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Biogeochemical phenomena induced by bacteria within sulfidic mine tailings

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SUMMARY

Mill tailings resulting from mining and metallurgical processes are usually disposed of into open-air impoundments, where they become subjected to chemical or microbial leaching. At the surface of the tailings, where oxic conditions prevail, acidophilic bacteria, such as thiobacilli, can oxidize sulfidic minerals (e.g. pyrite and pyrrhotite) and generate acidic metal-rich leachates as by-products of their metabolism. This, combined with chemical oxidation, leads to acid mine drainage (AMD). Biomineralization, whereby a proportion of the metal leachate is precipitated, can also occur in the oxidized tailings, often as a result of a close metal–bacteria interaction. Iron-rich precipitates are usually found on bacterial cell walls, and are thought to serve as nucleation sites for further mineralization within the tailings impoundments. As depth increases in mine tailings, oxygen depletion and the presence of water-saturated pores usually lead to anoxic conditions. Under such redox and chemical conditions, populations of sulfate-reducing bacteria (SRBs) can colonize the tailings. As a result of their metabolic activity, sulfate is reduced to hydrogen sulfide, which in turn can react with dissolved metals to form metal sulfide precipitates. Microbial sulfate reduction also generates alkalinity, although chemical dissolution of carbonate and oxide minerals probably also play an important role in the generation of alkaline conditions in mine tailings.

INTRODUCTION

In the United States, there are over 500 000 abandoned mines of which nearly half are now government owned, each with their associated mine tailing dumps and each with the potential of producing large volumes of toxic, heavy metal-laden discharges. In Canada, nearly 30 000 acres of these dumps (excluding those from gold, coal and uranium mining) contain approximately 350 000 000 tons of sulfidic and acid-producing mine tailings. Fine-grain sulfides (mainly pyrite (FeS_2) and pyrrhotite ($Fe_{1-x}S$)) remain after metal recovery and this material along with other gangue material (such as chlorite minerals, clays, quartz and traces of ore) are the main particulate components of sulfidic mine tailings. These particulates are generally mixed with sulfate-rich waters which have resulted from metallurgical processing. In tailings impoundments, sulfidic minerals undergo a series of microbial and chemical reactions that change the mineralogical and chemical composition of the sediments. These processes occur in two separate zones of the tailings which we refer to as the oxic and anoxic zones.

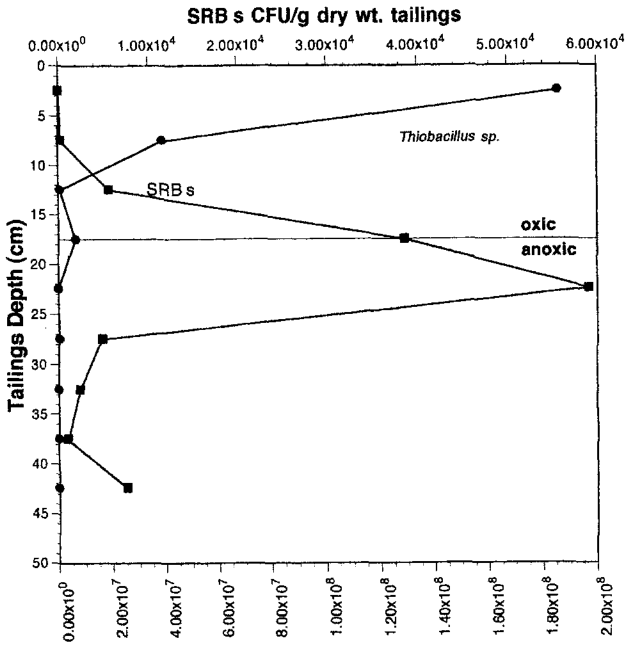

Fig. 1. Profiles of *Thiobacillus* sp. and sulfate-reducing bacteria (SRBs) populations present in the top 50 cm of the Kidd Creek Mine tailings. Note the occurrence of thiobacilli in the top oxic zone, while sulfate reducers only peak under reducing conditions. The horizontal line represents the interface between the oxic and anoxic zones.

In the oxic zone of the tailings, acidophillic chemolithotrophic and heterotrophic bacteria [32] (Fig. 1) oxidize iron sulfides to produce sulfuric acid and ferric iron as products of their metabolism [15,20]. Heavy metals associated with the tailings minerals (i.e. Fe, Cu, Zn, Pb, Cd, etc.) are leached and mobilized as acid accumulates. The leachates are frequently discharged as a natural consequence of water flow from the

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impoundments to threaten surrounding aquatic and terrestrial environments. The overall process is known as acid mine drainage (AMD). Biomineralization, whereby a variable proportion of the leachate interacts with and is complexed with the biota (mostly microbial), also occurs in the oxic zone. It follows the release of large amounts of ferric iron, which can precipitate as an oxide on the bacterial cell wall (Fig. 2) [36]. Because of AMD, many abandoned mines in the United States are considered by the Environmental Protection Agency as extremely hazardous toxic waste sites, with about sixty being designated as Superfund sites.

Oxidation of metal sulfides is usually confined to the surficial sediments where oxic conditions prevail. Particle size, precipitation and evapotranspiration rates affect the level of the water table, which often determine the depth of oxygen penetration into the tailings. In certain cases, the presence of hardpans will also limit the downward oxygen diffusion. Oxygen disappearance is gradual but usually coincides with the presence of water-saturated conditions, and eventually leads to strict anoxic conditions. Under such conditions, growth of thiobacilli populations and subsequent iron sulfide bioleaching are largely reduced [31]. Little is known about microbial activity in the anoxic zone of the tailings. Some researchers have looked at the formation and geochemistry of diagenetic minerals in tailings of impoundments [8,24], or those submerged under lakes [28–30], but there is little mention of anaerobic microbial processes such as sulfate reduction. However, in wetlands constructed for acid mine drainage treatment (i.e. those possessing effluents with high concentrations of H^+, Fe^{2+}/Fe^{3+} and SO_4^{2-}), microbial sulfate reduction has been reported [16,38]. In the tailings, sulfate reduction mediated by microorganisms and formation of authigenic metal sulfides should occur in the anoxic zone since it is a sulfate-rich environment capable of sustaining sulfate reducers. Our study of the Kidd Creek Mine tailings (located in Timmins, Ontario, Canada) indicates that sulfate-reducing bacteria (SRBs) are indeed present in the tailings, appearing just below the oxic/anoxic interface (Fig. 1). Microbial sulfate reduction produces hydrogen sulfide as an endproduct, which in turn reacts with dissolved metals to form metal sulfide precipitates [40]. Like the iron oxidizers in the oxic zone of the tailings, SRBs can change the geochemistry of the tailings and lead to the formation of authigenic minerals. A more detailed description of these biogeochemical processes which occur in both the oxic and anoxic zones of mine tailings follows.

Oxic zone

Kidd Creek Mine tailings as a physical example. Physically, the oxic zone of sulfidic mine tailings is characterized by bright orange-rust colored (oxidized) sediments at the surface, turning to darker shades of brown, and finally becoming a dark gray (unoxidized) with increasing depth. Oxidation of tailings depends on the mineralogy and hydrogeology of the containment site (i.e. sulfide content of the tailings, ground water movement and water table depth). The average depth of the oxic zone in sulfidic tailings in Canada is usually between 35–50 cm [6,36] but in the case of Kidd Creek Mine, it can be as shallow as 15 cm. Tailings disposal at Kidd Creek differs from the usual dispersal of finely ground material (diam. ≤30 μm) because these tailings are thickened prior to deposition (i.e. solid/liquid ratio is increased by addition of lime). This provides improved moisture retention thereby maintaining saturated conditions and a shallow water table [34].

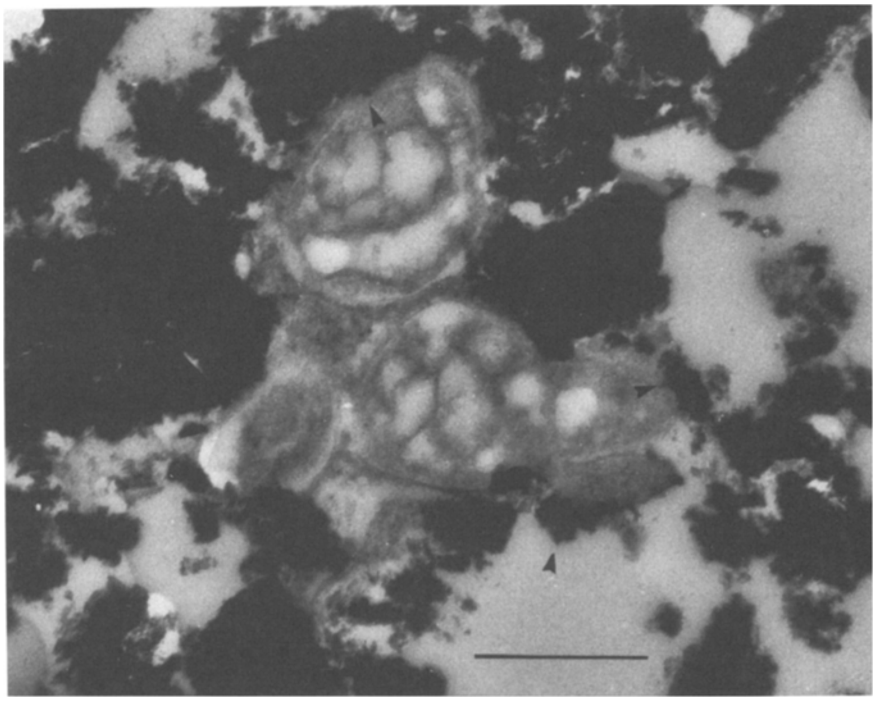

Fig. 2. Micrograph of a thin section of tailings showing *Thiobacillus ferrooxidans* cells. Note the presence of metal precipitates on the cell wall (see arrows). Scale bar is 200 nm.

It is the oxic zone of these impoundment sites that has attracted most attention regarding the source and amelioration of acid mine drainage (AMD). At Kidd Creek, pyrite and metal sulfides are oxidized by *Thiobacillus ferrooxidans* and produces a leachate of pH 2.0–2.5, containing iron (47 mmol L^{-1}), copper (39 μmol L^{-1}), zinc, lead and cadmium (Fig. 3). This leachate is collected in holding ponds which are chemically 'limed' to alkaline pH and precipitate toxic heavy metals before the outflow drains into the natural watershed. The net effect on the mineralogy in the oxic zone is their depletion of sulfides, their enrichment of silica/silcates and the precipitation of various Fe-oxyhydroxides (Fig. 4). Although chemical oxidation of Fe-sulfides has been suggested as the main cause of AMD, our studies and those of other researchers have shown that biochemical oxidation by chemolithotrophic Fe-oxidizing bacteria such as *T. ferrooxidans* is by far the main contributor to AMD [11,27]. Singer and Stumm [35] estimated that bacteria accelerated the oxidation of ferrous iron by a factor in excess of 10^6 as compared to the abiotic chemical rate.

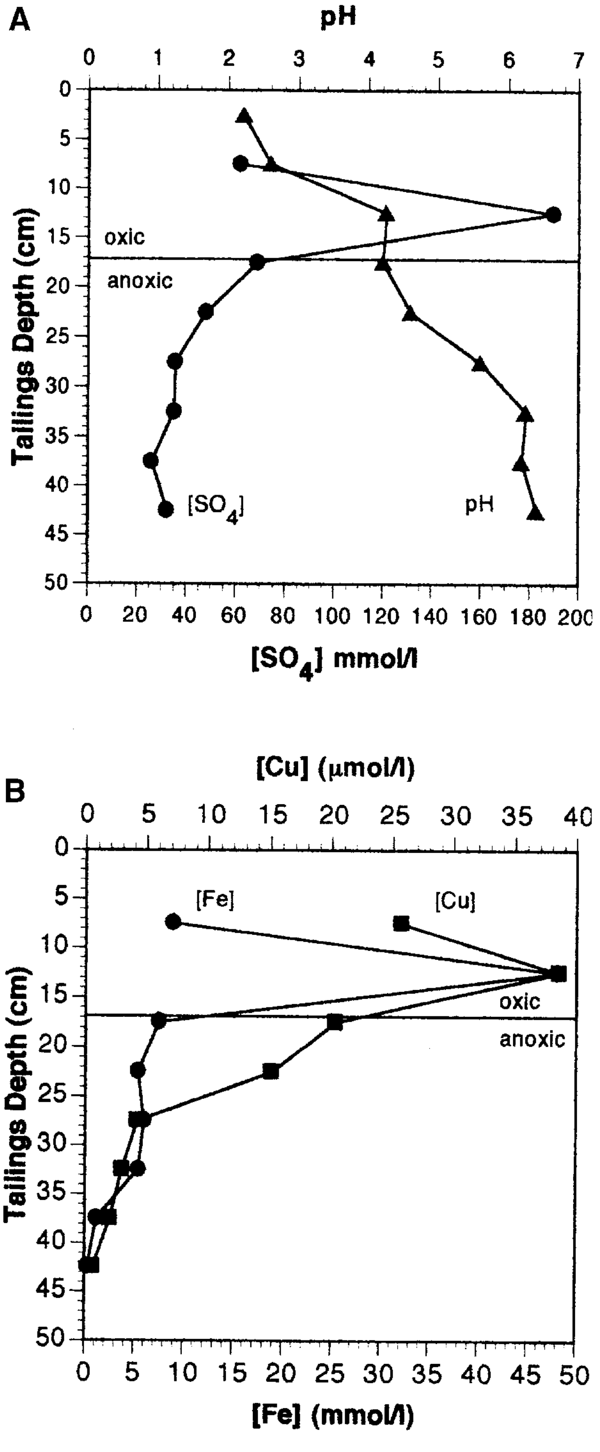

Fig. 3. (A) Pore-water pH, SO_4, and (B) Fe and Cu concentrations encountered in the top 50 cm of the Kidd Creek Mine tailings. Note the pulse of dissolved metals in the oxic zone as a result of the acidic conditions generated by the presence of large amounts of SO_4. The horizontal line represents the oxic/anoxic interface.

Thiobacillus ferrooxidans. This bacterium obtains cellular carbon through the fixation of atmospheric carbon dioxide via the Calvin cycle [20] and has the capacity to oxidize both ferrous iron (Fe(II)) and reduced sulfur compounds [37], producing sulfuric acid and ferric iron (Fe(III)) as metabolic byproducts [15,22]. Oxidation of these inorganic compounds as compared to oxidation of glucose by heterotrophs, yields only small quantities of free energy (ΔG) for cellular growth. Theoretically, the bioenergetics of *T. ferrooxidans* requires ca 22.4 moles of ferrous iron to fix 1 mole of carbon dioxide, giving a maximum growth yield of 0.53 g carbon mol^{-1} ferrous iron [20]. Consequently these bacteria are very slow growers, oxidizing considerable amounts of ferrous iron in order to survive.

Chemically, the oxidation of pyrite and sulfur by *T. ferrooxidans* is best described by the following chemical equations (modified from Singer and Stumm [35]):

$$FeS_2 + 7/2\,O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \qquad (1)$$

$$2Fe^{2+} + 1/2O_2 + 2H^+ \xrightarrow{\text{thiobacilli}} 2Fe^{3+} + H_2O \qquad (2)$$

$$14Fe^{3+} + FeS_2 + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad (3)$$

Equation (1) represents the initial reaction where ferrous iron is released by the inorganic oxidation of pyrite. Once Fe(II) is made available to *T. ferrooxidans*, it is then rapidly oxidized to Fe(III) (Eqn (2)), which in turn oxidizes additional pyrite, thereby regenerating additional Fe(II) for thiobacilli (Eqn (3)). This establishes a propagation cycle [35] between iron-oxidizing thiobacilli, degradation of sulfide minerals (Eqn (3)) and formation of heavy metal-rich, sulfuric acid leachates.

The indirect oxidation of the sulfides by microbially generated Fe(III) further compounds the complexity of leachate development. This releases additional toxic metal species into the tailings pore waters, while providing further energy sources for *T. ferrooxidans* (Eqn (3)). It is this direct and indirect mechanism of sulfide oxidation that allows the use of bioleaching as a cost-effective means of extracting economic metals from currently uneconomic, low-grade base and precious metal ore deposits [10,14,19,23].

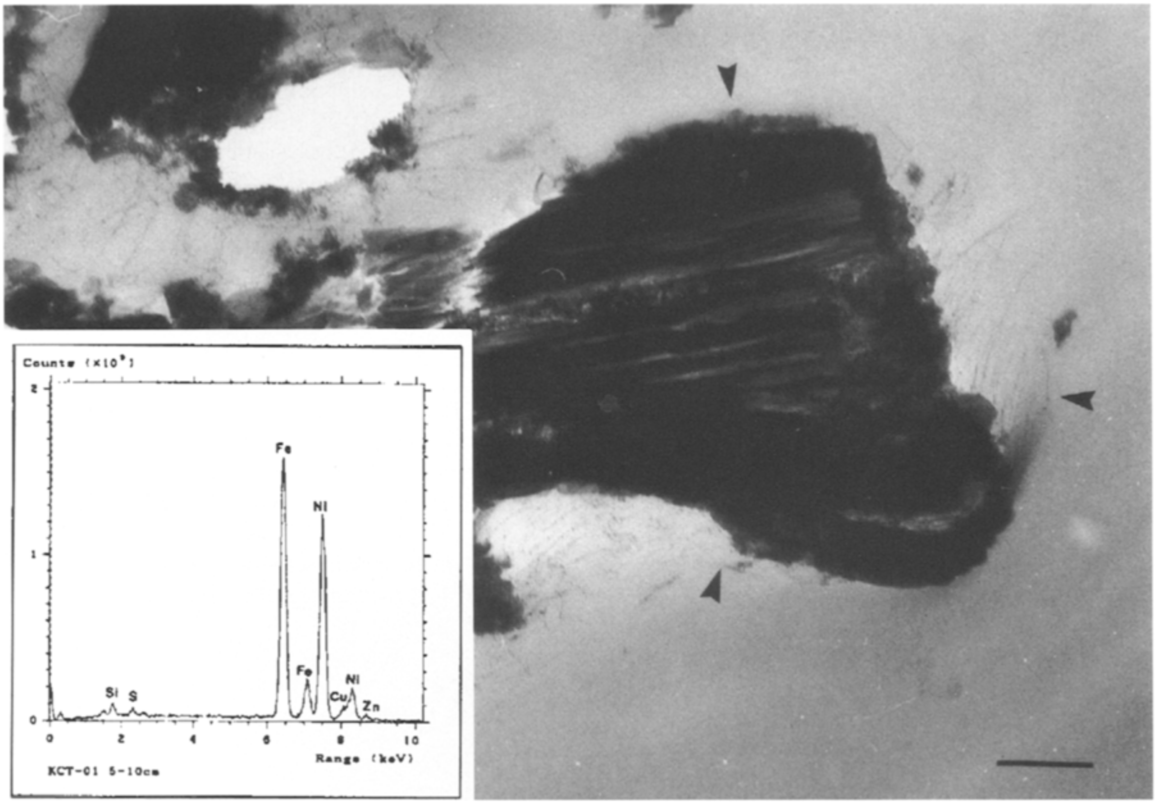

Fig. 4. Thin section of tailings showing the formation of authigenic amorphous and filamentous iron oxides (see arrows) encrusting silicate fragments (scale bar is 100 nm). EDS analysis of the iron-rich precipitate indicates the presence of minor elements, such as Cu, Zn, Si and S. The Ni peaks come from the supporting grid.

Biomineralization. Microbial metal accumulation and mineral precipitation can occur through active and/or passive processes, i.e. precipitation by microbial metabolism or by inherent metal-binding capacity of anionic constituents of the cell wall, respectively [3,4]. In natural environments these two processes can occur simultaneously and are not easily distinguishable [12]. In fact, viable and non-viable bacterial cells can act as templates for mineral formation [3].

The main indication of biomineralization at Kidd Creek appears to be the formation of Fe-oxide crusts occurring surficially and along vertical dessiccation cracks (up to 50 cm depth). These orange Fe-oxide crusts are analogous to hardpan, the latter typically found as cemented layers of iron and calcium sulfate and hydroxide precipitates overlying the water table [7]. Fe-oxide mineralization occurs within low pH tailings environments possessing relatively high thiobacilli (10^8 CFU g^{-1} dry wt tailings) populations. Presumably, these crusts are the result of supersaturated conditions of ferric iron and sulfate caused by thiobacilli, which themselves provide nucleating surfaces for mineral precipitation (Fig. 2). This mechanism of indirect biomineralization is not unrealistic since laboratory thiobacilli cultures grown in a ferrous iron-sulfate medium typically exhibit jarositic precipitates (potassium-ferric hydroxysulfates) on the walls of bacteria and culture test tubes once they reach pH 2–3. In addition, previous work with biofilms in acidic and neutral pH environments have shown heavy metal complexation by bacteria at pH 3.1 and increased metal adsorption with increasing pH [13].

Biomineralization when viewed on a micrometer scale could be explained by the development of a pH gradient between the *T. ferrooxidans*-sulfide mineral interface and the external milieu. This close bacteria–mineral interaction is still not fully understood, but is believed to be due to a combination of ionic and hydrophobic forces between the cell and the mineral face and plays a significant role in sulfide mineral colonization [36]. Oxidation of sulfides by *T. ferrooxidans* must create a pH gradient within the micro-environment, sufficient enough to maintain metals in solution along the bacteria–mineral interface at pH <2.5 [36], while allowing oxyhydroxides/sulfates to form on exposed bacterial and mineral surfaces at pH >2.5 [36]. This concommitant bioleaching and biomineralization is seen on sulfide mineral surfaces exhibiting bacteria-sized corrosion pits, grooves and holes. Some of these depressions contain attached *T. ferrooxidans* cells which are coated with metal sulfate residues (i.e. jarosite) [1,21]. Continued mineralization has been shown to significantly reduce, even to the point of terminating, further sulfide oxidation [21].

Due to the virtual absence of plant life on the Kidd Creek site, combined with *Thiobacillus* spp. as the dominant aerobic bacterium, it is believed the latter is a major source of SO_4 and dissolved organic carbon (DOC) percolating downwards into the anoxic zone, thereby sustaining an active SRB population. Seasonal variations in thiobacilli and SRBs populations, SO_4 concentration and DOC content are seen but remain directly proportional to one another, supporting the suggestion that SRBs are dependent on the oxic thiobacilli.

Anoxic zone

Redox stratification in the Kidd Creek Mine tailings. Anoxic conditions in these mine tailings corresponded to the presence of reduced sediments characterized by a dark gray color, and were correlated with the presence of water-saturation and the pE measurements. The oxygen diffusion coefficient in mill tailings depends on the moisture content of the sediments [33], but the disappearance of oxygen from the pore-waters was also related to its metabolic use by microorganisms and its consumption by sulfide–mineral oxidation reactions (see Eqns (1) and (2)). In anaerobic habitats such as Kidd Creek, different redox reactions and dissolution-precipitation reactions can occur, some of them being mediated by microorganisms. Of those potential reactions, microbial sulfate reduction is most likely to happen in these water-saturated sulfidic mine tailings.

Sulfate reduction. In tailings impoundments, microbial sulfate reduction rather than chemical sulfate reduction must predominate because the abiotic chemical reaction is believed to only happen above 200 °C [38]. As shown in Fig. 1, sulfate reduction mediated by bacteria is possible, since populations of sulfate reducers resembling *Desulfovibrio* spp. and *Desulfotomaculum* spp. were found in Kidd Creek Mine tailings. The basic biochemical reaction mediated by SRBs proceeds according to:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \qquad (5)$$

where CH_2O represents organic matter. Organic carbon necessary to sustain growth of sulfate reducers in tailings possibly originates from the run-off water draining into impoundments and the organic input from microbes in the oxic zone. As shown in Eqn (5), the reduction of SO_4^{2-} leads to the formation of H_2S which, once in solution, can easily react with dissolved metals (M^{2+}) to form metal sulfide precipitates, i.e.

$$M^{2+} + S^{2-} \rightarrow MS\ (s) \qquad (6)$$

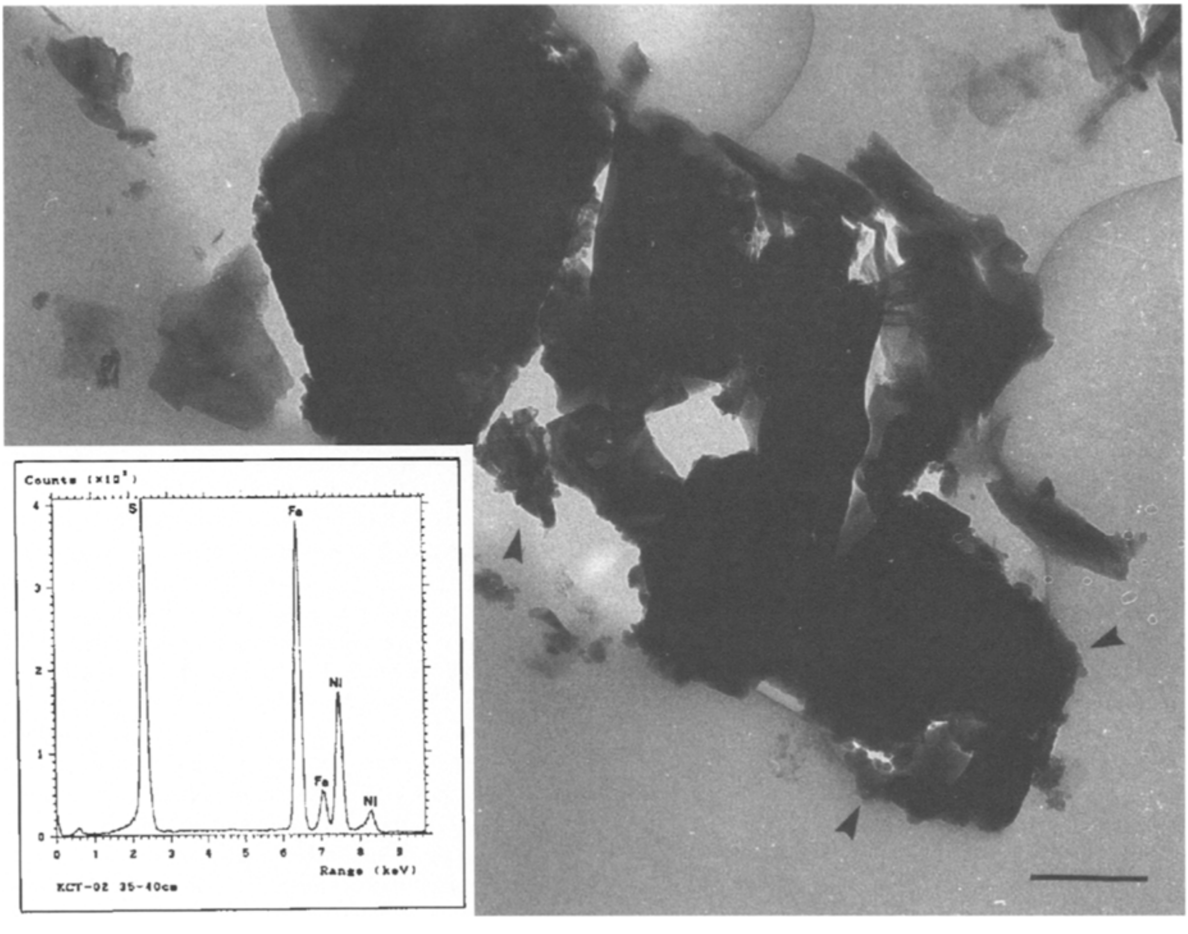

Fig. 5. Amorphous/granular iron sulfide (confirmed by EDS) precipitates (see arrows) coating silicate fragments in the anoxic zone of the Kidd Creek Mine tailings. Scale bar is 100 nm.

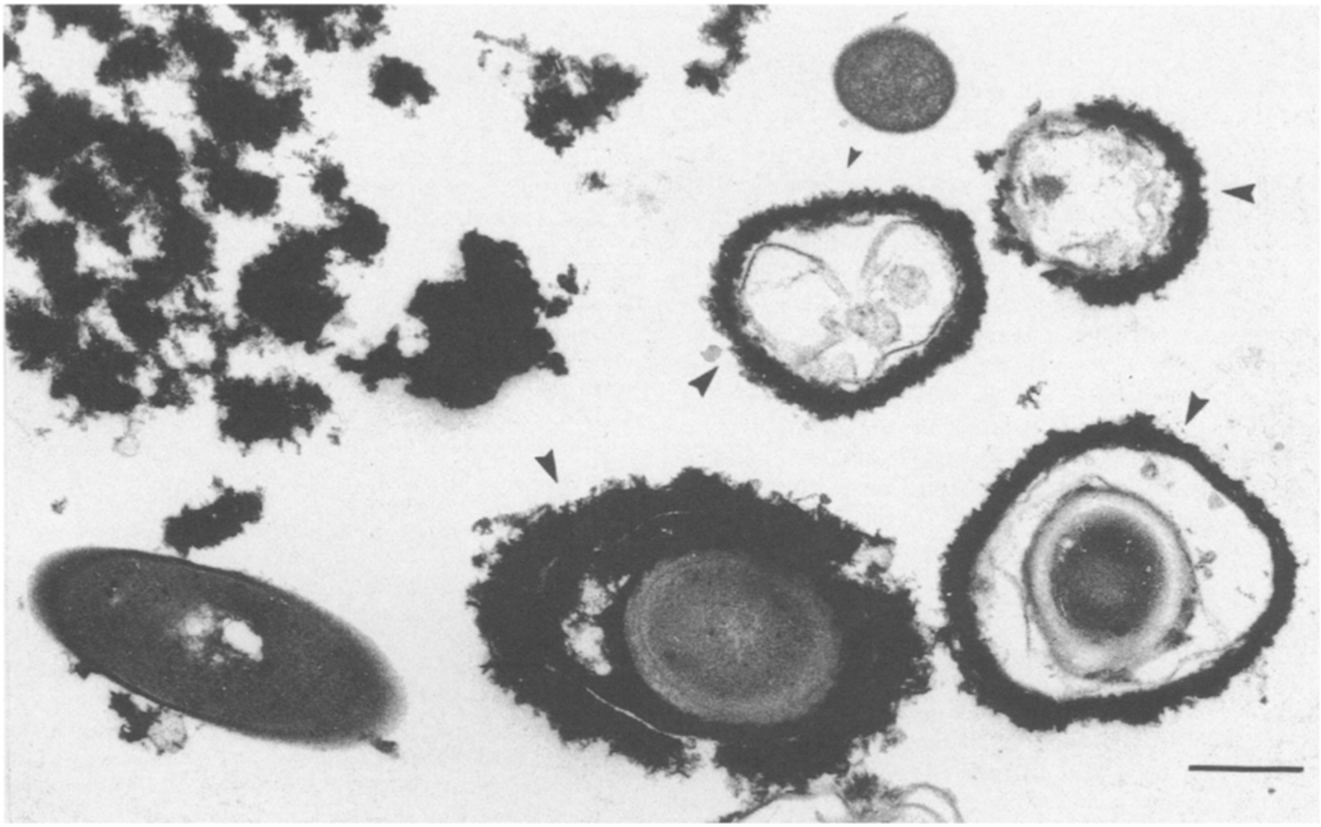

Fig. 6. Micrograph of a thin section of cells of a *Desulfotomaculum* sp. grown in the presence of Fe(II) showing the extent of precipitation of FeS on the cell wall (pointed by arrows). Scale bar is 0.5 μm.

Biomineralization. Obviously in tailings, there is an abundant supply of dissolved metals from the oxic zone that can contribute to metal sulfide precipitation (see Fig. 3). As seen in the Kidd Creek tailings, dissolved iron is the major metal from the oxic zone but a portion of Fe(II) can also originate from the reduction of ferric oxides in the anoxic part of the tailings [2]. As an example of Fe-sulfide precipitation, Fig. 5 shows amorphous authigenic iron sulfide precipitates coating silicate minerals from the Kidd Creek Mine tailings. Cell surfaces possibly act as the first nucleation sites for iron sulfide precipitation and lead to further mineralization [5]. Because of the extent of precipitation on certain cells (see Fig. 6; SRBs grown in laboratory), the minerals themselves become suitable interfaces to trap metals. Dissolved metals, such as Cu, Zn, Pb, Cd present in lesser amounts (Fig. 3), can then be adsorbed or coprecipitated with the most abundant iron sulfide or the most reactive one, such as mackinawite [26]. Besides the formation of amorphous Fe-sulfides in the Kidd Creek tailings, diagenetic iron sulfides having a chemical composition close to mackinawite (FeS_{1-x}) were also observed. The extent of FeS precipitation in mine tailings is not only a matter of hydrogen sulfide production, but also a function of the chemistry of the pore-waters. In any natural system, iron sulfide formation is usually affected by the presence of other ligands which compete for Fe(II) precipitation [25]. Of those ligands, carbonate ions (CO_3^{2-}) play an important role, since they can be introduced by the liming of the tailings, prior to their deposition. Another potential source of CO_3^{2-} is shown in Eqn (5), where HCO_3^- is an endproduct of sulfate reduction. Therefore, depending on the concentration of the reactants (Fe(II), CO_3^{2-} and SO_4^{2-}) and the pH of the pore-waters, Fe-sulfides (e.g. mackinawite, greigite, pyrrhotite or pyrite) and Fe-carbonates (siderite) can co-exist within the same environment [9,25]. Such mineral co-existence was indeed observed in the Kidd Creek tailings.

Neutralization of pH. As mentioned previously, microbial sulfate reduction generates alkalinity during the production of HCO_3^-. Accordingly, it neutralizes acidity in impoundments and wetlands receiving acid mine drainage [17,18,39,41]. However, little is known about sulfidic mine tailings. Pore-water pH profiles of mine tailings [8] often show a pH increase near the oxic/anoxic interface (Fig. 3). In the case of Kidd Creek Mine, this increase coincides with the presence of SRBs in the tailings (Fig. 1). However, other pH-buffering reactions, such as dissolution of carbonate minerals and iron hydroxides, can also produce alkaline conditions [8]. In addition, populations of sulfate reducers are far less important in number than the thiobacilli populations (Fig. 1), suggesting that the generation of alkalinity by microbial sulfate reduction is not entirely responsible for the pH increase observed below the oxic/anoxic interface of the Kidd Creek tailings.

CONCLUSION

In tailings impoundments, specific bacteria, such as thiobacilli and sulfate reducers, can actually affect the chemical stability of the gangue and ore material deposited in such sites. In the oxic part of the tailings, acidophilic thiobacilli populations generate acidic and metal-enriched leachates as by-products of their metabolism, which represent a major environmental problem. On the other hand, SRBs, generally active in the anoxic water saturated zone, produce hydrogen sulfide which reacts with dissolved heavy metals to form metal sulfide precipitates. Besides lowering metal and sulfate concentrations in the pore-waters, SRBs also generate alkalinity, which tend to minimize the effect of AMD. If the impoundments were to be maintained under water-saturated conditions in order to establish anoxic conditions, SRB populations could play an important role in the chemical stability of sulfidic mine tailings and could aid 'in situ' bioremediation processes.

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