colourless sulphur bacteria (e.g. *T. ferrooxidans*). The precise relationships require further elucidation of the significance of RNA analysis in taxonomy, after all, as pointed out recently (Woese 1991), there is greater difference in terms of RNA analysis between species in the same bacterial genus than there is between higher eukaryotes such as frogs and humans. Taxonomic problems aside, the ability to oxidize reduced sulphur compounds and gain energy from the reaction requires special enzymes, and the grouping thus has physiological significance. Table 5 provides examples of the many types found among the colourless sulphur bacteria, together with some of their environmental requirements. In addition to these, it appears that many symbiotic bacteria, including those of the spectacular hydrothermal vent worms, *Riftia*, are sulphide oxidizing bacteria related to *Thiomicrospira* (Lane et al. 1990).

The obligate and facultative autotrophs are of most obvious interest for wastewater treatment but, as will be seen below, chemolithoheterotrophs

(e.g. *Thiobacillus* Q (Gommers et al. 1988), *Hyphomicrobium* sp. (Suylen & Kuenen 1986)) can be important, particularly where organic sulphides are involved. The type of organism in the system will be primarily defined by the system itself – a wastewater treatment reactor running at 25°C, neutral pH and with a feed containing sulphide and nitrate will obviously enrich for bacteria resembling *Thiobacillus denitrificans*. Similarly, a reactor oxidizing pyrites in hot, acid solutions will probably give rise to a population similar to *Sulfolobus*. The selective pressures determining whether obligate or facultative autotrophs will dominate a community were investigated some time ago. Using chemostat cultures of *T. neapolitanus* (obligate autotroph), *Thiobacillus* A2 (now *T. versutus*, a facultative autotroph) and a spirillum, G7 (obligate heterotroph), Gottschal et al. (1979, 1981) showed that the final population (or community in some cases) was determined by the ratio between the turnover of inorganic and organic substrates in the feed to the chemostat, as outlined in Fig. 3. That this model was a simplification was emphasized by the fact that *T. neapolitanus* was found to excrete glycolate in sufficient quantities to support the growth of *T. versutus* in 3-membered cultures when it was expected to wash out. However, the model

Table 5. Survey of the metabolic types found among the colourless sulphur bacteria. Obl. = obligate; Fac. = facultative; *T.* = *Thiobacillus*; *Tms.* = *Thiomicrospira*; *Tsa.* = *Thiosphaera*; *Tx.* = *Thermothrix*; ^a = microaerophilic; ^b = also gains energy from iron; ^c = denitrifies heterotrophically; * = known to produce extracellular sulphur.

Representative species	Autotrophy		Denitrification		pH range	Temp. range
	Obl.	Fac.	to NO ₂ ⁻	to N ₂		
<i>T. neapolitanus</i> [*]	+	–	–	–	6–8	< 37°C
<i>T. thioparus</i> [*]	+	–	+	–	6–8	< 37°C
<i>T. denitrificans</i>	+	–	+	+	6–8	< 42°C
<i>Tms. denitrificans</i> [*]	+	–	+	+ ^a	6–8	< 37°C
<i>T. intermedius</i> [*]	–	+	–	–	5–7	< 37°C
<i>Tsa. pantotropha</i>	–	+	+	+	6–10	< 42°C
<i>Beggiatoa</i> sp.	–	+	–	–	7	< 37°C
<i>Beggiatoa</i> sp.	–	+	+	+	7	< 37°C
<i>T. ferrooxidans</i>	+ ^b	–	–	–	2–4	< 37°C
<i>T. acidophilus</i>	–	+	–	–	2–4	< 37°C
<i>T. tepidarius</i>	+	–	+	–	6–8	20–52°C
<i>Tx. thioparus</i>	–	+	+	+ ^c	6–8	40–80°C
<i>S. acidocaldarius</i> [*]	–	+	–	–	1–6	55–85°C

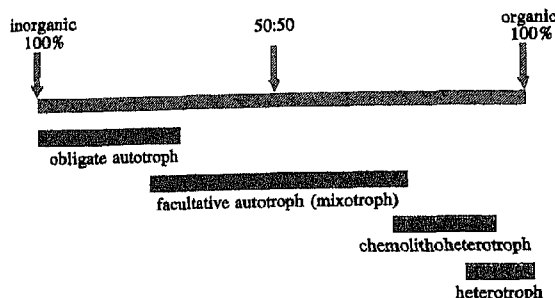


Fig. 3. The selection of different physiological types according to the organic:inorganic ratio in the medium supply (Gottschal et al. 1979).

also predicted that facultatively autotrophic bacteria such as *T. versutus* should dominate continuous cultures limited by equimolar amounts of organic and reduced sulphur compounds. This has now been observed for a number of chemostat enrichment cultures (see, for example, Beudeker & Kuenen 1982; Kuenen et al. 1985).

Possible solutions

There are some problems to which purely biological solutions will not be practical or even possible. Into this category might fall the super-heated, sulphide-containing gas exhausts from smelting. In other situations, a combined physico-chemical and biological treatment might be more feasible. Such systems might include the cleaning of refinery gases where a chemical method is simple and relatively cheap for the bulk of the sulphur, but biological treatment is necessary to attain required standards. Similarly, the cleaning of coal might efficiently combine separation techniques to remove the greatest part of the pyrites, with biological treatment removing the rest (Bos 1991). Such a system will be discussed briefly below. If the effluent contains toxic compounds, it may be necessary to remove them before biotreatment can take place. For example, high concentrations of sulphide which might be toxic for nitrification could be stripped off before the water is nitrified. In principle, the H_2S might then be reused for sulphuric acid production or, elsewhere, be converted biological-

ly or chemically into sulphur or sulphate. Alternatively, compounds can be removed for treatment elsewhere. For example, it might be simpler to treat the ammonia in photographic waste if the ammonia is first stripped from the effluent as the waste also contains thiosulphate, thiocyanate, cyanide and various complex organic compounds (Robertson & Kuenen 1984). Another major problem with photographic waste is the presence of quinones which not only resist treatment, but inhibit the conversion of other compounds. The simplest way to make their effluent easier to handle might be for the photographic industry to replace these compounds with something less recalcitrant and less toxic.

Biofilters (Diks & Ottengraf 1991) provide a simple solution for effluent gas streams that contain volatile reduced sulphur compounds. These may take the form of a peat filter, or a system where the gas flow is sparged through water, which is then treated elsewhere. For the treatment of aqueous, sulphur-containing waste streams, many options are available, depending on the type of waste to be treated and the requirements of the biomass. Systems using fluidized beds, in which the bacteria form biolayers on sand which is kept suspended in the reactor by the upward flow of the effluent, and biodiscs, in which the biomass is attached to surfaces which rotate in and out of the effluent stream, will be discussed below.

Research at Delft

Coal desulphurization

As mentioned above, the sulphur content of some coals can add enormously to atmospheric sulphur pollution. Methods of removing the sulphur from the coal before combustion have therefore been attracting a great deal of attention. There are various physical methods which can be used for the removal of large pyrites crystals, but biological oxidation using the acidophilic *Thiobacillus* or *Sulfolobus* species (ironically, the same species that can cause environmental problems such as acid mine water and pipe corrosion) is particularly suitable

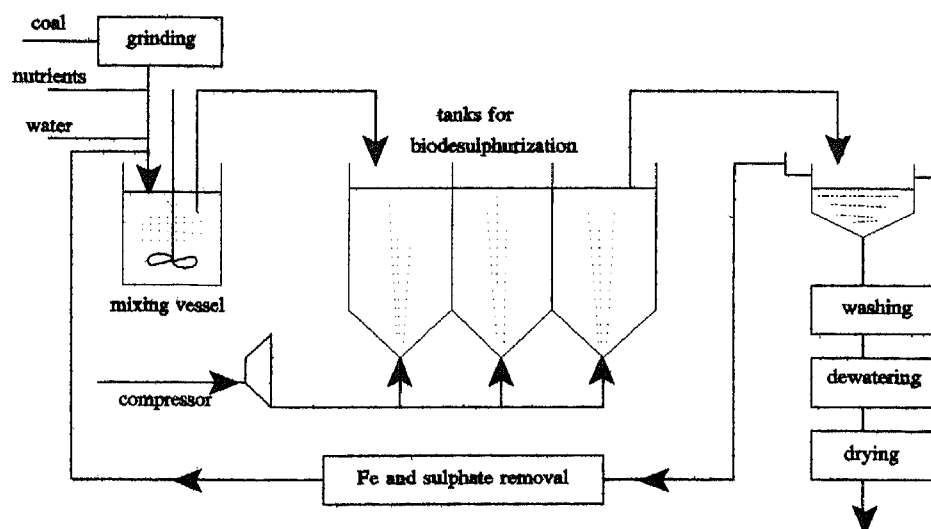


Fig. 4. Simplified system for the microbial desulphurization of coal in Pachuca tanks (biodesulphurization reactors) The coal is treated as a slurry of particles $< 100 \mu\text{m}$ in diameter in acid, nutrient solution (from Bos & Kuenen 1990).

for the treatment of coal containing finely distributed pyrite. It has been shown that such a process could be effective and feasible, especially for coals with a relatively low sulphur content (Bos et al. 1988; Bos & Kuenen 1989). Laboratory studies showed that the process was most effective if carried out in two steps. In the first stage, the pulverized coal suspension is mixed with a suspension of suitable bacteria growing on pyrite at a (very low) pH (around 1.8). This mixture is then pumped through a series of reactors to create a plug flow process where most of the pyrites would be oxidized. At the end of the process, the coal and process water are separated. The water can be recirculated a number of times, until the iron and/or salt concentration reach a critical level. A suitable reactor configuration for this type of process was described by Bos et al. (1988), and is shown in Fig. 4. The desulphurization reactors are of a type known as Pachuca tanks (in their simplest form, an inverted cone with aeration at the bottom of the tank). These are simple, relatively cheap and, because the up-flowing air keeps the suspension well mixed while maintaining aerobic conditions, do not require complex stirring equipment. It is essential that the first reactor has a residence time of at least 1 day to allow the inoculation of freshly introduced coal with bacteria attached to partially treated coal

particles. This residence time is based on the observed growth rate of *T. ferrooxidans* (0.06 h^{-1} , a doubling time of approximately 10 hours) in the system. Once a few bacteria are attached to the exposed surfaces of the pyrite crystals, growth and degradation can take place in the succeeding reactors. The obvious advantage of using a plug flow system is that treated and untreated coals are not mixed. Of course, the nature of the organisms involved demands a certain amount of care in choice of materials – *T. ferrooxidans* has been known to corrode stainless steel, and sulphur-containing concrete should clearly also be avoided. The efficiency of the process depends on the grinding of the coal to fine particles (maximum diameter $100 \mu\text{m}$) in order to expose the sulphide crystals to bacterial activity. Grinding the coal to this particle size is completely compatible with its later use as an oil-like slurry in the combustion process. This system is only effective against the inorganic sulphides, because organic sulphur compounds occur within the matrix, rather than as localized crystals. In order to prevent SO_2 being produced from organic sulphur in the coal, lime is added to the slurry. It is also necessary to treat the wastewater from the process to remove iron and other heavy metals, as well as to neutralize the acid.

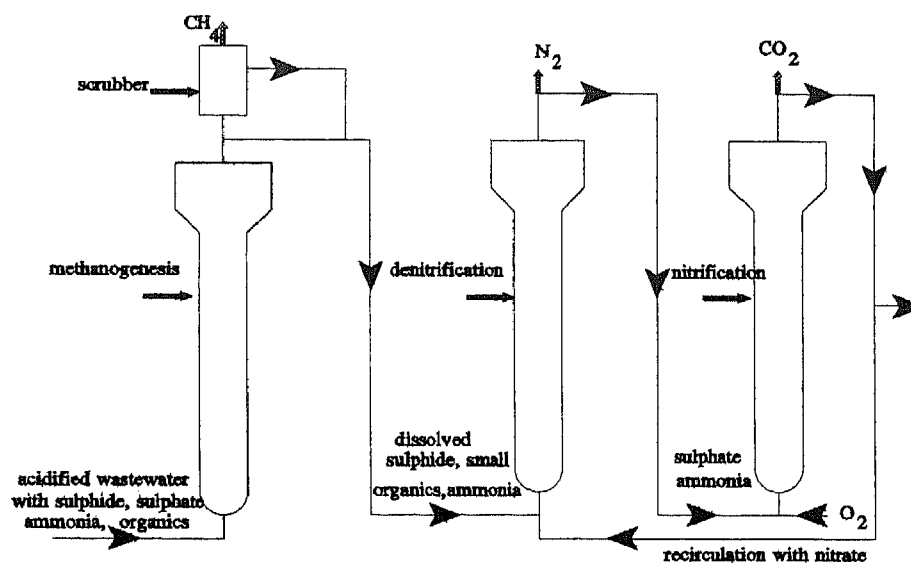
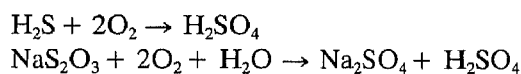


Fig. 5. A combination of reactors used to generate methane from organic industrial wastes, and then remove the nitrogen and sulphur content by means of nitrification and sulphide oxidation with denitrification.

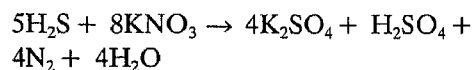
Wastewater treatment

Sulphate production with denitrification

Reduced sulphur compounds are an almost inevitable component in wastewater streams, especially if the system is completely or partially anaerobic and the wastewater contains significant amounts of sulphate. The most frequent (and conspicuous) sulphidic component in wastewater is sulphide, although thiosulphate and sulphur may also occur. As can be seen from the following reactions, oxidation of sulphide or thiosulphate with oxygen results in the equimolar production of sulphuric acid:



This problem can be overcome by using denitrifying bacteria to oxidize the sulphur compounds:



Using denitrifiers has the added advantage that the nitrogen content of the wastewater can be reduced at the same time, if it is present in an oxidized form.

A denitrifying, sulphide oxidizing, fluidized bed reactor was part of a system patented by a Dutch company in the early '80s, and illustrated in Fig. 5. The wastewater came primarily from bakers yeast production, and therefore had a high COD. This was reduced by methanogenesis after acetogenesis, both anaerobic processes which permitted the reduction of the sulphate in the wastewater to sulphide. To deal with the sulphide, and also remove any organics not consumed in the methanogenic reactor (e.g. small organic acids, protein from dying bacteria), a denitrifying reactor was next in the sequence. The necessary nitrate was provided by recirculation from the last reactor, in which the ammonia in the wastewater was oxidized by aerobic nitrifying bacteria. The overall performance of, and the microbial community in the denitrifying reactor received detailed attention in this laboratory over a period of about five years. Studies on the actual reactor appeared to support the model for microbial selection outlined in Fig. 3. The influent contained similar amounts of sulphide and small organic molecules (mostly acetate). A large proportion of the population was heterotrophic, but all of the sulphur bacteria isolated were facultative autotrophs (Robertson & Kuenen 1984). However, later experiments with a laboratory-scale

model of the column revealed that this was an over-simplification as obligate autotrophs were never isolated, even when the feed to the system was wholly inorganic (Robertson & Kuenen 1991). Sulphur balances made over long periods showed that a steady state was never reached by the system, there were periods of sulphur accumulation within the bed of the reactor, and periods of sulphate production. Although the reasons for this were never clear, it can be speculated that the fluctuations might be explained by the 'chaotic' behaviour of the hydraulic regime. All of the sulphide and small organics were consumed in the lowest part of the reactor, and the other, necessary, reactions took place higher up. For example, an accidental overload of the system resulted in the expansion of the sulphide and sulphur containing area within the bed, sometimes resulting in the production of N_2O rather than N_2 . This is most probably due to the inhibition of the last enzyme in the denitrifying pathway, N_2O reductase, which is known to be sulphide-sensitive.

The maximum sulphide and sulphur oxidizing capacities of the biomass after growth with different sulphide (or thiosulphate) to acetate ratios was measured, and the results are shown in Table 6. At all times, there was an over-capacity for sulphide oxidation to sulphur, but the sulphur-oxidizing capacity was only sufficient to cope with the load. A step-wise increase of the sulphide supplied to the column receiving the highest amount of acetate revealed that the biomass increased its sulphur and

sulphate forming activity to match the sulphide supply (Gommers et al. 1988). Further activity measurements revealed that the sulphide/sulphur oxidizing activity of the bed was stratified. Biomass taken from different heights in the fluidized bed was supplied with sulphide, acetate and nitrate, and the progress of the conversions followed. The sulphide was depleted within the first 20 minutes in all three samples, but the sulphate production rate was much slower. Clearly, during the rapid initial sulphide oxidation, sulphur was produced, and subsequently oxidized to sulphate. Only in the sample from the top of the bed was all of the expected sulphate produced. In the samples from the bottom and middle, only about 50% of the expected sulphate was found. These experiments explain the observation that, when the load on the reactor was increased above the sulphur oxidizing capacity of the biomass, elemental sulphur accumulated (temporarily) in the biofilm. Indeed, the ability to switch from complete sulphide oxidation to sulphate (producing 8 electrons) to sulphur accumulation (2 electrons) provided the system with a very high resilience for sulphide overloads.

To sum up, this research concluded that the biomass in the reactor could adapt to changes in its feed, and adjust its sulphur-oxidizing capacity to suit its requirements. In addition, it could adjust to deal with sulphide overloading. However, overloading with sulphide could result in N_2O production until adaption had taken place. Moreover, the nitrate supply to the system had to be carefully monitored because nitrite, if allowed to accumulate, was strongly inhibitory.

Sulphur production

As mentioned above, high sulphate levels are undesirable as they boost the natural sulphur cycle and can cause taste and alimentary problems in drinking water. It has been suggested that sulphur in wastewater could be recovered as S^0 (Butlin & Postgate 1954; Postgate 1982). If this was the case, it should be possible to prevent increased sulphate concentrations and reduce the sulphuric acid production by aerobic reactors if the sulphide oxidation could be stopped at S^0 rather than proceeding to sulphate:

Table 6. The maximum capacities of biomass grown at different S:acetate ratios in a denitrifying, fluidized bed reactor, together with the S-loads necessary to obtain the correct ratios. * = thiosulphate used in place of sulphide for experimental convenience. The thiosulphate oxidizing capacity was equivalent to that of sulphide.

Compound used	S:acetate ratio	S-load	Capacity $S^{2-} \rightarrow S^0$	Capacity $S^0 \rightarrow SO_4^{2-}$
S^{2-}	0.1	1.1	20	0.8
S^{2-}	1.1	2.6	25	2.6
S^{2-}	0.3	3.3	18	2.9
$S_2O_3^{2-*}$	9.0	10.3	54	28
$S_2O_3^{2-*}$	15	22	187	33

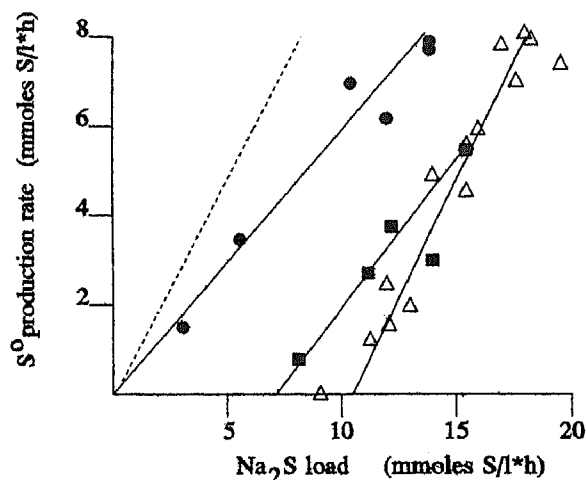
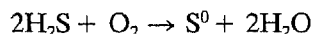


Fig. 6. The effect of the dissolved oxygen concentration on sulphur production by sulphide-oxidizing chemostat cultures of *Thiobacillus neapolitanus*. The dissolved oxygen concentrations quoted as percentage air saturation. Closed circles = oxygen limitation; closed squares = 20 % air saturation; open triangles = 50–60% air saturation; broken line = 'theoretical maximum' indicating conversion of all sulphide to sulphur, with no other products (G.C. Stefess, L.A. Robertson & J.G. Kuenen, unpublished results).



Research at Delft (as part of a combined project with the Agricultural University of Wageningen) has revealed that high sulphide loads and electron acceptor limitation both favour sulphur (rather than sulphate) production, even when strains of *T. neapolitanus* from a culture collection were used (Stefess & Kuenen 1989). Figure 6 shows the sulphur production obtained with steady state *T. neapolitanus* cultures at different dissolved oxygen concentrations. It can be seen that relatively high sulphide loads were necessary before the cultures grown at significant (20–60% air saturation) dissolved oxygen concentrations began to accumulate sulphur. Under oxygen limitation, sulphur was produced at all loads. The effect of varying sulphide loads on *T. neapolitanus* grown at 50–60% air saturation can be seen in Fig. 7. It can be seen that, as might be expected, sulphate production levels stabilized after sulphur accumulation started. At a critical load of about 17 mmol S·l⁻¹·h⁻¹, even the sulphur producing capacity of the culture was inad-

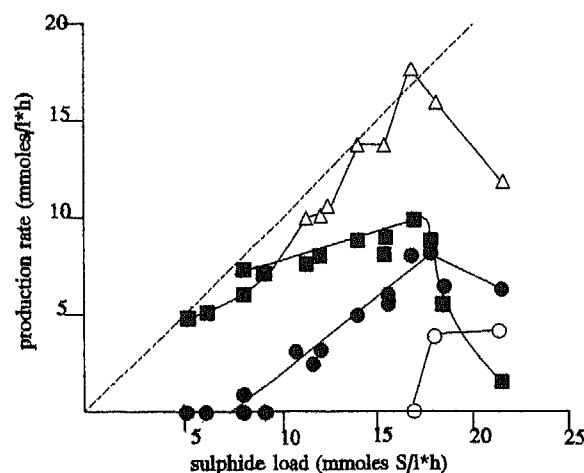


Fig. 7. The effect of increasing loads on sulphur production by sulphide-oxidizing chemostat cultures of *Thiobacillus neapolitanus*. Open triangles = total S calculated; closed squares = sulphate; closed circles = S⁰; open circles = H₂S stripping; broken line = 100% S recovery (G.C. Stefess, L.A. Robertson & J.G. Kuenen, unpublished results).

equated and sulphide began appearing in the off-gas (G.C. Stefess, L.A. Robertson & J.G. Kuenen, unpublished results). A pilot plant based on this reaction is being used to treat the effluent from a paper mill (Buisman 1989). The behaviour of a new autotrophic strain isolated from this reactor, currently designated *Thiobacillus* W1, was similar to that of *T. neapolitanus*, but differed in that above a certain sulphide load, sulphur production increased at the expense of sulphate production, rather than a constant level of sulphate being generated. Thus, sulphur production by *Thiobacillus* W1 under oxygen limitation could account for up to 70% of the sulphide added. With *T. neapolitanus* this never exceeded 50%. produced a greater proportion of sulphur.

The recovery of elemental sulphur is now being considered for integration in another process in which sulphate reducing bacteria are employed to generate sulphide from sulphate-rich wastewater. This sulphide is then used to precipitate dissolved metals. However, the sulphide generated from reduction of the sulphate is generally more than is required, and this must be dealt with. Sulphur production holds obvious attractions for this process,

as well as for others where the dissolved sulphate level must be reduced.

Treatment of organic sulphides

As mentioned above, organic sulphides contribute significantly to environmental problems. The bacteria able to metabolize these compounds and gain energy from the sulphide oxidation appear to fall into two groups, chemolithoautotrophs that fix their carbon via the Calvin cycle (e.g. *Thiobacillus thioparus*; Kanagawa & Kelly 1986), and chemolithoheterotrophs that use the serine pathway (e.g. *Hyphomicrobium* S; de Bont et al. 1981). Another *Hyphomicrobium* species, *Hyphomicrobium* EG was isolated from a sample from a paper mill waste treatment system, and studied in detail (Suylen & Kuenen 1986; Suylen et al. 1986). Despite the fact that the cultures were grown on methyl mercaptan, dimethyl sulphide or hydrogen sulphide, all three substrates remained toxic to the culture, apparently inhibiting the enzymes of organic sulphide breakdown (Suylen 1988). Sulphide oxidation was least affected (Table 7). pH was also critical, with the toxicity of DMS and MM increasing as the pH fell. Any wastewater treatment system employing such organisms should therefore be designed in such a way as to prevent sudden environmental changes or pulses of sulphide or overloads. For the same reasons, the reactor should be well mixed, a plug flow type would not be suitable. The treatment of gases containing these compounds may not be such a problem, provided that high concentra-

tions of biomass can be maintained in the reactor, because the gases will gradually dissolve as they pass through the water phase rather than entering the reactor as a concentrated solution. Indeed, such a system has been used on the laboratory scale to deodourize gases containing H₂S and organic sulphides (Kanagawa & Mikami 1989; Tanji et al. 1989).

Future prospects

The human race is steadily increasing, and with it our demands on the resources of the planet. If we are not to emulate a batch bacterial culture and perish in a mess of waste products, it is essential that we increase the efficiency of our activities, while ensuring that they have as little further impact on the environment as possible. The recent reports of holes in the ozone layer over the Northern as well as the Southern hemispheres and the apparent correlation between algal blooms and sea mammal mortality serve to emphasize that we can no longer see the oceans, the atmosphere, or the planet as a whole as bottomless pits into which our wastes can be dumped with impunity. It will be essential that renewable resources are used, and that 'clean technology' processes are developed and constructed as (almost) closed cycles. Of course, no process will ever be completely without waste products, and tailor-made, dedicated wastewater treatment systems will have to become a natural part of industrial operations, either within the cycle or as an 'end of the pipe' solution. To this effort, those working on the treatment of sulphidic wastes can greatly contribute, both in preventative measures and, when necessary, for cures.

Table 7. The effect of dimethyl sulphide (DMS), methyl mercaptan (MM) and hydrogen sulphide on the oxidation of dimethyl sulphoxide (DMSO), DMS, MM and H₂S by *Hyphomicrobium* EG (data from Suylen 1988).

Inhibitor	Maximum inhibition (as % of original)				Approx. concentration giving 50% of maximum inhibition (mM)			
	DMSO	DMS	MM	H ₂ S	DMSO	DMS	MM	Na ₂ S
DMS	60	40	40	35	0.5	1.25	1	0.5
MM	100	100	70	25	0.1	0.1	<0.1	<0.1
Na ₂ S	90	70	20	20	0.1	0.18	0.18	0.2

Coal and other minerals

As discussed, bacterial processes are feasible for the removal of inorganic sulphides from coal (for a review see Bos et al. 1993). The process described here is about to be tested on the pilot plant scale. The economic feasibility of the process, however, must be regarded as borderline. One of the stron-

gest economic factors is the cost of energy – as oil prices go up, coal cleaning becomes more attractive. It is especially appropriate for small-scale industrial use when coal-water mixtures are needed. The coal can be centrally treated and then transported. This has the added attraction of reducing the amount of corrosion likely to occur during transportation because of the activities of the sulphur oxidizing bacteria. Thermophilic bacteria have not proved appropriate because their environmental requirements also promote the formation of jarosite-like precipitates, defeating the object of the exercise. The solution to the problem posed by organic sulphides is likely to be more problematical. Although some promising results have been obtained with model substrates (for a review, see Bos et al. 1993), the dispersal of the sulphur within the coal macromolecules (making it inaccessible) remains a major difficulty.

Bacterial leaching of low quality ores appears to have a brighter future. Acidophilic sulphur pyrite-oxidizing bacteria have long been used for the extraction of copper. 15% of the copper produced in the USA comes from large scale leaching operations. (Brierly 1982) Sulphuric acid produced from pyrite by acidophiles is also used to leach uranium from low grade ores. Finally, a recent development is that acidophilic, pyrite oxidizing bacteria are being used to remove pyrites from gold-bearing ores, making the gold more accessible to chemical extraction.

Wastewater treatment and sulphur production

Sulphur-based denitrification is still occasionally mentioned in the literature, but the need to reduce sulphate output is making this less attractive. Similarly, sulphide removal by means of complete oxidation to sulphate is equally unattractive. Research into the possibilities of removing the sulphide as sulphur is continuing, together with investigations into the feasibility of removing sulphate from waste streams by conversion to sulphur via a sulphate reduction step. The problems associated with specialized waste streams (e.g. photographic waste) still

remain, and will probably require a less than simple solution (i.e. alternative formulations).

Research into the treatment of the organic sulphides is now attracting a great deal of attention, and a coordinated, inter-University research programme has begun in The Netherlands. This programme will look at both natural and anthropogenic organic sulphide generation, environmental impact and treatment.

Concluding remarks

Much remains to be done, and it is really up to governments at the national and international level to set realistic targets for controlling sulphur emissions. If this is not done, industry will not implement treatment procedures which will add to their costs, while their competitors do not. It is to the credit of some industrial concerns that they are already carrying out research into the further control of sulphur emissions, but it is now time to bring the others into line.

References

- Beudeker RF, Gottschal JC & Kuenen JG (1982) Reactivity versus flexibility in thiobacilli. *Antonie van Leeuwenhoek* 48: 39–51
- Bos P (1991) Microbial desulfurization of coal: a feasible process. In: Hecq W & Crine M (Eds) *Microbial Processes for Heavy Metal Recovery* (pp 63–78). University de Liège
- Bos P & Kuenen JG (1989) Microbial treatment of coal. In: Brierley C & Ehrlich H (Eds) *Microbial Metal Recovery* (pp 343–377). McGraw Hill Book Company, New York
- Bos P & Kuenen JG (1990) Microbial treatment of coal. In: Erlich H & Brierley C (Eds) *Microbial Mineral Recovery* (pp 344–377). McGraw-Hill Book Company, New York
- Bos P, Huber TF, Luyben KChAM & Kuenen JG (1988) Feasibility of a Dutch process for microbial desulphurization of coal resources. *Conservation and Recycling* 1: 279–291
- Bos P, Boogerd FC & Kuenen KG (1993) Microbial desulfurization of coal. In: Mitchell R (Ed) *New Concepts in Environmental Microbiology*. John Wiley & Sons, Chichester (in press)
- Brierly CL (1982) Microbiological mining. *Scientific American* 247 (August): 42–51
- Buisman CJN (1989) Biotechnological sulphide removal with oxygen. PhD Thesis, Agricultural University of Wageningen
- Butlin KR & Postgate JR (1954) The microbiological formation

- of sulphur in Cyrenaican lakes. In: Cloudsley-Thompson J (Ed) *Biology of Deserts* (pp 112–122). Institute of Biology, London
- de Zwart JMM & Kuenen JG (1992) C_1 cycle of sulfur compounds. *Biodegradation* 3: 37–59
- de Bont JAM, van Dijken JP & Harder W (1981) Dimethyl sulphoxide and dimethyl sulphide as a carbon, sulphur and energy source for growth of *Hyphomicrobium* S. J. *Gen. Microbiol.* 127: 315–323
- Dicks RM & Ottengraf SP (1991) Process engineering aspects of biological waste gas purification. In: Verachtert H & Verstraete W (Eds) *Environmental Biotechnology 1991* (pp 353–367). European Federation of Biotechnology
- Gommers PJF & Kuenen JG (1988) *Thiobacillus* strain Q, a chemolithoheterotrophic sulphur bacterium. *Arch. Microbiol.* 150: 117–125
- Gommers PJF, Bijleveld W, Zuidervijk FJM & Kuenen JG (1988) Simultaneous sulfide and acetate oxidation in a denitrifying fluidized bed reactor-II. Measurements of activities and conversion. *Water Res.* 22: 1085–1092
- Gottschal JC, de Vries S & Kuenen JG (1979) Competition between the facultatively chemolithotrophic *Thiobacillus* A2, and obligately chemolithotrophic *Thiobacillus* and a heterotrophic spirillum for inorganic and organic substrates. *Arch. Microbiol.* 121: 241–249
- Gottschal JC, Nanninga HJ & Kuenen JG (1981) Growth of *Thiobacillus* A2 under alternating growth conditions in the chemostat. *J. Gen. Microbiol.* 126: 85–96
- Isaksen ISA (1987) Is the oxidizing capacity of the atmosphere changing? In: Rowland FS & Isaksen ISA (Eds) *The Changing Atmosphere* (pp 141–158). John Wiley & Sons, Chichester
- Jørgensen BB (1982) Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. *Philosophical Transactions of the Royal Society of London* B298: 543–561
- Kanagawa T & Kelly DP (1986) Breakdown of dimethyl sulphide by mixed cultures and by *Thiobacillus thioparus*. *FEMS Microbiol. Lett.* 34: 13–19
- Kanagawa T & Mikami E (1989) Removal of methanethiol, dimethyl sulfide, dimethyl disulphide and hydrogen sulfide from contaminated air by *Thiobacillus thioparus* TK-m. *Appl. Environ. Microbiol.* 55: 555–558
- Kelly DP & Harrison AP (1989) The Genus *Thiobacillus*. In: Staley JT, Bryant MP, Pfennig N & Holt JG (Eds) *Bergey's Manual of Systematic Bacteriology* 3 (pp 1842–1858). Williams & Wilkins, Baltimore
- Kuenen JG (1989) Colorless sulfur bacteria. In: Staley JT, Bryant MP, Pfennig N & Holt JG (Eds) *Bergey's Manual of Systematic Bacteriology* 3 (pp 1834–1837). Williams & Wilkins, Baltimore
- Kuenen JG & Bos P (1989) Habitats and ecological niches of chemolitho(auto)trophic bacteria In: Schlegel HG & Bowien B (Eds) *Autotrophic Bacteria* (pp 53–80). Brock/Springer Series in Contemporary Bioscience, Science Tech. Publ., Madison, WI, Springer-Verlag, Berlin
- Kuenen JG, Robertson LA & van Gernerden H (1985) Microbial interactions among aerobic and anaerobic sulfur-oxidizing bacteria. *Adv. Microb. Ecology* 8: 1–60
- Kuenen JG, Robertson LA & Tuovinen OH (1992) The colourless sulphur bacteria In: Balows A, Truper HG, Dworkin M, Harder W & Schlefer KH (Eds) *The Prokaryotes*, Vol III (pp 2636–2657) Springer-Verlag, New York
- Lane DJ, Harrison AP, Stahl DA, Pace B, Giovannoni SJ, Olsen GJ, Heller DJ & Pace NR (1992) Evolutionary relationships among sulfur and iron oxidizing eubacteria. *J. Bacteriol.* 174: 269–278
- Legrand MR & Delmas RJ (1987) A 220-year continuous record of volcanic H_2SO_4 in the Antarctic ice sheet. *Nature* 327: 671–676
- Lein AYU (1983) The sulphur cycle in the lithosphere, part II, cycling. In: Ivanov MV & Freney JR (Eds) *The Global Biogeochemical Sulphur Cycle* (pp 95–128). John Wiley, Chichester
- Nanninga HJ (1987) Sulfate reduction and fermentation of amino acids in industrial waste water. PhD Thesis, University of Groningen, The Netherlands
- Postgate JR (1982) Economic importance of sulphur bacteria. *Philosophical Transactions of the Royal Society of London* B298: 583–600
- Ramanathan V (1987) The radiative and climatic consequences of the changing atmospheric concentrations of trace gases. In: Rowland FS & Isaksen ISA (Eds) *The Changing Atmosphere* (pp 159–186). John Wiley & Sons, Chichester
- Robertson LA & Kuenen JG (1984) Anaerobic and aerobic denitrification by sulphide oxidizing bacteria from waste water. In: van den Brink WJ (Ed) *Anaerobic Waste Water Treatment* (pp 3–12). AWWT Symposium Secretariat (pubs)
- Robertson LA & Kuenen JG (1992) Aerobic sulphur oxidizing bacteria. In: Balows A, Truper HG, Dworkin M, Harder W & Schlefer KH (Eds) *The Prokaryotes*, Vol I (pp 385–413). Springer-Verlag, New York
- Ryaboshapko AG (1983) The atmospheric sulphur cycle. In: Ivanov MV & Freney JR (Eds) *The Global Biogeochemical Sulphur Cycle* (pp 203–277). John Wiley, Chichester
- Stauffer BR & Neftel A (1987) What have we learned from the ice cores about the atmospheric changes in the concentrations of nitrous oxide, hydrogen peroxide and other trace species? In: Rowland FS & Isaksen ISA (Eds) *The Changing Atmosphere* (pp 63–78). John Wiley & Sons, Chichester
- Stefess GC & Kuenen JG (1989) Factors influencing elemental sulphur production from sulphide or thiosulphate by autotrophic thiobacilli. *Forum Microbiologie* 12: 92
- Suylen GMH (1988) Microbial metabolism of dimethyl sulphide and related compounds. PhD Thesis, Delft University of Technology, The Netherlands
- Suylen GMH & Kuenen JG (1986) Chemostat enrichment and isolation of *Hyphomicrobium* EG. A dimethyl-sulphide oxidizing methylotroph and reevaluation of *Thiobacillus* MS1. *Antonie van Leeuwenhoek* 52: 281–293
- Suylen GMH, Stefess GC & Kuenen JG (1986) Chemolithotrophic potential of a *Hyphomicrobium* species, capable of

- growth on methylated sulphur compounds. Arch. Microbiol. 146: 192–198
- Tanji Y, Kanagawa T & Mikami E (1989) Removal of dimethyl sulfide, methyl mercaptan and hydrogen sulfide by immobilized *Thiobacillus thioparus* TK-m. Journal of Fermentation and Bioengineering 67: 280–285
- WHO (1984a) Guidelines for drinking water quality. Vol 1. Recommendations. Pubs WHO, Geneva
- WHO (1984b) Guidelines for drinking water quality. Vol 2. Health criteria and other supporting information. Pubs WHO, Geneva
- Woese CR (1991) Prokaryote systematics: the evolution of a science. In: Balows A, Truper HG, Dworkin M, Harder W & Schlefer KH (Eds) The Prokaryotes, Vol I (pp 3–18). Springer-Verlag, New York