Microbiology of sulphur-oxidizing bacteria

Besides the role of sulphate-reducing bacteria in metal corrosion, that of sulphur-oxidizing bacteria should be considered. Especially the acidophilic members of the genus Thiobacillus have been reported frequently in this respect. Their corrosive effects may be attributed to their ability to produce sulphuric acid and therefore low pH values from inorganic reduced sulphur compounds. Some possess the ability to oxidize ferrous iron as well (e.g., Thiobacillus ferrooxidans and Sulfolobus species). They are found in environments with high concentrations of inorganic reduced sulphur compounds and low water activity, such as coal mines, acid soils and sulphur deposits.

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INTRODUCTION

When microbial corrosion is mentioned, it is the corrosion caused by sulphate-reducing bacteria which comes immediately to mind. Indeed, the damage caused by anaerobic, bacterial corrosion accounts for millions of pounds per year (Postgate, 1), and therefore the understanding and prevention of the microbial processes involved are of the utmost economical importance. However, by specifically stressing the importance of anaerobic corrosion by organisms such as Desulfovibrio desulfuricans one may overlook the general corrosive effects caused by many other types of organisms. In general, we may expect corrosion by organisms producing acids, the most effective being, of course, those that are not only acid producers, but also acidophilic or at least acid tolerant. These organisms are active at very low pH values, and will be effective in the dissolution of metals and other materials such as concrete.

The detrimental effects of fungi and especially sulphur oxidizing bacteria, such as members of the genus Thiobacillus, on metals and concrete have been described in the literature. The corrosive effects of the thiobacilli can mainly be attributed to their ability to produce sulphuric acid from the oxidation of inorganic reduced sulphur compounds or elemental sulphur, and occur especially in environments characterized by low pH. Obvious examples of this type of corrosion are those involving acid mine effluents or acid soils (Iverson, 2; Hughes, 3; Postgate, 4).

From the foregoing it may be concluded that within the scope of a symposium on microbial corrosion, attention to sulphur oxidizing bacteria and especially representatives of the genus Thiobacillus is justified. We will discuss the importance of sulphur oxidizing bacteria in the global sulphur cycle to demonstrate their ubiquity in nature, and then consider different types of sulphur oxidizing bacteria. Although a large variation in morphology, physiology and ecology exists, the number of sulphur oxidizing species relevant to metal corrosion is probably limited. These relevant species will be discussed in more detail in order to understand their corrosive activities. Special attention will be paid to their habitats and to the possible methods for detecting these bacteria.

THE ROLE AND IMPORTANCE OF SULPHUR OXIDIZING BACTERIA IN THE SULPHUR CYCLE

Before considering the role and importance of sulphur oxidizing bacteria in nature, it is important to realize that they comprise two groups:

- the colourless sulphur oxidizing bacteria,
- the coloured sulphur oxidizing bacteria.

The first group oxidizes sulphur compounds aerobically with oxygen or, sometimes anaerobically with nitrate as the terminal electron acceptor. The second group are photosynthetic, requiring light, and oxidize sulphur compounds anaerobically. The relevant chemical reactions are summarized in Table 1.

Fig. 1 shows a schematic sulphur cycle (Kuenen, 5). The main reservoirs of sulphur and sulphur compounds in nature are deposits of sulphidic minerals and elemental sulphur, whereas (dissolved) sulphates occur mainly in the marine environment. Both in soil and aquatic systems, hydrogen sulphide is produced from either the decay of sulphur-containing biological materials, or by sulphate-reducing bacteria. Sulphate reduction is particularly important in marine sediments. The hydrogen sulphide will either be precipitated as metal sulphides or will enter water, soil, or the air, and then may be spontaneously (chemically)
TABLE 1. Reactions catalyzed by types of sulphur oxidizing bacteria

<table>
<thead>
<tr>
<th>Aerobic sulphur oxidizers:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2\text{S} + 2\text{O}_2 \rightarrow \text{H}_2\text{SO}_4 )</td>
<td>(1)</td>
</tr>
<tr>
<td>or:</td>
<td></td>
</tr>
<tr>
<td>( 2\text{S}^2 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{SO}_4 )</td>
<td>(2)</td>
</tr>
<tr>
<td>Nitrate reducing sulphur oxidizers:</td>
<td></td>
</tr>
<tr>
<td>( \text{SH}_2 + 8\text{NO}_3^- \rightarrow 4\text{N}_2 + 5\text{SO}_4^{2-} + 4\text{H}_2\text{O} + 2\text{H}^+ )</td>
<td>(3)</td>
</tr>
<tr>
<td>Photosynthetic sulphur oxidizers:</td>
<td></td>
</tr>
<tr>
<td>( \text{CO}_2 + 2\text{H}_2\text{S} \rightarrow (\text{CH}_2\text{O}) + 2\text{S} + \text{H}_2\text{O} )</td>
<td>(4)</td>
</tr>
<tr>
<td>or:</td>
<td></td>
</tr>
<tr>
<td>( 2\text{CO}_2 + \text{H}_2\text{S} + 2\text{H}_2\text{O} \rightarrow 2(\text{CH}_2\text{O}) + \text{H}_2\text{SO}_4 )</td>
<td>(5)</td>
</tr>
<tr>
<td>cell material</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2. Colourless sulphur oxidizing bacteria
(adapted from Kuenen and Beudeker, 5).

<table>
<thead>
<tr>
<th>Obligate chemolithoautotrophs</th>
<th>Facultative chemolithoautotrophs</th>
<th>Chemolithoheterotrophs</th>
</tr>
</thead>
<tbody>
<tr>
<td>aerobes</td>
<td>aerobes</td>
<td>Thiobacillus peromutabilis</td>
</tr>
<tr>
<td><em>Thiobacillus neapolitanus</em></td>
<td><em>Thiobacillus intermedium</em></td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td><em>thiooxidans</em></td>
<td><em>nobilis</em></td>
<td>Beggiaota spp.</td>
</tr>
<tr>
<td><em>concretivorus</em></td>
<td><em>acidophilus</em></td>
<td>Heterotrophs</td>
</tr>
<tr>
<td><em>ferrooxidans</em></td>
<td><em>organonovus</em></td>
<td>Beggiaota spp.</td>
</tr>
<tr>
<td><em>kabobia</em></td>
<td><em>Sulfolobus acidocaldarius</em></td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td><em>Thiomicrospira pelophila</em></td>
<td><em>brierleyi</em></td>
<td></td>
</tr>
<tr>
<td>facultative anaerobes</td>
<td><em>sulfurarius</em></td>
<td></td>
</tr>
<tr>
<td><em>Thiobacillus denitrificans</em></td>
<td>facultative anaerobes</td>
<td>Unclassified</td>
</tr>
<tr>
<td><em>thiopara</em></td>
<td><em>Thiobacillus Al</em></td>
<td>Achromatium, <em>Novomonas</em>,</td>
</tr>
<tr>
<td><em>Thiomicrospira denitrificans</em></td>
<td><em>Thermotrichia thiopara</em></td>
<td>Thiobacillus, Thioploca,</td>
</tr>
<tr>
<td></td>
<td><em>Paracoccus denitrificans</em></td>
<td>Thiopira, Thiorthrix,</td>
</tr>
<tr>
<td></td>
<td><em>Thiomphaera pantotropha</em></td>
<td>Thiovulum.</td>
</tr>
</tbody>
</table>

TABLE 3. Characteristics of some acidophilic colourless sulphur oxidizing bacteria

<table>
<thead>
<tr>
<th>energy source</th>
<th>carbon source</th>
<th>mesophilic</th>
<th>thermophilic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobacillus thiooxidans</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ferrooxidans</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>acidophilus</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sulfolobus acidocaldarius</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>brierleyi</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>sulfurarius</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
or biologically oxidized. In anaerobic environments the oxidation of inorganic sulphur compounds will be predominantly biological and will be restricted to places where light or nitrate are available. In aerobic environments, biological oxidation must compete with spontaneous oxidation, and the high rate of spontaneous oxidation of relatively high concentrations of hydrogen sulphide observed have often led to the conclusion that biological oxidation would be unimportant in nature. This interpretation overlooks the fact that naturally occurring hydrogen sulphide concentrations in the presence of oxygen are usually low, in the order of 10^{-5} to 10^{-6} M. Chen and Morris [6] have shown that with 10^{-4} M oxygen and hydrogen sulphide, the half life for spontaneous oxidation is about 30 to 60 minutes. Thus, with concentrations of 10^{-5} to 10^{-6} M, the half life would be even higher. By means of continuous cultures of Thiobacillus species, Beudeker et al. [7] have shown that, at these concentrations, the biological oxidation of hydrogen sulphide proceeds at a very high rate with virtually no interference by spontaneous oxidation. Under such conditions, hydrogen sulphide is completely oxidised to sulphuric acid and no intermediary products such as elementary sulphur are formed.

Jørgensen [8] has shown that at oxygen - hydrogen sulphide interfaces in aquatic systems, the bacterial sulphide oxidation accounts for up to 10% of the total oxidation. The oxidation rate of sulphide in the presence of the sulphur oxidizing organism Thiovulum was about 25,000 times higher than would be expected from the spontaneous oxidation which would proceed very slowly at the observed concentrations of about 5.10^{-5} M for both oxygen and hydrogen sulphide.

We might conclude that the colourless sulphur bacteria, especially at the interface where reduced inorganic sulphur compounds must oxygen must play an important role in the recycling of sulphur compounds. For that reason they are ubiquitous in nature, and can be isolated from almost everywhere in the aquatic system. Although highly interesting from the microbiologists point of view, the coloured photosynthetic sulphur bacteria are of less importance, since they need both light and anaerobic conditions for growth, and these will occur only in shallow sediments and in some special habitats as stratified lakes.

**THE COLOURLESS SULPHUR OXIDIZING BACTERIA**

Kuenen and Beudeker [9] have recently reviewed the colourless sulphur bacteria. Table 2 shows the genera and species involved. As mentioned before, virtually all are aerobic, using oxygen for the oxidation of sulphur compounds. Some are facultative anaerobes, able to use nitrate as an alternative to oxygen. Most of the organisms in Table 2 are able to obtain energy from sulphur oxidation, but it is known that a few do not. It has been suggested that these latter organisms benefit from the oxidation of hydrogen sulphide from their environment. In Beggiatoa, the sulphur oxidation may not only be useful in the generation of energy, but also in the formation of intracellular globules of sulphur. These can be used as an electron acceptor for the oxidation of organic compounds in anaerobic conditions (Nelson and Castenholz, 10).

Quite a number of the genera and species mentioned in Table 2 have not been studied in detail. Most members of the genera Achromatium, Macromonas, Thiobacterium, Thioploca, Thiospira, Thiocystis, Thiovulum and many Beggiatoa species have not been obtained in pure culture (Le Riévere & Schmidt, 11; Wiessner, 12). Most of these organisms are found in places where low concentrations of hydrogen sulphide and oxygen are present. They all have an unusual morphology and are very large compared to most other prokaryotes. They are all characterized by conspicuous intracellular sulphur globules which may disappear when the cells are exposed to oxygen without sulphide. In the presence of sulphide the number of sulphur globules may increase, and in nature are invariably present. They are only found in environments which contain sulphide. Therefore their ability to oxidize sulphide is not doubted. However, very little is known of their special growth requirements. For that reason appropriate enrichment techniques are still not available.

All of the genera so far mentioned are found in aquatic environments at fairly neutral pH-values (6-8) and therefore they do not seem to be involved in the substantial reduction of the pH in their environment. However, they may precondition the environment by lowering the pH to the point where the acidophilic species may start to grow. Their role in corrosion is unknown.

Members of the genus Thiobacillus (Kuenen and Tuvvinen, 13) and related genera such as Thiomicrospira (Kuenen and Tuvvinen, 13) and Thiosphaera (Robertson and Kuenen, 14) are of most interest. The genus Sulfolobus (Brock, 15) should also be mentioned. Their morphology is far from spectacular. They are small rods, spirillii, or cocci, without inclusions visible under the light microscope. They lack photosynthetic pigments. This group of organisms is characterized by its diversity and heterogeneity. It comprises both aerobic and facultative anaerobes that are able to use nitrate as alternative to oxygen. Most are mesophilic (optimum temperature between 20 and 35°C), and some thermophilic isolates are known.

Sulfolobus species are true thermophiles able to grow at temperatures up to about 90°C. The sulphur oxidizers may prefer dissolved oxygen concentrations ranging from close to saturation. Most prefer neutral pH values, but some are acidophilic. Their common characteristic is their ability to oxidize reduced inorganic sulphur compounds and/or elemental sulphur. Almost every natural ecosystem, depending on its conditions, may be expected to contain an appropriate sulphur oxidizer. This might be illustrated by the following data (Beudeker et al., 7).

In the genus Thiobacillus a wide variety of physiological types exist (Table 2). The spectrum ranges from obligate chemolithoautotrophs, via facultative chemolithoautotrophs and mixotrophs, to chemolithohterotrophs.

An obligately chemolithoautotroph derives its energy from the oxidation of inorganic sulphur compounds and CO₂ serves as the carbon source. This is a highly oxidizing type of organism, unable to grow on organic compounds, of which Thiobacillus neapolitanus is an example. It has a high maximal specific growth rate of 0.35 h⁻¹ and can respire sulphur compounds at a correspondingly high rate. The maximal oxygen uptake rate found with thioplloca was 5 μl O₂ min⁻¹ mg dry weight⁻¹. This is roughly ten times faster than found for heterotrophic bacteria. It should be
The sulphur cycle

**FIGURE 1. The sulphur cycle**

mentioned here that in the laboratory thiosulphate is used as a convenient alternative to sulphide, as the former will show no spontaneous oxidation. The rate at which the known thiobacilli oxidise thiosulphate is identical to that of sulphide.

*Thiobacillus A2* is a facultative chemolithoautotroph. Both inorganic sulphur and organic compounds can serve as energy sources, and either CO₂ or organic compounds can act as carbon sources. *Thiobacillus A2* can also grow mixotrophically, when it uses organic and sulphur compounds simultaneously. During growth on thiosulphate alone, its maximal growth rate reaches 0.1 h⁻¹ and the maximal oxygen uptake rate 0.8 μM O₂ min⁻¹ mg dry weight⁻¹. During mixotrophic growth on thiosulphate and acetate the maximal growth rate doubles.

It turned out to be possible to predict the selection and/or coexistence of these two metabolic types in nature. On the basis of pure and mixed cultures studies with these two organisms in the laboratory it was hypothesized that in nature, in environments where the rate of sulphide supply and metabolism is high compared to that of organic compounds, the more specialized organisms such as *Thiobacillus neapolitanus* could compete successfully with the more versatile *Thiobacillus A2*. In turn, organisms such as *Thiobacillus A2* would be favoured in environments which are supplied simultaneously with sulphur and organic compounds in quantities of similar order of magnitude (Beudeker et al., 7). This hypothesis has been shown to hold in continuous flow systems such as chemostat enrichment cultures and sewage plants for the treatment of sulphide rich effluents (Kuenen and Robertson, 16).

The chemolithoheterotrophic thiobacilli can obtain energy from the oxidation of reduced sulphur compounds, but lack the ability to grow autotrophically (London and Rittenberg, 17). They need an organic source of carbon, which may also be used as energy source. Thus these organisms are essentially heterotrophs. An often quoted representative of this group is *Thiobacillus permutans*. Although it has recently been reported that this particular organism is able to grow autotrophically, there is little doubt that such organisms not only exist in nature (Cuttle, 18; Kelly and Kuenen, 19), but may also play a dominant role in the
turnover of reduced inorganic sulphur compounds. Organisms of the *Thiobacillus perometabolis* type would fit into a niche where the ratio between the turnover rates of reduced inorganic sulphur compounds and of organic compounds is low (Kuenen and Beudeker, 9).

**ACIDOPHILIC COLOURLESS SULPHUR OXIDIZING BACTERIA**

All colourless sulphur oxidizing bacteria produce sulphuric acid from reduced inorganic sulphur compounds and elemental sulphur. Most of them will become inactive if the pH drops too far. Their role can then be taken over by acidophilic sulphur oxidizing bacteria, and these are especially important in connection with corrosion. In Table 3, some characteristics of the acidophilic organisms *Thiobacillus ferroxidans* (Colmer et al., 20), *Thiobacillus thiooxidans* (Starkey, 21), *Thiobacillus acidophilus* (Gauy and Silver, 22), *Sulfobulbus acidocaldarius* (Brock et al., 23), *Sulfolobus brierleyi* and *Sulfolobus sulfuriferus* (Zillig et al., 24) are given. Some are obligate chemolithoautotrophs, others are facultative chemolithotrophs. An acidophilic chemolithoautotroph has not yet been isolated. The pH optimum of the acidophiles is around pH 2.5. In shaken cultures the pH may eventually drop to pH 1, and cultures of *Thiobacillus thiooxidans* have been reported to survive even at pH 0 (Harmsen, 25).

*Thiobacillus ferroxidans* can not only derive its energy for growth from the oxidation of reduced sulphur compounds, but it can also obtain metabolic energy from the oxidation of ferrous to ferric iron, a potent corrosive agent. This iron oxidizing ability is unique among the members of the genus *Thiobacillus*. *Sulfobulbus acidocaldarius*, *Sulfolobus brierleyi*, and *Sulfolobus sulfuriferus* also combine the ability to derive their energy from both ferrous iron and inorganic sulphur oxidation (Brock, 15). In the microbial world there are several genera of non-sulphur oxidizing bacteria that share the ability to oxidize iron, e.g. Gallionella (Hanert, 26). The latter live at neutral pH. Although they may be involved in detrimental effects on iron, they are beyond the scope of this paper. The three Sulfolobus species have been isolated from hot sulphur springs by Brock et al. (23), Brierley and Brierley (27) and Zillig et al. (24). Their optimal temperature ranges from 70 to 80°C. This is much higher than that of *Thiobacillus ferroxidans*. However, it should be stressed that besides the mesophilic *Thiobacillus ferroxidans* strains, thermophilic iron oxidizing thiobacilli also have been reported (Brierley et al., 28). The physiological resemblance between *Thiobacillus ferroxidans* and Sulfolobus species as far as iron and sulphur oxidation is concerned is strong, but the organisms are unrelated. Sulfolobus belongs to the Archaeabacteria (Brock, 15) whereas *Thiobacillus* belongs to the Eubacteria.

When considering the energetics of the microbial oxidation of ferrous iron it will be obvious that the benefit given by this process is marginal (Inglewed, 29). This is of course not only due to the low energy yield of iron oxidation, but also to the fact that these organisms must assimilate CO₂ for the synthesis of cell material. In order to fix CO₂ at an appropriate rate, enormous quantities of ferrous iron must be oxidized to produce a small amount of biomass. We have found that for 1 gram of biomass, about 150 grams of ferrous iron must be oxidized. The yield of biomass with sulphur as the energy source is much higher since, per sulphide ion oxidized, 8 electrons are available for respiration. Although growth rates observed are rather low (generation times 4–6 hours under optimal laboratory conditions), the ferrous iron oxidation rates are high. In our laboratory we have found oxygen uptake rates of about 1 μmol O₂ min⁻¹ mg⁻¹. A rapidly growing heterotroph such as *Bacterium coli* growing on glucose has a maximal oxygen uptake rate of about 0.25 μmol O₂ mg⁻¹ min⁻¹. The sulphur oxidizing bacteria often grow much faster than the iron oxidizers. It was mentioned before that these organisms may respire oxygen at rates which are even 10 times faster than heterotrophs such as *Bacterium coli*.

**THE NATURAL HABITATS OF ACIDOPHILIC COLOURLESS SULPHUR OXIDIZING BACTERIA**

*Thiobacillus ferroxidans*, *Thiobacillus thiooxidans* and *Thiobacillus acidophilus* have been found to be the major components of the microbial populations in some low pH sulphur-containing environments such as acid mine drainage water and acid soils (Lundgren et al., 30) Langworthy, 31; Arkesteyn, 32).

**Pyrite containing habitats**

Acid mine drainage water has long been an environmental problem, especially in the eastern parts of the United States. Drainage water from mines with pyrite rich coals has an extremely low pH. This water is characterized by a high iron content and brownish yellow precipitates of basic ferric sulphates. This heavily polluted water causes severe environmental problems, for example in some regions of the Allegheny Mountains. The up-flow parts of the Susquehanna are notorious for the damage to flora and fauna by these acid streams (Martin and Hill, 33).

The formation of acid mine water is initiated by spontaneous oxidation of pyrite present in the coal and wetted by drainage water. If the ratio between water and pyrite is comparatively low and no carbonates are present in the environment, the pH will fall rather rapidly. Once the pH has dropped below 5, spontaneous oxidation slows and a microbial population with the ability to obtain energy from the oxidation of pyrite will develop. This population can compete successfully with the spontaneous oxidation. The most important organism in acid mine water is *Thiobacillus ferroxidans* which has the ability to oxidize both the sulphur and the ferrous moiety of pyrite, according to the following equation:

\[
4\text{FeS}_2 + 15\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe}_2\text{(SO}_4)_3 + 2\text{H}_2\text{SO}_4
\]  

(6)

A direct contact mechanism in which the *Thiobacillus* cells are attached to the surface of the pyrite has been described (Marr, 34). The ferric iron formed may act as an oxidizing agent on the remaining pyrite. From pyrite ferrous iron, sulphate and elemental sulphur will be formed:

\[
\text{Fe}_2\text{S}_3 + 2\text{Fe}_2\text{(SO}_4)_3 \rightarrow 3\text{Fe}_2\text{SO}_4 + 2\text{O}_2
\]  

(7)

*Thiobacillus ferroxidans* can reoxidize the ferrous iron into ferric iron, which again starts to oxidize pyrite. Other sulphide minerals present in the coal can also be attacked in a similar way. Copper can be dissolved according to the following equation:

\[
\text{CuFeS}_2 + 2\text{Fe}_2\text{(SO}_4)_3 + \text{CuSO}_4 + 5\text{Fe}_2\text{SO}_4 + 2\text{O}_2
\]

(8)
Thiobacillus ferrooxidans is always accompanied in acid mine water by other acidophilic sulphur oxidizing bacteria such as Thiobacillus thioxidans and Thiobacillus acidophilus. These cannot oxidize iron and must probably oxidize the elemental sulphur formed in reactions 7 and 8 (Karavaiko and Noshikova, 35).

Another important habitat for acidophilic thiobacilli is acid soil. Recently Arkesteijn (32) studied the role of Thiobacillus ferrooxidans in the formation of acid soil. Some soil, especially sediments in coastal regions, will accumulate high amounts of pyrite. In mangrove forests in tropical coastal areas, the sediments will be supplied by large amounts of organic matter and sulphate from the sea water forced into the sediments by tidal movements. If iron is present and hydrogen sulphide is formed by the activity of sulphate-reducing bacteria, iron sulphide will be formed. Occasional exposure to oxygen results in partial oxidation of the sulphide and elemental sulphur can be formed. From elemental sulphur and ferrous sulphide, pyrite will be formed.

If pyritic soils are drained and become aerated, the same processes as described in the formation of acid mine drainage water will start. The initial spontaneous oxidation of pyrite results in a low pH, favourable for the development of Thiobacillus ferrooxidans and related acidophiles.

Precipitates of basic iron sulphates (jarosites) are also formed, turning these sediments brownish yellow. In some parts of the Netherlands which have been reclaimed from the sea, acid soils may occur. Because of their brownish yellow colour, they are called ‘cat clays’. These soils are characterized by their low pH and metals present will be susceptible to corrosion. This might be due to the activity of Thiobacillus ferrooxidans which is present in high numbers in these soils, as well as to the low pH. This will be discussed later.

**Sulphur deposits**

Natural deposits of elemental sulphur occur all over the world. Whenever these deposits are exposed to water and air, organisms such as Thiobacillus thiioxidans will eventually start to grow and produce very acid conditions. Artificial deposits, for example pyrites, can be enriched with natural gas, may also show the presence of an active population of Thiobacillus thiioxidans. As a result of vigorous acid production, the drainage water may strongly pollute the environment.

**THE ACIDOPHILIC THIOBACILLI AND CORROSION**

Acidophilic colourless sulphur oxidizing bacteria may generally be expected in those environments where comparatively high concentrations of reduced inorganic sulphur compounds are present and the amount of water is limited. By biological or sometimes spontaneous oxidation the pH of the water can fall below 5 and enrichment conditions for acidophilic sulphur bacteria are created if oxygen is available.

For example, in 1945 Parker (36) reported corrosion by a thiobacillus (Thiobacillus concretivorus) in concrete sewerage pipes, due to the biological oxidation of hydrogen sulphide from the sewage present in water condensing on the roof of the pipe.

For that reason it is also quite logical that sulphur containing materials will suffer from the detrimental effects of these organisms if they are exposed to air and humidity. Corrosion of sulphur containing stone constructions (Krumbein and Pochon, 37), degradation of sulphur containing rubber (Thaysen et al., 38), and metallic corrosion may also be expected in such environments. Metallic corrosion and acid formation can be attributed to the pH fall due to sulphuric acid production, but without sulphur present, microbial corrosion seems still to be possible. Recently Kino et al. (39) demonstrated rust removal from iron surfaces in cultures of Thiobacillus ferrooxidans. They suggested a scheme for the dissolution of metallic iron by Thiobacillus ferrooxidans. Ferrous iron in equilibrium with metallic iron will be removed by the biological oxidation, resulting in ferric iron which oxidizes the metal. Thus Thiobacillus ferrooxidans recycles the ferric ions required for the initial attack. An iron plate immersed in the culture liquid at room temperature for 20 hours showed pin holes with diameters of over 40 μ. However, these results have been obtained in the presence of extreme high ferric ion concentration (44 g/l).

Metal corrosion by acidophilic thiobacilli has been reported rather frequently. Some data are related to acid mine water (e.g. Retman, 40; Rawat et al., 41), others with corrosion in acid soils (Schwarzbegere, 42). Andreyuk et al. (43) showed that sulphur oxidizing bacteria had been involved in the corrosion of subway equipment. Rivière (44) demonstrated the corrosion of iron supports in vineyards due to the activity of Thiobacillus ferrooxidans. The conditions suited the organisms since sulphur powder had been sprayed as a fungicide. As a result, localized high concentrations of sulphur with low humidity were created, which in turn could lead to low pH values.

A potential source of corrosion lies in the future. We may expect an increase in coal utilization. New coal technologies have been developed in recent years to burn coal more efficiently, one of the options being the use of pulverized coal. In addition, the transport and combustion of coal-water slurries has been suggested. These slurries will provide suitable conditions for the growth of Thiobacillus ferrooxidans and related organisms because coal often contains considerable amounts of sulphidic minerals, especially pyrite. Simply by pulverizing the coal, pyrite hidden in the coal matrix will become accessible to spontaneous and biologically assisted oxidation. For that reason we may predict corrosion problems with the coal handling and storing equipment if the appropriate measures are not taken. When considering the habitats of acidophilic sulphur oxidizing bacteria, it should be stressed that the isolation of Thiobacillus ferrooxidans from the marine environment has never been reported. Notwithstanding their remarkable versatility and tolerance to heavy metals and low pH values, the strains of Thiobacillus ferrooxidans known today are all sensitive to chlorine (Lazaroff, 45). The occurrence of Thiobacillus thioxidans in the marine environment is not very well documented. It should be noticed that marine environments are highly buffered, and almost never show low water activity. Thus marine environments with pH values below 4 as required for Thiobacillus thiioxidans may be rare.

**PRACTICAL APPLICATIONS OF THE ACIDOPHILIC COLOURLESS SULPHUR OXIDIZING BACTERIA**

The mechanisms underlying the formation of acid mine water and acid soils are nowadays applied in
hydrometallurgy. With mixed cultures of Thiobacil-
lus ferroxidans and related acidophiles, low
grade ores can be leached (Kelly et al., 46;
Norris and Kelly, 47). About 10% of the copper pro-
duction in the United States is achieved by means
of microbial leaching (Brierley, 49). Uranium ores
can also be processed with bihydrometallurgical
techniques (Brierley, 49). The leaching is mediated
by ferric ions formed by the bacterial oxidation of
ferrous iron:
\[
\text{UO}_2 + \text{Fe}_2\left(\text{SO}_4\right)_3 \rightarrow \text{UO}_2\text{SO}_4 + 2\text{FeSO}_4
\]  
(9)

In the microbial leaching of ores, mixed cultures appear
to be more effective than pure cultures of
Thiobacillus ferroxidans (Norris and Kelly, 47).
The role of the other acidophilic sulphur oxidizers
has been discussed in the foregoing chapter. In
these mixed cultures acidophilic heterotrophs are
also present (Wichlacz and Unz, 50; Harrison, 51),
but little is known of their role. Perhaps they
contribute to the removal of traces of toxic pro-
ducts. It is known that acidophiles are poisoned
by low concentrations of organic acids (Rao and
Barger, 51). When the pH is low these compounds
can easily enter the cells as they are undissocia-
ted and can pass through the hydrophobic bacterial
membranes without active transport.

A new application of acidophilic thiobacilli, and
especially Thiobacillus ferroxidans is in the
microbial desulphurization of coal. In the future
we may expect an increase in coal utilization, and
can therefore predict enormous environmental pro-
blems if sulphur dioxide emissions from energy
plants are not reduced. In microbial desulphuri-
ization processes pyrite, the most important in-
organic sulphur compound in coal, will be removed
by the oxidation of Thiobacillus ferroxidans.
The coal must be pulverized and mixed with water
containing mineral salts to provide the organism with
essential elements. Oxygen and carbon dioxide
are supplied by aerating the coal-water slurry
(Dagan and Apel, 53; Dettz and Barvinchak, 54;
Hoffmann et al., 55).

In bihydrometallurgical processes and in the
microbial desulphurization of coal, the respon-
sible organisms come into contact with high con-
centrations of heavy metals. The extraordinarily
high tolerance of the acidophilic organisms for
heavy metals has probably resulted from selection
because of the high concentrations of these metals
in their natural environment (Tuovinen and Kelly,
56).

In the bihydrometallurgical processes, Thiobacil-
lus ferroxidans can be replaced by Sulfolobus
species. Members of this genus are thermophilic,
and more efficient leaching than that produced by
Thiobacillus ferroxidans has been reported
(Brierley and Brierley, 57).

THE COLOURED PHOTOSYNTHETIC SULPHUR OXIDIZING
BACTERIA

Although the coloured sulphur oxidizing bacteria
seem to be quantitatively less important in the
sulphur oxidation in their colourless counterparts,
to complete this survey they will be discussed
briefly.

The microbiology of anaerobic sulphur oxidizing
bacteria has recently been reviewed by Tröper and
Fischer (58). An up to date survey of the charac-
terization and isolation of the most important
representatives of this group has been given by
Tröper and Pfennig (59). Anaerobic oxidation of
reduced inorganic sulphur compounds and elemental
sulphur is performed by photosynthetic bacteria
belonging to the Chlorobiaceae (green sulphur
bacteria) and the Chromatiaceae (purple sulphur
bacteria). Some Rhodospirillaceae and cyanobacteria
are also able to utilize sulphide and/or sulphur
as an electron donor in photosynthesis. If hydro-
gen sulphide serves as such, elemental sulphur can
be produced as an intermediary or end product.

Sulphur is found as extracellular globules in the
Chlorobiaceae, or as intracellular globules in the
Chromatiaceae. These organisms may be found, for
example, in both fresh and marine habitats such as
lakes, ditches and shallow marine sediments,
especially in the upper anaerobic layers where
some light can penetrate. They contain high concen-
trations of photosynthetic pigments and are most
effective in trapping energy from light. Dense
blooms of these photosynthetic bacteria may deve-
lop in stratified water bodies with an anaerobic
sulphide-containing hypolimnion into which light
can penetrate. Especially in the late summer, when
the layer of sulphide containing water reaches its
highest level these blooms take place. This will
lead to spectacular colouration of the water. Mass
accumulation of these organisms may also be found
in the effluents of sulphur springs which are
riches in hydrogen sulphide.

High light intensities will result in an over-
growth by other photosynthetic organisms such as
cyanobacteria and algae. The coloured sulphur
bacteria can coexist with sulphate-reducing bacte-
ria (Jannasch, 60, Postgate, 4). In such a sys-
tem, a very high growth rate of sulphate-reducing
bacteria may be observed, even in the presence of
only trace amounts of sulphate, since the sulphide
produced will be recycled by reoxidation by the
photosynthetic bacteria (van Gemerden, 61). One
might speculate that corrosion mediated by sulphate-
reducing bacteria under sulphate limiting condi-
tions might be enhanced by the presence of photo-
synthetic bacteria. To our knowledge such effects
have never been reported.

ENUMERATION AND MEASUREMENT OF THE ACTIVITY OF
(ACIDOPHILIC) SULPHUR BACTERIA

A number of methods for the measurement of the
activity of sulphur oxidizing bacteria exist. The
presence of relatively high numbers of certain
bacteria often means that they are indeed active
in a given environment. However, one should rea-
лизize that high numbers of a certain metabolic type
are the result of a process which may already have
stopped. On the other hand, activity may be high
in spite of low numbers if the bacteria are rapid-
ly removed from an environment by protozoa or by
washing out.

The methods for the assessment of the invol-
vement of colourless sulphur bacteria in the
breakdown of sulphur compounds in any given envi-
rionment can roughly be divided into two categories:
the enumeration methods and the methods for the
measurement, either directly or indirectly, of
their activity.

Enumeration

In principle, some of the conspicuous colourless
sulphur bacteria can be recognized under the
microscope, even when they occur in complex micro-
bial populations. However, the more common types
such as the thiobacilli cannot be discriminated in this way from many other morphologically similar organisms. A sophisticated method involves the use of fluorescent antibodies in order to stain specifically the organelles where oxygen uptake is occurring for. This technique has been applied to enumerate Thio-
 bacillus ferrooxidans (Aeppli et al., 62). Other enumeration methods are based on the assumption that active cells should be able to reproduce and that each individual cell should be able to pro-
duce a subpopulation as a colony, or as a suspen-
sion of organisms after a suitable dilution of samples, and proper incubation of the bacteria on suitable selective media, colonies or dilutions showing growth can be counted. The latter method is known as the most probable number (MPN) method. For the choice of suitable media the reader is referred to the review by Kuenen and Tuovinen (13).

Activity measurements

Indirect methods for measuring the activity of sulphur oxidizing bacteria in samples involve the detection of acid (H₂SO₄) or SO₂-production, and the measurement of oxygen uptake. This method can be more sensitive and meaningful when radioactive ³²P compounds are used as a substrate. After incubation, the remaining labelled and un-
labelled substrates, together with the sulphur compounds can be analyzed and used for the assess-
ment of activity of the sulphur oxidizers. In most cases sulphate is not the only product. Substanti-
tial amounts of thiosulphate, tetrathionate, elemental sulphur and sulphite may be formed.

A very indirect method is based on the measure-
ment of sulphide-dependent dark CO₂ fixation. This method is based on the assumption that sul-
phur oxidizing are all autotrophic, and this is not necessarily the case, as we have shown in the paragraph on the colourless sulphur oxidizing bac-
teria. At the same time, the interpretation of the results of this method is troubled by the fact that all heterotrophic bacteria can fix some CO₂ as a part of heterotrophic carbon metabolism (Kuenen, 5). Jørgensen (8) approached the problem by using microelectrodes sensitive for O₂, pH, and sulphide to measure gradients and fluxes of these compounds in natural sediments. This is a very promising method which has been developed for the analysis of natural habitats which are very heterogenous having anaerobic pockets of less than 1 mm in diameter in an otherwise aerobic en-
virenment. These anaerobic areas would permit the growth of sulphate reducers and it is obvious that microelectrodes may be crucial in their detection. Once pockets of sulphur compounds such as pyrite or ferrous sulphide have been formed, these in turn be able to act as a potential source of acid production.

Concluding remarks

Although anaerobic sulphate-reducing bacteria cause most sulphuric corrosion, the role of sulphur oxidizing bacteria is substantial. In this respect, especially, the aerobic colourless sulphur oxidiz-
ing bacteria belonging to the genus Thiobacillus and physiologically related genera should be men-
tioned. They are ubiquitous in nature. Within these genera there is a large variation in physio-
logical types in temperature and pH requirement, etc. Corrosion by these organisms may be attributed mainly to the production of sulphuric acid from sulphur or reduced sulphur compounds. The acido-
philes are most harmful because they create low pH environments (up to pH 1). Some of them are also able to oxidize iron (Thiobacillus ferrooxi-
dans and Sulfolobus species). These enhance cor-
rosion by their ability to produce ferric iron, a potent corrosive agent.

The acidophilic sulphur oxidizing bacteria have been found in environments characterized by a comparatively high reduced sulphur compound or elemental sulphur content and low water activity e.g. acid mine water and acid soils. Corrosion by thiobacilli in such environments has been reported frequently. In the future, with the reintroduction of coal in the production of energy, coupled with new technologies in which pulverized coal-water slurry will be used, we may expect a substantial increase in the corrosion of equipment for coal handling and transport, caused by acidophilic sul-
phur oxidizing bacteria.

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