Investigations for the quantification of microorganisms in sulfidic mine waste dumps

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Zusammenfassung

Bergbau-Abfälle werden als Abraum oder als mit Metallen abgereicherte Aufbereitungsrückstände (Tailings) deponiert. Bergbauhalden enthalten oft Sulfidminerale wie Pyrit oder Pyrrhotit, die chemisch und biologisch unter Freisetzung von Schwefelsäure und Schwermetallen oxidiert werden. Die sauren, mit Schwermetallen angereicherten Sickerwässer dieser Halden werden als acid mine oder rock drainage (AMD/ ARD) bezeichnet. Um AMD/ ARD bildende Prozesse zu verstehen, abzuschätzen und zu kontrollieren, ist es nötig, die Mikrobiologie und Biogeochemie von sulfidischen Bergbauhalden zu untersuchen.

In dieser Arbeit wurden mikrobielle Gemeinschaften in drei verschiedenen sulfidischen Tailings-Halden quantitativ analysiert unter Verwendung der molekularbiologischen Quantifizierungstechniken Catalyzed Reporter Deposition – Fluorescence *in situ* Hybridization (CARD-FISH), FISH und quantitative real-time PCR (Q-PCR) im Vergleich mit SYBR Green Direktzählungen und der most-probable-number (MPN) Kultivierungsmethode. Gesamtzellzahlen, Zahl der Bacteria, Archaea, Eukarya und der spezifischen Bakterien *Acidithiobacillus* sp., *At. ferrooxidans*, *At. caldus*, *Leptospirillum* sp., *L. ferrooxidans*, *Sulfobacillus* sp., *Acidiphilium* sp., *Acidimicrobium* und Verwandte, Geobacteraceae, Sulfat-Reduzierer (*dsrA*) sowie MPN-Zahlen von acidophilen Fe(II)- und Schwefel-Oxidierern wurden bestimmt. Tiefenprofile dieser Zellzahlen zeigten, dass die Zusammensetzung der mikrobiellen Gemeinschaft an den drei Standorten sehr unterschiedlich ist und auch stark zwischen den Zonen der oxidierten und nicht-oxidierten Tailings-Halden variiert. Zonen mit hohen Zellzahlen korrelieren mit hohen mikrokalorimetrisch gemessenen Pyrit und Pyrrhotit Oxidationsaktivitäten, ebenso wie mit Lagen von Verkrustungen, die relevant sind für den natürlichen Rückhalt von Schwermetallen und Arsen (natural attenuation). Maximale Zellzahlen mit bis zu 10^9 Zellen g^{-1} Trockengewicht wurden in den Pyrit oder Pyrrhotit Oxidationszonen detektiert, während die Zellzahlen in den Zonen der nicht oxidierten Tailings deutlich geringer waren. Bacteria dominierten über Archaea und Eukarya an allen untersuchten Standorten. Unter den Bacteria dominierte an zwei Standorten der acidophile Fe(II)- und Schwefel- oxidierende *Acidithiobacillus* sp. über den acidophilen Fe(II)-oxidierenden *Leptospirillum* sp., während beide Gattungen gleichstark am dritten Standort vertreten waren. Der acidophile Fe(II)- und Schwefel-oxidierende *Sulfobacillus* sp. war generell in geringerer Zahl zu finden und der Fe(II)-oxidierende *Acidimicrobium* und Verwandte konnten gar nicht nachgewiesen werden. Der acidophile Fe(III)-reduzierende *Acidiphilium* sp. wurde nur an einem Standort gefunden, während die neutrophilen Fe(III)-reduzierenden Geobacteraceae, ebenso wie das *dsrA* Gen von Sulfat-Reduzierern an allen Standorten quantifiziert wurden. FISH Analysen lieferten zuverlässige Daten nur für Tailings-Zonen mit hoher mikrobieller Aktivität, während CARD-FISH, Q-PCR, SYBR Green

Direktzählungen und MPN sich für eine quantitative Analyse von mikrobiellen Gemeinschaften von Tailings-Halden im Allgemeinen eigneten.

Außerdem erwiesen sich FISH und Q-PCR als sinnvolle Methoden für die Überwachung von Zellzahlen in einem Experiment zur biologischen Metalllaugung (Biomining). Es konnte gezeigt werden, dass Biomining eine Option für die Bio-Sanierung (Bioremediation) von Tailings-Halden und die Extraktion von wertvollen Metallen aus Bergbauabfällen ist.

Schlagworte: Tailings, Acid Mine Drainage, *Acidithiobacillus*

Summary

Mine waste material is dumped as waste rock or as metal-degraded waste from ore processing (tailings). Waste dumps often contain sulfide minerals such as pyrite or pyrrhotite which are chemically and biologically oxidized and therewith generate sulfuric acid and mobilize heavy metals. The acidic, heavy metal enriched effluents from such dumps are called acid mine or rock drainage (AMD/ ARD). To understand, estimate and control AMD/ ARD forming processes it is necessary to investigate the microbiology and biogeochemistry of sulfidic mine waste dumps.

In this thesis the microbial communities in three different sulfidic mine waste tailings were quantitatively analyzed using the molecular quantification techniques catalyzed reporter deposition – fluorescence *in situ* hybridization (CARD-FISH), FISH and quantitative real-time PCR (Q-PCR) in comparison with SYBR Green direct counting and most-probable-number (MPN) cultivation. Total cell numbers, numbers of Bacteria, Archaea, Eukarya, and of the particular bacteria *Acidithiobacillus* sp., *At. ferrooxidans*, *At. caldus*, *Leptospirillum* sp., *L. ferrooxidans*, *Sulfobacillus* sp., *Acidiphilium* sp., *Acidimicrobium* and relatives, Geobacteraceae, sulfate reducer (*dsrA*) as well as MPN-numbers of acidophilic Fe(II)- and sulfur-oxidizers were determined. Depth profiles of these cell numbers showed that the composition of the microbial communities is highly different at the three sites, and also strongly varied between zones of oxidized and unoxidized tailings. Zones with high cell numbers correlate with a high pyrite or pyrrhotite oxidation activity measured by microcalorimetry as well as with cemented layers relevant for natural attenuation of heavy metals and arsenic. Maximum cell numbers with up to 10^9 cells g^{-1} dry weight were determined in the pyrite or pyrrhotite oxidation zones whereas the cell numbers were significantly lower in the zones of unoxidized tailings. Bacteria dominated over Archaea and Eukarya at all tailings sites. Among the Bacteria, the acidophilic Fe(II)- and sulfur-oxidizing *Acidithiobacillus* sp. dominated over the acidophilic Fe(II)-oxidizing *Leptospirillum* sp. at two sites, while both genera were equally abundant at the third site. The acidophilic Fe(II)- and sulfur-oxidizing *Sulfobacillus* sp. was generally less abundant and the Fe(II)-oxidizing *Acidimicrobium* and relatives were not detected at all. The acidophilic Fe(III)-reducing *Acidiphilium* sp. could only be found at one site while the neutrophilic Fe(III)-reducing Geobacteraceae as well as the *dsrA* gene of sulfate reducers were quantifiable at all three sites. FISH analysis provided reliable data only for tailings zones with high microbial activity but CARD-FISH, Q-PCR, SYBR Green direct counting and MPN were suitable methods for a quantitative microbial community analysis of tailings in general.

In addition, FISH and Q-PCR turned out to be suitable methods for monitoring cell numbers in a biomining experiment. Biomining is shown to be an option for tailings bioremediation and extraction of valuable metals from mine waste.

Keywords: tailings, acid mine drainage, *Acidithiobacillus*

1. Introduction

1.1 Metal mining

Mining operations are conducted throughout the world to extract valuable mineral resources. Many sulfide minerals are economically important as metal ores. Metals such as lead, cobalt, copper, nickel, zinc, silver or gold are recovered from sulfide ore deposits. For example, about 66% (ca. 2.23 million tons) of the global recovered lead ores are produced in China, Australia and the USA. In 2006 Russia, Canada, Australia and Indonesia provided about 60% of the worldwide recovered nickel ores (1.4 million tons nickel). Chile is the biggest producer of copper with 5.4 million tons in 2006 and yielded 30% of the global production followed by the USA, Peru, Australia and China. Furthermore 2006 approximately 10.2 million tons zinc were recovered worldwide. 65% of this production was extracted in China, Australia, Peru, the USA and Canada (Rohstoffwirtschaftliche Länderstudien, 2006).

The demand for metals highly increased during the last years. In the 1980s and 90s an adequate supply, partly an oversupply of mining resources could be offered. The prices for the most resources reached a historical low in the 1990s. Since 2003/ 2004 resources ran short and supply bottlenecks occurred. This was not a result of the depletion of the worldwide natural resources but a consequence of a surprising fast and strong increase of demands without an adequate supply. In 2003 the demand of mining resources rose rapidly due to a global economic boom and especially the industrial production in China but also in India and further oriental newly industrializing countries which exceeded their recovery. This caused an upsurge of prices to never reached highest levels of many resources in 2004 to nowadays. As for example the global prices for copper reached a historical low in 2001, they rose from less than 3000 US-$ per ton at the beginning of 2004 to approximately 6000 US-$ per ton in 2006 and to more than 7000 US-$ in spring of 2007. The enormous increased demand of steel caused a high demand in zinc since 2004 which continued undiminished in 2006 and 2007. In 2006, due to the scarcity of the supply the world prices rose by approximately 80% and reached their highest value ever (Der Fischer Weltalmanach 2008).

All predictions assume that it will take several years until a balance between demand and supply is established by increasing the extraction of mining resources preconditioned that the economic growth of the most important countries of consumption prolong in the present extent. According to present geoscientific findings in near to middle future mining resources will not run short due to exhaustion of the deposits. The extent of actual reserves is predominantly dependent on the price and the state of the extracting and producing mining techniques. It is comparative which mineral concentration is sufficient to consider a rock as metal ore. Periodically the amount of available resources grows with increasing prices because the extraction of previously disregarded low-grade deposits becomes reasonable.

Furthermore the exploration and recovery of so far unknown or not economically viable resources results from this development (Der Fischer Weltalmanach 2008).

In this context biomining bears a special meaning for extracting metals from ores (Donati and Sand 2007; Rawlings and Johnson 2007a). Biomining is a general term and is describing applied bioleaching and biooxidation (Rawlings, 2002). Both terms describe oxidation processes. Bioleaching is the conversion of an insoluble metal (usually a metal sulfide, e.g. CuS, NiS, ZnS) into a soluble form (usually the metal sulfate, e.g. $CuSO_4$, $NiSO_4$, $ZnSO_4$) enhanced by microbial activity. Adjacently, the metal is recovered from solution. The microbial oxidation process may also be termed biooxidation. However, the term biooxidation is usually used to describe a process in which the recovery of a metal is enhanced by microbial decomposition of the mineral, but the metal being recovered is not solubilized but more accessible for extraction. An example is the recovery of refractory gold from sulfide ores. After biooxidation of the gold-bearing sulfide, e.g. arsenopyrite, the gold is extracted from the mineral suspension by cyanide treatment.

The advantage of biomining is the economically feasible extraction of metals from low-grade ore deposits especially for copper recovery (Clark et al., 2006; Rawlings and Johnson 2007a). The conventional metallurgical extraction process i.e. flotation in combination with roasting or smelting requires a sufficient sulfide content of the ore and is environmentally harmful by producing sulfur dioxide and other toxic gaseous emissions (Rawlings et al., 2003). Although biomining processes require longer reaction times than conventional metallurgical processes, the increasing demand of metals, the decreasing proportion of high-grade ore deposits and the nowadays demanded remediation of mine waste from conventional ore processing lead to an increased application of biomining especially for copper recovery. For this reason the development of e.g. molecular biological methods for monitoring and improving biomining processes as well as the investigation of the associated microbial communities as done in this study is of special meaning.

1.2 Sulfidic mine waste dumps

Mining operations are accompanied by high amounts of mine waste material which are accumulated and dumped as waste rock consisting of not recoverable minerals from mining or as tailings which are metal-degraded ore materials from ore processing. In case of metal mining from sulfide ores, both types of mine waste dumps, waste rock or tailings, may still contain sulfide minerals.

The different mine waste dumps can be classified by the material's capacity for acid generation or, conversely, the material's capacity for neutralizing acidity (Blowes et al., 2003; Höglund and Herbert, 2004). The Acid-Base Accounting (ABA) is a method for determining

the acidification potential (AP) and the neutralization potential (NP) of a waste material. With this method the balance between acid producing and buffering capacity of material is estimated (British Columbia Acid Mine Drainage Task Force, 1989). The difference between NP and AP is the net neutralization potential (NNP) and is an indication for the potential impact of a deposit for the environment. The effluents from sulfidic mine waste dumps are often extremely acidic and contain high amounts of iron and other metals and are known as acid mine drainage (AMD) or acid rock drainage (ARD). An example is given in Fig. 1.

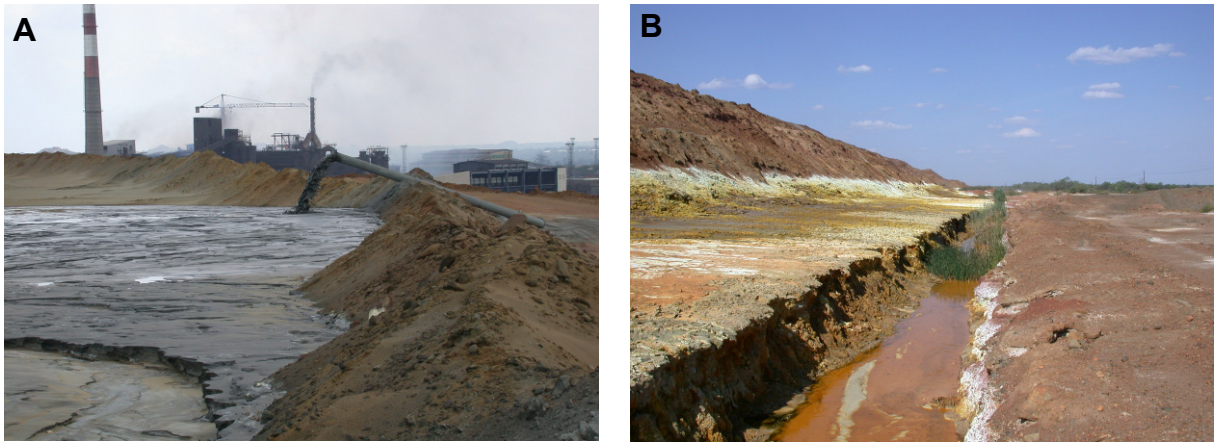

Fig. 1. *Pyrrhotite-containing mine waste tailings dump near Selebi-Phikwe in Botswana in 2003. A: Tailings material is dumped as sludge from ore processing. The ore processing plant is located in the background. B: A trench for the collection of acid mine drainage (pH ~2.3) surrounds the mine waste dump (Pictures: Axel Schippers, BGR Hannover).*

1.3 Biogeochemical processes in sulfidic mine waste dumps

Several biogeochemical processes occur in sulfidic mine waste dumps. Most important are processes related to sulfide weathering. Chemical and biological oxidation of the sulfide minerals such as pyrite or pyrrhotite generates sulfuric acid and soluble iron and in addition mobilizes other heavy metals (AMD/ ARD). The sulfide oxidation processes in mine waste dumps are influenced by temperature, pH, humidity, and the availability of oxygen in the dumps, which is mainly controlled by diffusion. Furthermore the microbial abundance and activity of acidophilic Fe(II)- and metal sulfide-oxidizing microorganisms strongly impacts metal sulfide oxidation. The biological pyrite oxidation rate was shown to be up to two orders of magnitude higher than the chemical rate (Nordstrom and Southam, 1997; Höglund and Herbert, 2004).

Two oxidizing pathways for metal sulfides can be distinguished: the thiosulfate and polysulfide mechanism (Schippers and Sand, 1999). Acid-insoluble metal sulfides like pyrite

(FeS_2), molybdenite (MoS_2) or tungstenite (WS_2) are attacked by Fe(III) ions according to the thiosulfate mechanism as shown in equations 1 and 2:

$$FeS_2 + 6\ Fe^{3+} + 3\ H_2O \longrightarrow S_2O_3^{2-} + 7\ Fe^{2+} + 6\ H^+ \quad [1]$$

$$S_2O_3^{2} + 8\ Fe^{3+} + 5\ H_2O \longrightarrow 2\ SO_4^{2-} + 8\ Fe^{2+} + 10\ H^+ \quad [2].$$

The main sulfur intermediate of this reaction is thiosulfate.

Acid soluble metal sulfides like pyrrhotite ($Fe_{1-x}S$, x ranges between 0 and 0.125), sphalerite (ZnS) or chalcopyrite ($CuFeS_2$) are attacked by Fe(III) ions and/ or by protons. Here, the main sulfur intermediates are polysulfides and elemental sulfur (eq. 3-5):

$$MS + Fe^{3+} + H^+ \longrightarrow M^{2+} + 0.5\ H_2S_n + Fe^{2+} \quad (n \geq 2) \quad [3]$$

$$0.5\ H_2S_n + Fe^{3+} \longrightarrow 0.125\ S_8 + Fe^{2+} + H^+ \quad [4]$$

$$0.125\ S_8 + 1.5\ O_2 + H_2O \longrightarrow SO_4^{2-} + 2\ H^+ \quad [5].$$

Bacteria generate the sulfuric acid by sulfur-oxidation and therewith supply protons for hydrolysis attack and/ or keep the iron ions in an oxidized state (Fe(III)) for an oxidative attack by Fe(II)-oxidation. The oxidation of Fe(II) is highly accelerated by the activity of Fe(II)-oxidizing prokaryotes in comparison to the pure chemical reaction (Singer and Stumm, 1970). Fe(II) is oxidized to Fe(III) which may precipitate as Fe(III)(hydr)oxide and thereby producing protons:

$$Fe^{2+} + 0.25\ O_2 + 2.5\ H_2O \longrightarrow Fe(OH)_3 + 2\ H^+ \quad [6].$$

High amounts of heavy metals can precipitate with or adsorb to Fe(III)(hydr)oxides but also to Mn(IV)(hydr)oxides and therefore are immobilized (Schippers, 2003). This process is of special meaning for natural attenuation in sulfidic mine waste dumps because these immobilized heavy metals are not released to the environment as AMD/ ARD.

Besides the described aerobe oxidation of metal sulfides, anaerobic biogeochemical processes occur in mine waste dumps as well. Fe(III) and sulfate generated by the activity of Fe(II)- and/ or sulfur compound-oxidizing Bacteria or Archaea can be used by Fe(III)- and sulfate-reducing prokaryotes for anaerobic respiration. While some microorganisms can grow with sulfur compounds as electron donor and Fe(III)-ions as electron acceptor, Fe(III)-oxides can also serve as the final electron acceptor for the oxidation of organic carbon [eq. 7] (Schippers, 2007; Fortin and Praharaj, 2005):

$$Fe(OH)_3 + 0.25\ CH_2O + 2\ H^+ \longrightarrow Fe^{2+} + 0.25\ CO_2 + 2.75\ H_2O \quad [7].$$

As described above heavy metals can adsorb to Fe(III)(hydr)oxides. Therefore the microbial reduction of Fe(III)(hydr)oxides [eq. 7] releases soluble Fe(II) and precipitated or adsorbed metals in solution and additionally consumes protons. Hence, solubilized heavy metals can flow out of the sulfidic mine waste dump as AMD/ ARD. In contrast, the increased pH counteracts AMD/ ARD generating processes.

The reduction of sulfate to sulfide is carried out by sulfate-reducing microorganisms according to following equation [eq. 8] (Fortin and Praharaj, 2005):

$$2\ CH_2O + SO_4^{2-} \longrightarrow 2\ HCO_3^- + H_2S \quad [8],$$

in which CH_2O represents the organic matter. H_2S easily reacts with dissolved metal species such as Fe(II) and other heavy metals and precipitates as metal sulfides [eq. 9] (Fortin and Praharaj, 2005; Fortin et al., 1995):

$$M^{2+} + S^{2-} \longrightarrow MS\ (s) \quad [9].$$

Furthermore sulfide mineral formation may occur when elemental sulfur is microbiologically disproportionated to sulfide and sulfate in the presence of Fe(III)- or Mn(IV)(hydr)oxides (Schippers, 2003):

$$3\ S^0 + 2\ FeOOH \longrightarrow SO_4^{2-} + 2\ FeS + 2\ H^+ \quad [10].$$

These microbial formations of sulfide minerals [eq. 9, 10] are called biomineralization and is another metal immobilizing process in sulfidic mine waste dumps relevant for natural attenuation.

The rates of biogeochemical processes in mine waste dumps correlate with the microbial activity. So far, methods such as standardized column experiments and humidity cells or the less frequently applied microcalorimetry and *in situ* oxygen consumption measurements were used for determining biogeochemical processes (Schippers and Kock, 2007). Column experiments and humidity cell tests are laboratory approaches for accelerating and continually control weathering processes for estimating potential AMD formation. The rate of metal sulfide oxidation reactions is measured by microcalorimetry via the reaction energy and by *in situ* oxygen consumption measurements via the decrease of the oxygen content [eq. 5,6] over time in a reaction chamber in the tailings. Investigations of pyritic uranium mine waste heaps near Ronneburg in Germany clearly showed a correlation

between cell numbers and microbial activity measured by microcalorimetry (Schippers et al., 1995). A comparative study of an Arctic Canadian pyritic mine tailings pond yielded consistent pyrite oxidation rates for two methods, *in situ* oxygen uptake rates and microcalorimetric measurements. There, oxygen uptake rates were approximately 40 moles O_2 m^{2-} day^{-1} in ½ and 6 years old, well-drained tailings, and only approximately 0.005 moles O_2 m^{2-} day^{-1} in 6 years old nearly water-saturated tailings due to lower oxygen diffusion rates (Elberling et al., 2000). The oxidation rate measurements of metal sulfides provide an instrument for predicting time periods of AMD formation and for estimating and controlling remediation concepts as well as natural attenuation processes (Blowes et al., 2003; 2004).

1.4 Remediation measures for sulfidic mine waste dumps and natural attenuation

The release of AMD/ ARD from sulfidic mine waste requires a treatment of AMD after release and/ or remediation measures to prevent AMD generation in the waste dump itself. The fundamental idea of all remediation measures applied to waste dumps is to reduce oxidation processes and to immobilize metals either by reducing the oxygen diffusion rate, increasing the pH and/ or inhibiting metal sulfide-oxidizing microorganisms. So far, remediation treatments with chemicals or biocides, underwater storage, dry covering or replanting of the dump were applied to mine waste (Ledin and Pedersen, 1996; Schippers and Bosecker, 2002; Johnson and Hallberg, 2005). The combination of different treatments can improve the results of remediation. Nevertheless, the individual situation at each mine waste dump influences the success of the attempts and requires adjustment of the measures. Therefore, it is necessary to investigate and to observe ongoing processes in the mine waste dumps before, while and after remediation.

An alternative to active treatment of mine waste or AMD is natural attenuation of the release of toxic compounds (e.g. metals and arsenic) to the environment. As mentioned in the previous chapter several biogeochemical processes may contribute to natural attenuation. The ongoing processes of oxidation and reduction in mine waste tailings affect the alteration of the tailings material, i.e. weathering of reactive mineral phases, precipitation of secondary mineral phases and gels, which may coat particles, agglutinate them, and reduce the porosity and therewith forms hardpans and cemented layers (chapter 4.2.5: Graupner et al., 2007). Cemented layers and hardpans in mine waste dumps effect a temporary natural attenuation of the toxic compounds, a restriction of oxygen permeation and therewith a restriction of the downward movement of the oxidation front and a reduction of erosion events on the dump surface by wind and water (McGregor and Blowes, 2002; Gilbert

et al., 2003). Therefore natural attenuation and the formation of cemented layers and hardpans are beneficial processes which can complement other remediation measures.

1.5 Microbial ecology of sulfidic mine waste dumps

The microbial ecology comprises the relationship of microorganisms with each other as well as with their environment. The diversity of microorganisms, their quantity and their activity represent the ecology of a certain environment. As described in chapter 1.3, the determination of metal sulfide oxidation rates provide information about the microbial activity in sulfidic mine waste dumps. In the following, the diversity of microorganisms so far identified in these environments is presented. Afterwards, the methods used for quantification of the microbial communities and their representatives in environmental samples are elaborated and an overview on previous studies about sulfidic mine waste dumps is given.

1.5.1 Microbial diversity of sulfidic mine waste dumps

Different microorganisms are involved in the oxidation and reduction processes in mine waste dumps. The microbial diversity comprised aerobic and anaerobic species which are autotrophic (CO_2-fixation) or heterotrophic (C_{org} as carbon source) as well as lithotrophic (inorganic compounds as energy source) or organotrophic (C_{org} as energy source).

In several previous studies Fe(II)-oxidizing Bacteria such as *At. ferrooxidans*, *Leptospirillum ferrooxidans, L. ferriphilum* and *Sulfobacillus* sp. (Selenska-Pobell et al., 2001; Demergasso et al., 2005a; Bruneel et al., 2005; Diaby et al., 2007) were detected in sulfidic mine waste dumps. While representatives of the genera *Acidithiobacillus* and *Sulfobacillus* are additionally able to oxidize sulfur compounds as well as to reduce Fe(III) under anaerobic conditions, *Leptospirillum* only uses Fe(II) as substrate. In contrast, the acidophiles *At. thiooxidans*, *Thiobacillus* sp. and *Thiomonas* sp. detected in mine waste dumps oxidize sulfur compounds but not Fe(II) (Schippers et al., 1995, 1996, 2000; Wielinga et al., 1999; Dill et al., 2002; Bruneel et al., 2005; Diaby et al., 2007). Furthermore, the heterotrophic bacteria *Acidiphilium* sp. and *Pseudomonas* sp. may grow via oxidation of sulfur compounds and additionally are able to reduce Fe(III) under anaerobic conditions (Groudev and Groudeva, 1993; Macur et al., 2001; Selenska-Pobell et al., 2001; Diaby et al., 2007). An exclusively organotrophic Bacterium detected in this environment is *Acidobacterium* (Diaby et al., 2007). The contribution of organo- and heterotrophic microorganisms within the community are presumably synergistic interactions with bacteria by removing metabolic byproducts of the autotrophs as implicated in different studies (Pronk

and Johnson, 1991; Hallmann et al., 1993; Schippers et al., 1995; Baker and Banfield, 2003). As representatives of anaerobic, sulfate-reducing bacteria *Desulfosarcina* sp. and *Desulfotomaculum* sp. were detected (Schippers et al., 1995; Blowes et al., 1995; Fortin et al., 1996, 2000; Fortin and Beveridge, 1997; Wielinga et al., 1999; Benner et al., 2000; Bruneel et al., 2005; Diaby et al., 2007).

But not only Bacteria could be detected in mine waste dumps. The acidophil, facultative mixo- and organotrophic archaeum *Ferroplasma acidiphilum* was detected by Demergasso et al. (2005a) and is capable to oxidize Fe(II) aerobically and to reduce Fe(III) under anaerobic conditions. As representative of an eukaryotic organism the fungus *Aspergillus* sp. (Harrison, 1978) was found by cultivation techniques in tailings samples.

So far, for biomining applications and AMD effluents more studies were carried out for the identification and quantification of microorganisms than for mine waste tailings. Additionally to the above mentioned microbes, in these environments acidophilic metal sulfide-oxidizing Bacteria such as *Acidimicrobium ferrooxidans*, "*Ferrimicrobium acidiphilum*" as well as further strains of the genus *Acidithiobacillus* and acidophilic metal sulfide-oxidizing Archaea such as *Metallosphaera* spp., *Sulfolobus* spp. and *Acidianus* spp. were detected (Baker and Banfield, 2003; Johnson and Hallberg, 2003; Schippers, 2007; Rawlings and Johnson, 2007b).

1.5.2 Quantification of microorganisms in environmental samples

For the investigation of biomining applications, AMD effluents, and mine waste dumps several identification and quantification methods were used. While, so far, prokaryotes in sulfidic mine waste dumps primarily were quantified by cultivation techniques, several qualitative molecular biological approaches were applied to investigate the microbial diversity in biomining environments. Generating clone libraries and sequencing DNA as well as the application of methods like denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) or amplified ribosomal DNA restriction enzyme analysis (ARDREA) are just few approaches which were used for the investigation of these environments (Schippers, 2007). These qualitative molecular biological investigations provide valuable information about the microbial diversity but little or no information about the microbial activity and the quantities of the different microbial groups or species.

The quantification of microorganisms in environmental samples can be done by classical cultivation techniques such as the most–probable-number technique (MPN) or agar plates which yield cell numbers just according to microbial physiological properties. So far, several studies enumerated or isolated prokaryotes involved in oxidation and reduction processes in biomining environments by cultivation techniques. For instant, acidophilic Fe(II)-oxidizing microorganisms could be detected with highest cell numbers of up to 10^8 cells g^{-1} dry weight

(dw) in sulfidic mine tailings in Quebec and Ontario in Canada (Southam and Beveridge, 1992; Blowes et al., 1995) but also in smaller numbers in uranium, sulfidic mine waste rock heaps in Germany (Schippers et al., 1995) and in a porphyry copper tailings impoundment in Chile (Diaby et al., 2007). Furthermore sulfur-oxidizing microorganisms were detected in cell numbers of up to 10^3 cells g^{-1} dw in the above mentioned tailings dump in Ontario, Canada and in the mine waste heaps in Germany. Also sulfate- and Fe(III)-reducing bacteria were found in the mine waste heaps in Germany as well as in sulfidic mine tailings in Ontario, Canada and in mining-impacted sediments in Idaho, USA in cell numbers up to 10^6 cells g^{-1} dw (e.g. Schippers et al., 1995; Fortin et al., 1996; Wielinga et al., 1999; Cummings et al., 2000). Additionally, the growth of microorganisms such as sulfur compounds-, ammonia- and nitrite-oxidizers, nitrate- and manganese-reducers as well as aerobic and anaerobic chemoorganotrophic bacteria in adequate media was performed in different studies of sulfidic mine waste tailings (e.g. Southam and Beveridge 1992; Schippers et al. 1995; Wielinga et al., 1999; Diaby et al., 2007).

There are different molecular biological methods for quantifying microorganisms. Nucleic acid staining fluorochromes such as SYBR Green, acridine orange or DAPI were used to determine total cell numbers. This technique was applied to sulfidic mine waste tailings located in Namibia and Chile and provided total cell numbers of up to 10^8 and 10^9 cells g^{-1} dw, respectively (Dill et al., 2002; Diaby et al., 2007).

In the case of fluorescence *in situ* hybridization (FISH) and catalyzed reporter deposition - fluorescence *in situ* hybridization (CARD-FISH) 16S ribosomal RNA (rRNA) probes are used to quantify specific groups of microorganisms. So far, these epifluorescence methods were applied to AMD effluents and biomining applications but not to sulfidic mine waste dumps. Bacteria and Archaea were quantified in an industrial heap bioleaching operation in Chile by FISH as well as CARD-FISH (Demergasso et al., 2005b). The biooxidation of pyrite by defined mixed cultures in bioreactors was investigated by using specific FISH-probes detecting microorganisms such as *Leptospirillum* sp., *Acidithiobacillus caldus*, *Acidimicrobium ferrooxidans* or *Ferroplasma* sp. (Okibe and Johnson, 2004). Furthermore several sites of AMD effluents were investigated by FISH analyses. Eukarya, Bacteria, Archaea as well as beta-proteobacteria and microorganisms of the genera *Acidithiobacillus*, *Leptospirillum*, *Sulfobacillus*, *Acidiphilium*, *Acidimicrobium*, *Ferroplasma* or *Thermoplasma* were quantified in AMD effluents from the Richmond Mine at Iron Mountain, California or from Pynydd Parys, Anglesey in North Wales as well as in the Tinto River in Spain (Edwards et al., 1999; Bond and Banfield, 2001; González-Toril et al., 2003; Hallberg et al., 2006).

Quantitative real-time PCR (Q-PCR) applies specific primers for 16S rRNA genes for identification and quantification of microorgansims. This highly sensitive method was successfully applied to pure cultures of Bacteria and Archaea involved in biomining and in

samples from biomining operations (Liu et al., 2006; Remonsellez et al., 2007; Zammit et al., 2007) but not to samples from sulfidic mine waste dumps. Specific assays were developed for detecting microorganisms such as *Acidithiobacillus* sp., *Acidithiobacillus caldus*, *Leptospirillum* sp., *Sulfobacillus* sp., *Sulfolobus* sp. or *Ferroplasma* sp..

1.6 Aims of the thesis

The aims of this thesis were:

1. Evaluation of the applicability of the molecular quantification techniques FISH, CARD-FISH and Q-PCR to solid samples from mine waste tailings dumps and a biomining experiment, and the comparison with the established methods direct counting of total cells using SYBR Green staining and MPN cultivation
2. Quantitative microbial community analysis of sulfidic mine waste tailings dumps, considering different depths layers in the dumps especially a comparison of oxidized and unoxidized zones
3. Comparison of the microbial communities in three chemically and mineralogically different sulfidic mine waste tailings dumps located in different climate zones
4. Investigation of the role of the microbial communities for biogeochemical processes in the sulfidic mine waste dumps, i.e. potential metal sulfide oxidation rates and natural attenuation processes leading to cemented layer formation in the dumps

Samples for the studies were obtained from one biomining experiment run in the geomicrobiology laboratory of the Federal Institute for Geosciences and Natural Resources (BGR), Hannover, and from three different sulfidic mine waste tailings dumps. The first tailings dump was located in semi-arid climate near Selebi-Phikwe in Botswana, was still in operation at the time of sampling and dominated by the metal sulfide pyrrhotite (Fig. 1, 2A). The second one was located in cold and wet climate near Kristineberg in northern Sweden (Fig. 2B). This tailings dump was remediated by a soil cover and the main metal sulfide was pyrite. The third investigated tailings dump was unremediated and located in temperate climate near Freiberg in Germany (Fig. 2C). The main metal sulfides in these tailings dump were pyrite, arsenopyrite, sphalerite and galena.

Fig. 2. *In this thesis investigated mine waste tailings dumps. A: The drilling vehicle on top of the tailings dump located near Selebi-Phikwe in Botswana which was still in operation 2003 (see also Fig. 1). B: The drill rig on top of the remediated tailings dump near Kristineberg in Sweden. C: The outcrop on the unremediated tailings dump near Freiberg in Germany (Pictures A, B: Axel Schippers, BGR Hannover).*

2. Results and Discussion

2.1 Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste dam near Selebi-Phikwe in Botswana

The first manuscript is concerned with geochemical and geomicrobiological analyses of the sulfidic tailings dump near Selebi-Phikwe in Botswana. For a remediation strategy within the mine closure plan the AMD potential of this mine waste dump was quantified.

The AMD generating tailings dump consisted at the year of sampling 2003 of waste from about 32 years of Ni-, Cu-, Zn- and Co-sulfidic ore processing, was approximately 40 m high and covered an area of ca. 1 km^2. Because the tailings material was dumped as sludge, the surface of the central part of the dump was water covered (Fig. 1, 2A). In the dry periphery three holes were drilled through the water unsaturated down to the saturated zone at about 25 m depth and 65 solid samples were taken in 1 m intervals. Brown precipitates of Fe(III)(hydro)oxides could be found throughout the entire unsaturated zone which had a paste pH in the range of 3-4. The total sulfur content in the tailings ranged between 2-8% and mainly consisted of reduced inorganic sulfur. The main metal sulfide was pyrrhotite while pyrite, Ni- and Co-sulfides occurred in minor amounts.

The whole water unsaturated zone of 25 m depth at the periphery was colonized by microorganisms in high numbers. The mean cell number of total microorganisms detected by SYBR Green direct counting was 8 x 10^7 cells g^{-1} dw. A high proportion of this number could be detected as Bacteria by CARD-FISH (1 x 10^7 cells g^{-1} dw). These Bacteria are considered to be alive since the method detects ribosomal RNA which is quickly degraded in dead cells in the environment (Schippers et al., 2005). Cultivable, Fe(II)-, and metal sulfide-oxidizing *At. ferrooxidans*-like microorganisms were present in high numbers, too (mean value of MPN: 3 x 10^6 cells g^{-1} dw). These high cell numbers indicate a high bacterial activity in the tailings dump. Surprisingly, a decrease of cell numbers with depth could not be observed, as found in other sulfidic tailings (Silver, 1991; Elberling et al., 2000). Furthermore the numbers of cultivable metal sulfide-oxidizing microorganisms were significantly greater in the here investigated tailings dump than in other tailings (Silver, 1991; Blowes et al., 1995, 1998; Elberling et al., 2000; Schippers et al., 2000).

The high cell numbers in the tailings dump of this study correlated with high potential pyrrhotite oxidation rates of up to 1 mmol m^{-3} s^{-1} for all depths. A half of the pyrrhotite oxidation was chemically the other half biologically as determined by microcalorimetric measurements. A mean potential pyrrhotite oxidation rate related to the mineral surface of 1.9 x 10^9 mol pyrrhotite m^{-2} s^{-1} was determined which was in the range of laboratory

pyrrhotite oxidation tests (Janzen et al. 2000) but several times higher than rates determined for other sulfidic tailings (Elberling and Nicholsen, 1996; Elberling et al., 2000). Assuming constant pyrrhotite oxidation rates over time, the pyrrhotite in the tailings dump of this study would be oxidized within 80-140 years. These findings clearly showed the high microbial activity and high AMD potential of the tailings dump in Botwana and the requirement of remediation measures. For the reduction of AMD formation during mining operations, metal sulfides like pyrrhotite should be separated from the tailings material and separately deposited e.g. under a cover to prevent oxidation by oxygen in the air.

2.2 Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage

In this paper the mine waste tailings located in Selebi-Phikwe, Botswana and in Kristineberg, Sweden were investigated and compared. In addition to the first manuscript (see 2.1), further microbiological analyses of the site in Botswana were performed for studying the microbial impact on metal sulfide oxidation on acid mine drainage and comparing it with the second site in Sweden.

The tailings in Sweden covered an area of 0.1 km^2 and were 6-8 m high (Fig. 2B). This tailings dump consisting of waste from Cu- and Zn-sulfide ore processing was in operation from the 1940s until the early 1950s and left unremediated. Sulfide oxidation occurred in distinct depth layers (oxidized tailings) characterized by low sulfide contents (1-2%) and brown precipitates of Fe(III)(hydr)oxides. In the unoxidized tailings sulfide contents of 10-30% occurred, totally dominated by pyrite. The mean pH in the unoxidized zone was 4.9, and 3.5 in the oxidized zone. In 1996 the tailings dump was covered with an approximately 2 m thick soil cover. Three boreholes were drilled and 30 subsamples from the oxidized and unoxidized tailings were taken for laboratory analysis in 2003.

Microorganisms were quantified using four different methods (SYBR Green direct counts, CARD-FISH, Q-PCR and MPN). Additionally, the potential pyrite or pyrrhotite oxidation rates in the two mine waste tailings were determined by microcalorimetry.

The geomicrobiological analyses in the uncovered, pyrrhotite-containing tailings in Botswana and the covered tailings impoundment in Sweden showed significant differences for both tailings. In the tailings dump in Botswana the whole water unsaturated zone of 25 m depth at the periphery was colonized by microorganisms in high numbers. The cell numbers detected by SYBR Green direct counts provided highest values of 8 x 10^7 cells g^{-1} dw in average. The Q-PCR values for total prokaryotes and total Bacteria are somewhat lower (mean values: 6 x 10^7 cells g^{-1} dw and 3 x 10^7 cells g^{-1} dw, respectively). The mean number of living Bacteria detected by CARD-FISH was 1 x 10^7 cells g^{-1} dw. A high proportion of 30%

of the living Bacteria could be detected via MPN cultivation of acidophilic Fe(II)-oxidizing microorganisms. The average potential pyrrhotite oxidation rate was 3.4 x 10^{-4} mol pyrrhotite m^{-3} tailings s^{-1} at 25°C while about half of this oxidation activity was biologically catalyzed.

In contrast, in the tailings in Sweden, distinct differences in cell numbers in the oxidized and unoxidized zones could be observed. Acidophilic Fe(II)-oxidizing microorganisms were only detected in the zone of oxidized tailings (mean value: 5 x 10^5 cells g^{-1} dw). The number of Bacteria was 3 x 10^8 cells g^{-1} dw in average (Q-PCR). Total cell numbers detected by SYBR Green are considerably higher in the oxidized than in the unoxidized zone and identical to the cell number of Bacteria (Q-PCR). Up to 100% of the pyrite oxidation was biologically catalyzed and the average potential pyrite oxidation rate was 1.6 x 10^{-5} mol pyrite m^{-3} tailings s^{-1} at 10°C. In both mine waste tailings Archaea could not be detected by Q-PCR and CARD-FISH which indicates the dominance of Bacteria in the tailings.

For the first time microorganisms were quantified in sulfidic mine tailings using four different methods. All methods provided reliable results which clearly showed the differences of the microbial community composition of these two mine waste tailings. In Botswana throughout the entire unsaturated zone a high proportion of the SYBR Green and Q-PCR detectable cells could be detected by CARD-FISH and MPN. This indicated that a high proportion of the microorganisms was alive because the cells were cultivable by MPN and detectable by CARD-FISH targeting ribosomal RNA which is quickly degraded in dead cells in the environment (Schippers et al., 2005). The high potential pyrrhotite oxidation rate and the high proportion of biological oxidation demonstrate the high AMD generation potential in these tailings and the huge impact of microorganisms on metal sulfide oxidation. By contrast, in the tailings impoundment in Sweden, only a minor proportion of less than 1% of acidophilic Fe(II)-oxidizing microorganisms was detected in the oxidized zone and the potential pyrite oxidation rate was one order of magnitude lower than the rate for the pyrrhotite-containing tailings in Botswana. These results can be explained with reduced molecular oxygen diffusion due to the soil cover in Sweden and the lower reactivity of pyrite in comparison with pyrrhotite (Belzile et al., 2004).

2.3 Quantitative microbial community analysis of three different sulfidic mine tailings dumps generating acid mine drainage

A comparative quantitative microbial community analysis for three different tailings dumps is given in the third manuscript. Additionally to the already described uncovered and covered tailings dumps in Botswana and Sweden, respectively (chapter 2.1 and 2.2), the uncovered tailings dump near Freiberg, Germany was part of this study (Fig. 2C). This tailings dump was up to 30 m high and 0.06 km^2 large, unremediated and a soil cover of less

then 0.2 m could partly be found at the time of sampling 2006. The tailings was in operation from 1955 to 1968 and consisted of waste from Pb- and Zn- sulfidic ore processing. The main metal sulfides of the tailings material were pyrite, arsenopyrite, sphalerite and galena. The oxidized zone between the soil cover and 0.6 m depth was characterized by metal sulfide contents ≤ 0.1% and a pH of 4-5. In 0.6-0.63 m depth three thin cemented layers (pH 3-4) delimited the zone of active oxidation processes below (metal sulfide content ≤ 1%; pH ~3). Below 0.85 m depth the unoxidized zone was located with metal sulfide contents of 1% and pH values between 5 and 7. From an outcrop-profile in the center of the tailings dump down to 1.2 m 21 samples were taken (Fig. 3).

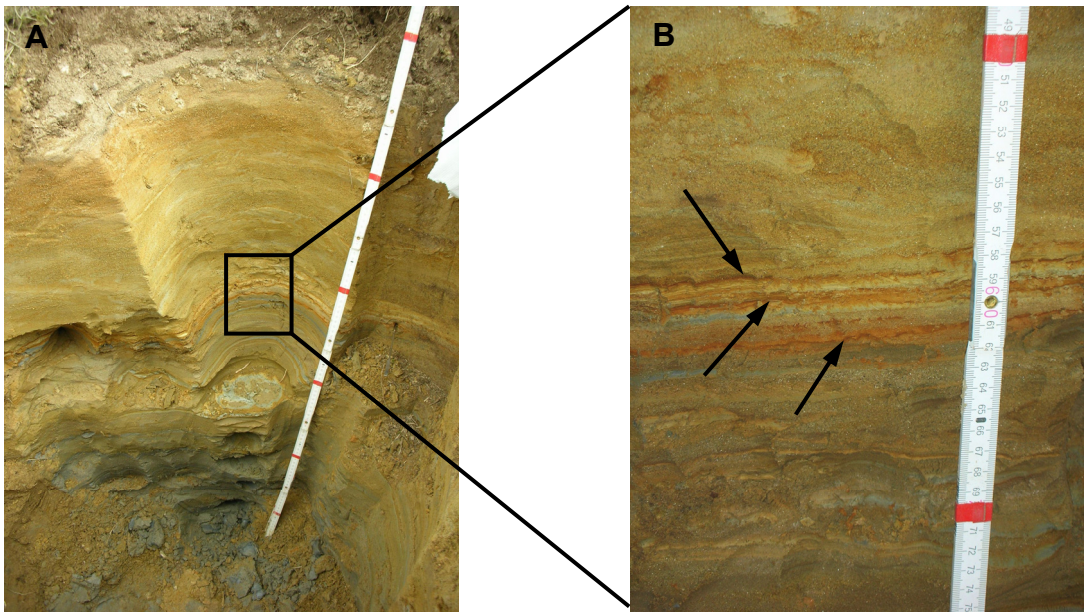

Fig. 3. *(A) Depth profile of oxidized tailings (<85 cm) with cemented layers (60-63 cm) in a tailings dump in Freiberg, Germany. The oxidation zone was located at depths of ~60-85 cm. (B) Magnification of three distinct 0.2-0.25 cm thick cemented layers (arrows), underlain by altered silt layers in the depth range of ~60-63 cm. Red marks on scale define 20 cm.*

The five quantification methods Q-PCR, FISH, CARD-FISH, SYBR Green II direct counting and MPN were used to investigate the microbial community in the different sulfidic and acidic mine waste tailings dumps. The depth profiles of the three sites showed significant differences in cell numbers and in the composition of the microbial communities as well as strong variations between zones of oxidized and unoxidized tailings. SYBR Green II total cell counts provided highest cell numbers in all three tailings of up to 10^9 cells g^{-1} dw in the oxidation zones in Botswana and Germany whereas significant lower cell numbers were detected in unoxidized tailings. Bacteria were detected as the dominating microorganisms at all here investigated sites. Archaea and Eukarya were less abundant. The physiologically more versatile *At. ferrooxidans* dominated over the exclusively Fe(II)-oxidizing acidophilic

Leptospirillum sp. in the samples from the tailings in Botswana and Germany. In the tailings dump in Sweden both genera were detected in equal numbers. Other quantified and less abundant, acidophilic Fe(II)- and sulfur-oxidizing organisms belonged to the genus *Sulfobacillus*. Cell numbers of *Acidimicrobium* and relatives were below detection limit in all investigated samples. Besides Fe(II)- and sulfur-oxidizing microorganisms, Fe(III)-reducing representatives were detected in different numbers at the three sites. Besides *At. ferrooxidans* and *Sulfobacillus*, which also reduce Fe(III), the acidophilic *Acidiphilium* sp. could only be found in the tailings in Botswana, but the neutrophilic Geobacteraceae were present in all three tailings. Sulfate-reducers could be quantified by detecting their specific functional gene *dsrA* in all three tailings as well.

With exception of FISH all used methods in this study were shown to be applicable to mine waste tailings in general and provided suitable results. In the case of FISH only in samples from zones of high microbial activity as found in the tailings in Freiberg (Fig. 4), comparable cell numbers could be detected than quantified with the more sensitive method CARD-FISH.

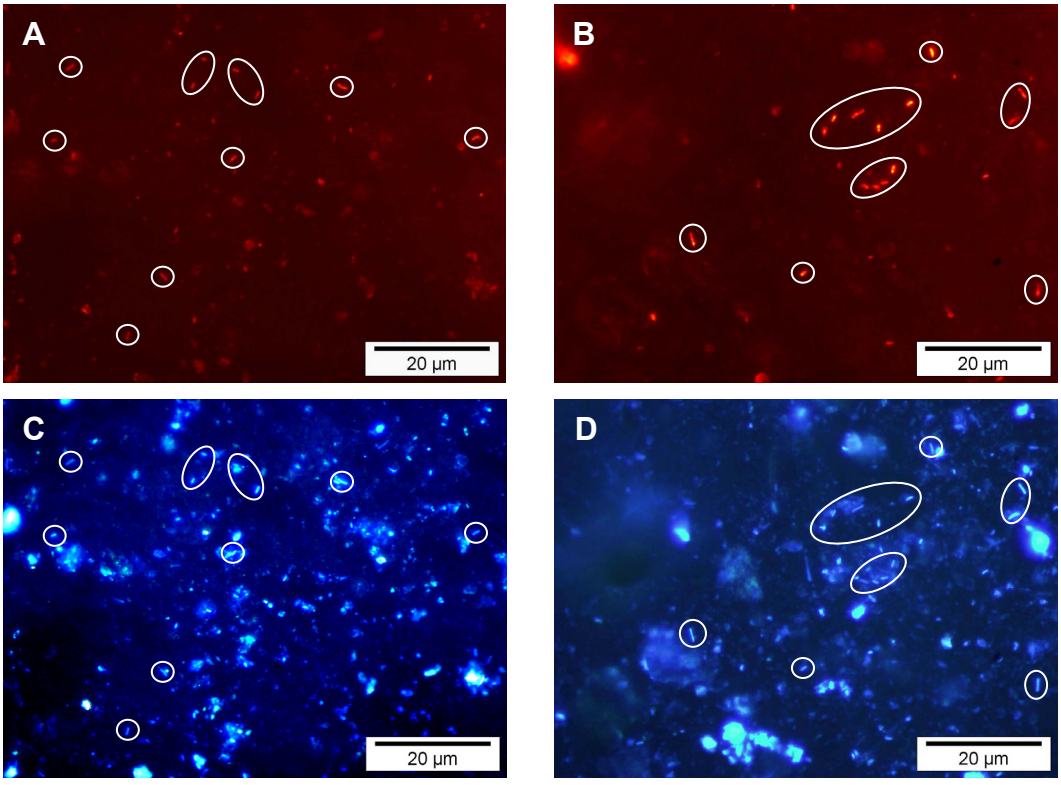

Fig. 4. *Quantification of microorganisms by FISH (A) and CARD-FISH (B) in a tailings sample from a mine waste dump in Freiberg, Germany. Living cells with weak FISH- and bright CARD-FISH-signals detected with the EUB338 probe specific for Bacteria are shown. Additional, total cells are counterstained with the blue fluorescing DNA-dye DAPI (C, D). Each DAPI picture shows the same detail than shown in the FISH and CARD-FISH picture, respectively. Some cells are depicted by encircling.*

The results of this study as well as of the investigation of a porphyry copper mine in Chile (Diaby et al., 2007) demonstrated that the composition of the Fe(II)- and sulfur-oxidizing bacterial community largely varied at different tailings sites. Fe(III)- and sulfate-reducing microorganisms were detected in this study as well as in other studies of sulfidic mine waste (Schippers et al. 1995; Wielinga et al., 1999; Cummings et al., 2000). The anaerobic and presumably neutrophilic Bacteria might be protected from acidity and oxygen by living in microenvironments (Fortin et al., 1996; Schippers et al., 1995). Archaea seem to play a minor role in sulfidic mine tailings which presumably can be explained by the fact that species relevant for iron- and sulfur-cycling described are extremophiles which grow under very acidic conditions with high iron and sulfate concentrations or at high temperatures which usually do not prevail in mine tailings.

For the first time the microbial communities in sulfidic mine waste tailings were extensively quantified by molecular biological methods in this study. The intense colonization by Fe(II)- and sulfur-oxidizing as well as Fe(III)- and sulfate-reducing microorganisms in the here investigated tailings dumps and elsewhere show, that biogeochemical iron- and sulfur-cycling are predominant processes mediated by microbial activity.

2.4 Quantification of microorganisms involved in cemented layer formation in sulfidic mine waste tailings (Freiberg, Saxony, Germany)

The aim of this study was to investigate the contribution of microorganisms to mineral weathering and cemented layer formation in the mine waste tailings near Freiberg, Germany (see also 2.3). Microorganisms were quantified by molecular biological and cultivation methods in a high resolution depth profile. Highest cell numbers were detected in the zone of cemented layers (0.6-0.63 m) and the oxidation zone below. Total cell numbers of $>10^9$ cells g^{-1} dw were found by SYBR Green direct counting. Bacterial cell numbers detected by Q-PCR throughout the whole investigated depth profile were in average an order of magnitude lower, while Archaea were only detected (by Q-PCR but not by CARD-FISH) in the oxidized zone above the cemented layers (up to 10^8 cells g^{-1} dw). Numbers of living Bacteria and cultivable Fe(II)-oxidizing microorganisms detected by CARD-FISH and MPN reached highest values of 10^9 cells g^{-1} dw in the zone of cemented layers and the oxidation zone underneath. Cell numbers of cultivable sulfur-oxidizing microorganisms (MPN) showed a similar depth profile but distinct lower cell numbers than those of cultivable Fe(II)-oxidizing microorganisms.

In particular, the high numbers of Fe(II)-oxidizing microorganisms detected in the zone of cemented layers and the oxidation zone below demonstrate the high microbial metal

sulfide oxidation activity in these zones. Secondary mineral phases of cemented layers are formed by oxidation processes. Extracellular polymeric substances (EPS) formed by microorganisms are able to bind metals in complexes and further may serve as nucleation site for mineral formation (Sand et al., 2001; Ferris et al., 1989; Southam and Beveridge, 1992). The high microbial cell numbers in these tailings indicate a significant role of microorganisms in cemented layer formation by microbial mineral weathering and formation of secondary mineral phases.

2.5 Formation of sequences of cemented layers and hardpans within sulfide-bearing mine tailings (mine district Freiberg, Germany)

This study with geochemical, mineralogical and geophysical focus was applied to investigate the mechanism of the formation of cemented layers in an AMD generating tailings dump and the natural attenuation of metals in the cemented layers. The site was the sulfidic mine waste tailings dump near Freiberg, Germany (see also 2.3 and 2.4). A high resolution profiling combining geochemical and mineralogical with geomicrobiological analyses was carried out to describe the process of weathering of reactive mineral phases, to determine the position of the oxidation front as well as to reveal the mechanisms of cementation of tailings.

The significant change in cell numbers of metal sulfide-oxidizing microorganisms at the recent position of the oxidation front correlated with the strong changes in geophysical and geochemical data and corresponded with the location of the cemented layers. This indicates the importance of biogeochemical processes such as microbial metal sulfide oxidation for the formation of cemented layers. The main effects of the cemented-layer formation are the natural attenuation of the toxic As and Pb species in the secondary phases, a slowing of the downward movement of the oxidation front due to reduced porosities and dense silt/ clay layers as well as a reduction of the extent of the erosion of the surface of the tailings dump by wind and water. Furthermore these investigations showed that the potential of a tailings dump to form cemented layers is greatly enhanced by a heterogeneous distribution of grain sizes and reactive materials in the upper zone and also by the presence of sulfide-rich tailings on top of slightly permeable layers.

2.6 The use of FISH and real-time PCR to monitor the biooxidation and cyanidation for gold and silver recovery from a mine tailings concentrate (Ticapampa, Peru)

The aim of this study was to verify if biomining is an option for tailings bioremediation and extraction of valuable metals from mine waste and if quantitative molecular methods are suitable for monitoring of biomining. In this study, gold and silver recovery from an acid mine drainage generating sulfidic mine tailings dump near Ticapampa, Peru, via biooxidation and cyanidation was demonstrated. Refractory gold (up to 316 g/t) is hosted in As-rich zones of some arsenopyrites. An adapted mixed culture of *At. ferrooxidans*, *At. thiooxidans* and *L. ferrooxidans* was applied for biooxidation of a tailings concentrate. During biooxidation, arsenopyrite was preferentially dissolved. The following cyanidation of the biooxidized concentrate showed a recovery of 97% and 50% for gold and silver, respectively. The values were 56% and 18% for the untreated concentrate.

The biooxidation process was monitored by using FISH, CARD-FISH, SYBR Green direct counting and Q-PCR. The total cell numbers showed bacterial growth over the experimental time and an increase from about 10^6 cells mL^{-1} to about 10^8 cells mL^{-1}. Cell numbers detected by FISH were slightly lower than cell numbers detected by the more sensitive method CARD-FISH. By FISH and Q-PCR analyses *At. ferrooxidans* was detected as dominating bacterium in the biooxidation experiment while *Leptospirillum* sp. was quantified in distinct lower cell numbers.

In conclusion, the here applied methods FISH, CARD-FISH and Q-PCR are suitable methods for monitoring numbers of metal sulfide-oxidizing bacteria during biooxidation and bioleaching processes. The high proportion of the recovered precious metals indicates that biomining of tailings offers a low-cost and efficient bioremediation measure especially if the present high market prices for metals are considered.

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4. Publications

4.1 Contributions to the manuscripts presented in this thesis

1. **Schippers, A., D. Kock, M. Schwartz, M. E. Böttcher, H. Vogel, and M. Hagger.** 2007. Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste tailings dam near Selebi-Phikwe in Botswana. Published in *J. Geochem. Explor.* **92**: 151-158.

   Concept development by A.S., microbiological analyses by D.K., oxidation rates, geochemical analyses and site description by A.S., M.S., M.E.B., H.V. and M.H., writing of the manuscript by A.S. with editorial help by the co-authors.

2. **Kock, D., and A. Schippers.** 2006. Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage. Published in *Hydrometallurgy* **83**: 167-175.

   Concept development by A.S., analyses and presentation of results by D.K., writing of the manuscript by D.K. and A.S.

3. **Kock, D., and A. Schippers.** 2008. Quantitative microbial community analysis of three different sulfidic mine tailings dumps generating acid mine drainage. Published in *Appl. Environ. Microbiol.* **74**: 5211-5219.

   Concept development and analyses by D.K., writing of the manuscript by D.K. with editorial help by A.S.

4. **Kock, D., T. Graupner, D. Rammlmair, and A. Schippers.** 2007. Quantification of microorganisms involved in cemented layer formation in sulfidic mine waste tailings (Freiberg, Saxony, Germany). Published in *Advanced Materials Research* **20/21**: 481-484.

   Concept development by D.K., analyses by D.K., writing of the manuscript by D.K. with editorial help by the co-authors.

5. **Graupner, T., A. Kassahun, D. Rammlmair, J. Meima, D. Kock, M. Furche, A. Fiege, A. Schippers, and F. Melcher.** 2007. Formation of sequences of cemented layers and hardpans within sulfide-bearing mine tailings (mine district Freiberg, Germany). Published in *Appl. Geochem.* **22**: 2486-2508.

   Concept development by T.G., microbiological analyses by D.K., geophysical analyses by M.F., geochemical and mineralogical analyses by T.G., A.K., J.M., A.F. and F.M. writing of the manuscript by T.G. with editorial help by the co-authors.

6. **Schippers, A., A. A. Nagy, D. Kock, F. Melcher, and E.-D. Gock.** 2008. The use of FISH and real-time PCR to monitor the biooxidation and cyanidation for gold and silver recovery from a mine tailings concentrate (Ticapampa, Peru). Published in *Hydrometallurgy* **94**: 77-81.

   Concept development by A.S., microbiological analyses by D.K. and A.S., microprobe analyses by F.M., bioleaching experiments by A.S., cyanide leaching experiments by A.A.N. and E.D.G., writing of the manuscript by A.S. with editorial help by the co-authors.

4.2 Publications

4.2.1 Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste tailings dam near Selebi-Phikwe in Botswana

Schippers, A., D. Kock, M. Schwartz, M. E. Böttcher, H. Vogel, and M. Hagger

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Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste tailings dam near Selebi-Phikwe in Botswana

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Abstract

An acid mine drainage (AMD) generating tailings dam consisting of waste from about 32 years of Ni-, Cu-, Zn- and Co-sulfidic ore processing near Selebi-Phikwe, Botswana, was investigated to quantify the AMD generation potential for developing a remediation strategy within the mine closure plan. The climate in the region is semiarid with an average annual temperature of 21 °C. The approximately 40 m high dam covers an area of ca. 1 km^2. The surface of the central part of the dam is water covered, but the periphery is dry. Three holes were drilled through the water unsaturated periphery down to the saturated zone at about 25 m depth. Altogether 65 solid samples were taken in 1 m intervals and geomicrobiologically and geochemically analyzed. Brown precipitates of iron(hydro)oxides were discernible throughout the entire unsaturated zone with a paste pH in the range of 3–4. The tailings had sulfur contents in the range of 2–8 wt.% (average 4%). The total sulfur consisted mainly of reduced inorganic sulfur. Pyrrhotite was the main metal sulfide, pyrite, Ni- and Co-sulfides occurred in minor amounts. Metal sulfide oxidizing *Acidithiobacillus ferrooxidans*-like bacteria were present in high numbers (mean value of most-probable-number method: 3×10^6 cells g^{-1} dw) throughout the whole unsaturated zone. Mean numbers of living bacteria (CARD-FISH) and total microorganisms (SybrGreen direct counts) were 1×10^7 cells g^{-1} dw and 8×10^7 cells g^{-1} dw, respectively. The average potential pyrrhotite oxidation rate measured by microcalorimetry was 1.9×10^{-9} $mol_{pyrrhotite}$ m^{-2} s^{-1}. The ratio of biological to chemical pyrrhotite oxidation was ca. 50:50. For the assumed 0.1 m thickness of the oxidation zone within the 25 m unsaturated zone the potential average pyrrhotite oxidation rate was 3.4×10^{-5} mol m^{-2} s^{-1} (94 kg FeS m^{-2} $year^{-1}$). At this rate, all pyrrhotite in the tailings dam would be oxidized within 80 years.

Keywords: Pyrrhotite; Tailings; Oxidation rate; Acid mine drainage; Bacteria

1. Introduction

Acid mine drainage (AMD) is a strongly acidic solution containing high amounts of heavy metals and sulfate that threaten groundwater quality. AMD is

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generated by chemical and biological oxidation of pyrite, pyrrhotite and other metal sulfides in mine waste heaps or in tailings from sulfidic ore processing (Johnson et al., 2000; Jambor et al., 2003; Schippers, 2004). The pyrite oxidation rate depends on temperature, pH, humidity and the availability of oxygen in the tailings, which is mainly controlled by diffusion. In addition, the oxidation rate strongly depends on the abundance of acidophilic Fe(II)- and metal sulfide oxidizing microorganisms, which accelerate the kinetics of pyrite oxidation 30–300-fold (Nordstrom and Southam, 1997; Nordstrom and Alpers, 1999). Geomicrobiological and geochemical studies of pyrite and pyrrhotite containing mine tailings showed the important role of acidophilic Fe (II) oxidizing microorganisms, such as *Acidithiobacillus ferrooxidans* for the generation of AMD (Silver, 1991; Blowes et al., 1995, 1998; Elberling et al., 2000; Schippers et al., 2000; Dold and Fontboté, 2001). Only a few studies have included the measurement of pyrite or pyrrhotite oxidation rates (Elberling and Nicholson, 1996; Elberling et al., 2000; Elberling and Damgaard, 2001). Due to pyrrhotite oxidation, sulfuric acid is formed according to Eq. (1) (Seal and Hammarstrom, 2003):

$$Fe_{1-x}S + (2-x/2)O_2 + xH_2O \rightarrow (1-x)Fe^{2+} + SO_4^{2-} + 2xH^+ \quad (1)$$

Based on the mineralogical composition, x ranges between 0 and 0.125.

Fe(II) may be further oxidized to Fe(III)hydroxide according to Eq. (2):

$$Fe^{2+} + 0.25O_2 + 2.5H_2O \rightarrow Fe(OH)_3 + 2H^+ \quad (2)$$

The kinetics of chemical pyrrhotite oxidation, which is critical for AMD generation, have been studied in the laboratory and reaction rates of up to 3.5×10^{-8} mol m^{-2} s^{-1} at pH 2.75 were determined (Janzen et al., 2000). The chemical pyrrhotite oxidation rate was in the order of 20–100 times those measured for pyrite (Belzile et al., 2004). Biological pyrrhotite oxidation rates have not been determined to our knowledge.

In the present study, the pyrrhotite containing tailings dam at Selebi-Phikwe, Botswana was geochemically and geomicrobiologically analyzed, including the use of microcalorimetric measurements for the estimation of potential pyrrhotite oxidation rates. The rates allowed us to calculate a time period of AMD formation for the tailings dam which is crucial for the development of a remediation strategy within the mine closure plan.

2. Methods

2.1. Site description and sampling

The climate in Selebi-Phikwe, Botswana, is semiarid with an average annual temperature of 21 °C. The mine waste tailings dam near Selebi-Phikwe generates high amounts of AMD, which are collected in a drainage ditch surrounding the dam. To prevent contamination of surface water, AMD is purified in a plant by addition of limestone to increase the pH and to precipitate metals. The tailings dam consists of waste from about 32 years of Ni-, Cu-, Zn- and Co-sulfidic ore processing. Approximately 204 t dry solids/h from the ore processing plant (concentrator) are deposited on the tailings dam. This material consists of about 35% solid material with an average grain size diameter of about 0.1 mm. The solid material contains about 11% pyrrhotite, about 1.5% other metal sulfides, and hornblende and feldspar as major gangue minerals. The approximately 40 m high dam in 2003 covers an area of ca. 1 km^2. The final height shall be 50 m in the year 2014. Currently, the surface of the central part is water covered, whereas the periphery of the dam surface is dry. Three holes (B-H1 = core 1, B-H2 = core 2, B-H3 = core 3) were drilled through the dry periphery to the saturated zone at about 25 m depth.

Brown precipitates of iron(hydrox)oxides due to pyrrhotite oxidation occurred throughout the entire unsaturated zone. Altogether 65 solid samples were taken in 1 m intervals. The analysis of polished sections of 25 samples from core 1 showed that the average surface area of the grains was approximately 1 m^2/g and that the fraction of oxidized pyrrhotite varied between 5% and 50% (average 20%), and did not correlate with depth.

2.2. Geochemical analysis

The paste pH was measured with an electrode after shaking of 5 g sample in 12.5 ml 1 M KCl for 1 h. Water content was determined as weight difference after drying. The elemental composition of the solid material was determined by XRF analysis (Philips PW 2400). Organic carbon (C_{org}) and the total amount of sulfur (S_{total}) were measured with the instrument LECO CS 200 after acid removal of carbonates. Total reduced inorganic sulfur (TRIS) was determined as chromium reducible sulfur following the procedure described by Fossing and Jørgensen (1989). Samples were freeze dried and homogenized in an agate mortar. The powder was distilled for 2 h in acidic Cr(II) chloride solution and the evolved hydrogen sulfide was precipitated quantitatively as ZnS in a Zn acetate trap. The sulfide concentration was determined with

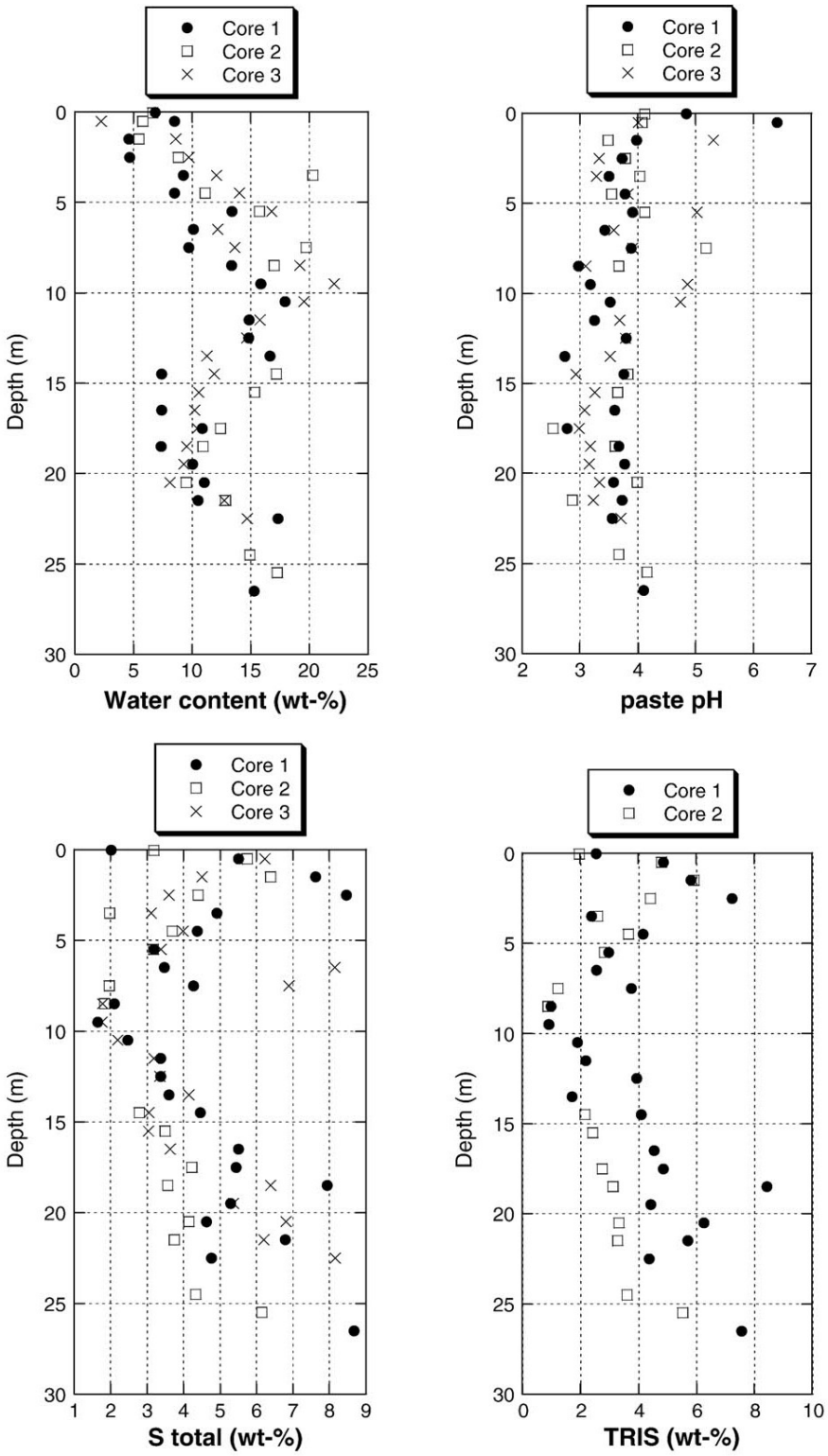

Fig. 1. Depth profiles of pH, humidity, S_{total} and total reduced inorganic sulfur (TRIS) for different cores from the tailings dam Selebi-Phikwe.

Table 1
Mean values and standard deviation of geochemical parameters

	Mean value	Standard deviation	Analyzed samples
Water content	12%	4%	65
pH	3.8	0.7	65
C_{org}	0.05%	0.02%	64
S_{total}	4.4%	1.8%	64
TRIS	3.8%	1.8%	42
SiO_2	44.3%	2.4%	65
Fe_2O_3	19.9%	3.4%	65
Al_2O_3	12.9%	0.9%	65
MgO	7.5%	1.0%	65
CaO	6.0%	0.6%	65
Na_2O	1.7%	0.1%	65
K_2O	0.9%	0.1%	65
TiO_2	0.4%	0.0%	65
MnO	0.1%	0.0%	65
P_2O_5	0.1%	0.0%	65
Ni	1864 mg/kg	931 mg/kg	65
Cu	731 mg/kg	731 mg/kg	65
Cr	255 mg/kg	31 mg/kg	65
Ba	149 mg/kg	16 mg/kg	65
Zn	112 mg/kg	7 mg/kg	65
V	110 mg/kg	10 mg/kg	65
Co	99 mg/kg	44 mg/kg	65
Zr	77 mg/kg	9 mg/kg	65
Sr	76 mg/kg	6 mg/kg	65

the methylene blue method by spectrophotometry (Cline, 1969). All photometric measurements were run in duplicate. Results agreed within ±5%. Pore water was obtained using a pore water press and analyzed for its elemental composition by ICP-OES (Jobin Yvon Emission 166 Ultrace HR 1000).

2.3. Microbiological analysis

The total number of microorganisms (including living and dead cells) was determined by counting of SybrGreen stained cells under a fluorescence microscope according to Weinbauer et al. (1998). The number of living Bacteria was determined by catalyzed reporter deposition–fluorescence in situ hybridization (CARD-FISH) as previously described (Pernthaler et al., 2002). The number of metal sulfide oxidizing *A. ferrooxidans*-like bacteria was quantified by the "most probable number technique" (MPN; McCrady, 1915; De Man, 1983) using a medium for the enrichment of acidophilic Fe(II) oxidizers (Leathen et al., 1951).

2.4. Potential pyrrhotite oxidation rate

The potential pyrrhotite oxidation rate at 25 °C at atmospheric oxygen partial pressure was determined by microcalorimetry (Schippers et al., 1995; Rohwerder et al., 1998; Elberling et al., 2000) because the reaction rate correlates with the heat output. A Thermal Activity Monitor Thermostat type 2277 (Thermometric; Järfälla, Sweden) equipped with 20 ml Ampoule Micro Calorimetric Units (type 2230-000) was used to measure the heat output (μW) from exothermal pyrrhotite oxidation in 10 g samples. The heat output caused by chemical pyrrhotite oxidation only was measured in a second experiment after inactivation of microorganisms at 65 °C for 6 h. The difference between the values of the two measurements was taken as the heat output due to biological pyrrhotite oxidation in μW. A complete oxidation of FeS to Fe(III) and sulfate produces a reaction energy $\Delta_f H^0$ of −940 kJ mol^{-1}. Using this value, the molecular mass of FeS of 0.088 kg mol^{-1}, the measured heat output a (μW) and the sample weight w (g), the combined pyrrhotite oxidation rate r was calculated according to the following equation:

$$r\ (\mu g\ kg^{-1}\ s^{-1}) = -0.94^{-1}\ (mmol\ kJ^{-1})0.088 \times (kg\ mol^{-1})a\ (\mu W)w^{-1}\ (g^{-1})$$

The rate was converted to μmol m^{-3} s^{-1} using an estimated tailings density of 1700 kg m^{-3}. Since the microcalorimetric measurements were done at atmospheric and not at *in situ* oxygen partial pressure the obtained value is considered as a potential pyrrhotite oxidation rate.

3. Results

3.1. Geochemical data

Depth profiles of pH, humidity, S_{total} and total reduced inorganic sulfur (TRIS) for two or three different cores from the tailings dam Selebi-Phikwe are shown in Fig. 1. Throughout the whole depth profile, the pH was in the acidic range with values around 3 to 4. The water content mainly fluctuated between 5 and 20 wt.%. The decrease of the water content towards the surface may be explained by evapotranspiration. The average total sulfur concentration was 4.6 wt.% in drill hole B-H1 and 4.3 wt.% in hole B-H2. The average ratio between TRIS and S_{total} was identical for both holes (0.86:1). Mean values and standard deviations of these and other geochemical parameters are given in Table 1. Organic carbon (0.05%) only plays a minor role in the tailings dam. Amounts of Fe, Ca and Mg were high.

Pore water was obtained from only one sample from B-H1 at a depth of 10 m and the metal concentrations in the pore water were (mg/l): Al 8.24, Ba<0.10, Ca 526, Cd<0.01, Co 0.33, Cr<0.10, Cu<0.10, Fe 1516, K

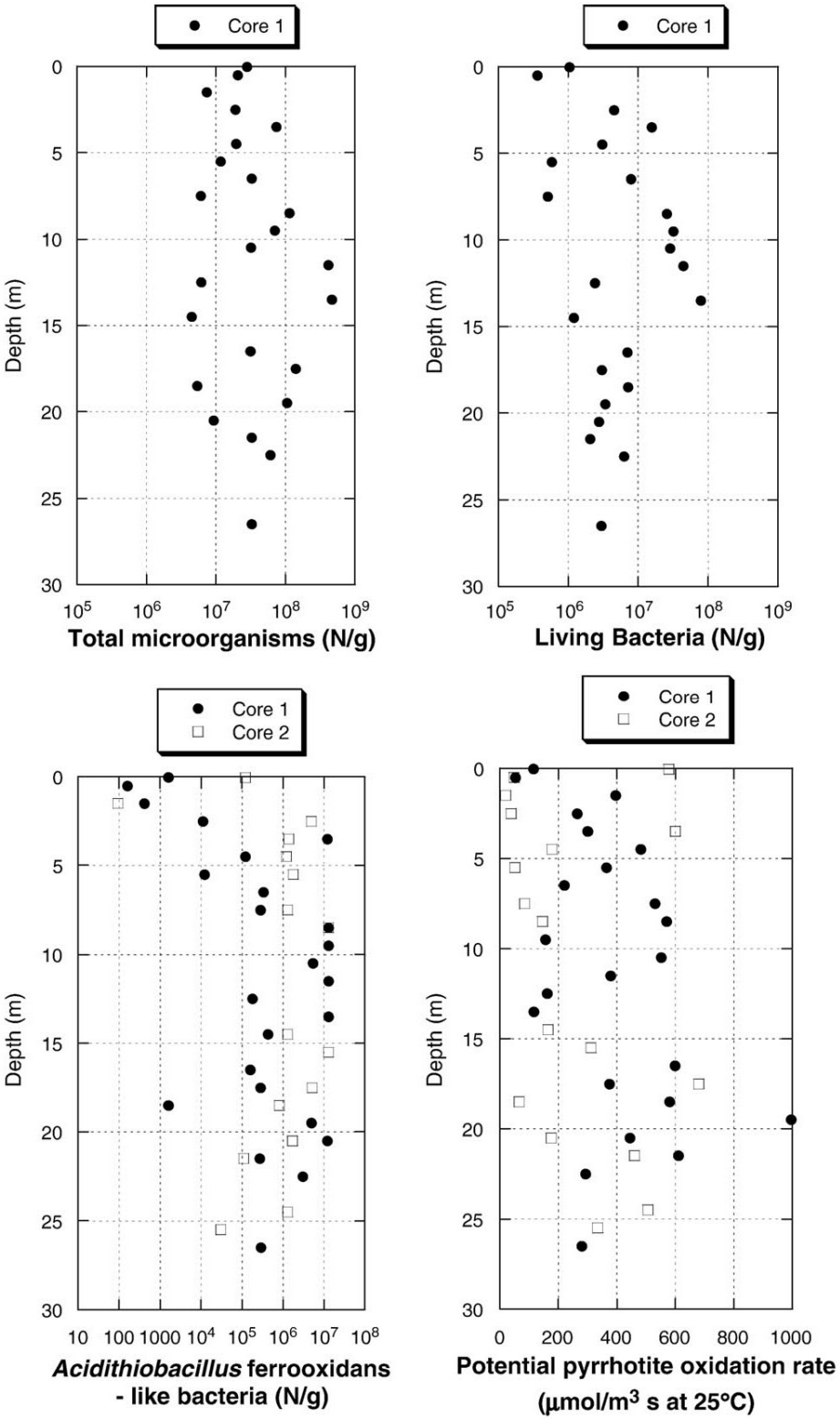

Fig. 2. Depth profiles of numbers of total microorganisms, living bacteria, metal sulfide oxidizing *Acidithiobacillus ferrooxidans*-like bacteria and potential pyrrhotite oxidation rate for different cores from the tailings dam Selebi-Phikwe.

Table 2
Mean values and standard deviation of microbiological parameters

	Mean value	Standard deviation	Analyzed samples
Potential pyrrhotite oxidation rate at 25 °C at atmospheric oxygen pressure related to the pyrrhotite surface	1.9×10^{-9} $mol_{pyrrhotite}$ m^{-2} s^{-1}	1.3×10^{-9} $mol_{pyrrhotite}$ m^{-2} s^{-1}	41
Proportion of biological pyrrhotite oxidation	52%	32%	41
Total number of microorganisms	8×10^{7} cells g^{-1} dw	1×10^{8} cells g^{-1} dw	24
Number of living bacteria	1×10^{7} cells g^{-1} dw	2×10^{7} cells g^{-1} dw	24
Number of *Acidithiobacillus ferrooxidans*-like bacteria	3×10^{6} cells g^{-1} dw	5×10^{6} cells g^{-1} dw	41

393, Li 1.09, Mg 472, Mn 44.8, Na 469, Ni 68.1, Pb<0.10, Sr 2.10 and Zn 4.04. The high values for Al, Fe and Zn are typical for AMD.

3.2. Microbiological data

Depth profiles of numbers of total microorganisms, living bacteria, metal sulfide oxidizing *A. ferrooxidans*-like bacteria and potential pyrrhotite oxidation rates for one or two cores from the tailings dam near Selebi-Phikwe are given in Fig. 2. Mean values and standard deviations of the microbiological parameters are given in Table 2.

Throughout the whole depth profile, high numbers of total microorganisms, living bacteria and *A. ferrooxidans*-like bacteria were detected. The numbers of total microorganisms and of living bacteria did not show any trend with depth. The numbers of *A. ferrooxidans*-like bacteria were significantly lower at the tailings surface than at deeper layers, but a depth trend could not be observed. About one eight of the number of total microorganisms were detected as living bacteria. Of these, about one third were *A. ferrooxidans*-like bacteria. These high numbers of metal sulfide oxidizing bacteria were consistent with high potential pyrrhotite oxidation rates up to 1 mmol m^{-3} s^{-1} for all depths. The ratio of biological to chemical pyrrhotite oxidation was about 50:50 based on the microcalorimetric measurements. A mean potential pyrrhotite oxidation rate of 1.9×10^{-9} $mol_{pyrrhotite}$ m^{-2} s^{-1} was determined, as all TRIS was sulfur in pyrrhotite for which the formula FeS was used for the calculations.

4. Discussion

The geochemical and geomicrobial data of the tailings dam near Selebi-Phikwe, Botswana, clearly show that pyrrhotite is chemically and biologically oxidized at high rates in the water unsaturated zone. A high proportion of the originally deposited pyrrhotite has been already oxidized within the first years of tailings deposition.

Metal sulfide oxidizing bacteria, such as *A. ferrooxidans*-like bacteria, were present in high numbers of 3×10^{6} cells g^{-1} dw in the whole of the unsaturated zone of the tailings dam near Selebi-Phikwe and generate acid mine drainage. Other, not determined species of the living bacteria (1×10^{7} cells g^{-1} dw) may also contribute to the overall metal sulfide oxidation activity. Surprisingly, the abundance of microorganisms did not decrease with depth in the unsaturated zone, as found in other pyrite containing tailings (Silver, 1991; Elberling et al., 2000). Numbers of *A. ferrooxidans*-like bacteria determined by the most-probable-number cultivation technique were significantly greater in the tailings dam near Selebi-Phikwe than in other tailings (Silver, 1991; Blowes et al., 1995, 1998; Elberling et al., 2000; Schippers et al., 2000).

The mean potential pyrrhotite oxidation rate of 1.9×10^{-9} $mol_{pyrrhotite}$ m^{-2} s^{-1} determined for the tailings was in the range of 3.5×10^{-8} to 6×10^{-10} mol m^{-2} s^{-1} for laboratory pyrrhotite oxidation tests (Janzen et al., 2000). The rates determined in this study were potential rates, because the microcalorimetric measurements were done at atmospheric oxygen content and not in the tailings dam at the *in situ* oxygen content, which varies with depth. The *in situ* pyrrhotite oxidation rate in the tailings is limited by oxygen diffusion, which also determines the thickness of the oxidation zone at the top of the tailings.

For other tailings, depletion of molecular oxygen within the top 0.2 to 0.6 m was measured, strongly dependent on the sulfide content and the water content of the tailings material (Elberling and Nicholson, 1996; Blowes et al., 1998; Elberling et al., 2000; Blowes et al., 2001). For the Selebi-Phikwe tailings dam, a thickness of the oxidation zone of only 0.1 m was assumed because of the high oxygen consumption due to the high pyrrhotite oxidation rate. In this case, the potential average pyrrhotite oxidation rate would be 3.4×10^{-5} mol m^{-2} s^{-1} dam surface (94 kg FeS m^{-2} $year^{-1}$). This rate is about 10 times higher than the maximum pyrrhotite oxidation rate determined (as *in*

situ O_2 consumption rate) for a pyrrhotite-rich tailings pond in the Sudbury area, Ontario, Canada (Elberling and Nicholson, 1996), and about 30 times higher than a previously measured pyrite oxidation rate in an Arctic tailings impoundment in northern Canada (Elberling et al., 2000). The latter study has shown that *in situ* O_2 consumption rates and microcalorimetrically determined rates are almost identical.

The different rates determined for different tailings may be explained by differences in molecular oxygen diffusion, temperature, metal sulfide reactivity and abundance of metal sulfide oxidizing bacteria. The diffusion of molecular oxygen is mainly controlled by the water content. Consequently, variations in pyrite oxidation rates by a factor of 100 were found depending on the water content at different sampling sites of an Arctic tailings impoundment (Elberling et al., 2000). Temperature differences explained variations in pyrite oxidation rates by a factor of 2 in case of a temperature shift from 10 °C to 20 °C (Elberling et al., 2000). The reactivity of different metal sulfides varies greatly. The chemical pyrrhotite oxidation rate was in the order of 20–100 times those measured for pyrite (Belzile et al., 2004). Metal sulfide oxidizing bacteria such as *A. ferrooxidans* have been shown to increase the chemical pyrite oxidation rate by a factor of 30 to 300 (Nordstrom and Southam, 1997; Nordstrom and Alpers, 1999).

However, according to the microcalorimetrically determined potential average pyrrhotite oxidation rate of 94 kg FeS m^{-2} $year^{-1}$, an annual sulfate production rate of approximately 100 kg m^{-2} dump surface $year^{-1}$ was estimated, which equals an annual sulfate production of 100,000 t for the whole tailings dam near Selebi-Phikwe. Based on the mean TRIS value, a total sulfate formation potential of 8×10^6 t could be calculated. Assuming a constant pyrrhotite oxidation rate over time, all pyrrhotite would be oxidized within 80 years.

Somewhat lower annual sulfate production rates of 56–66 kg m^{-2} $year^{-1}$ dump surface were determined for the Selebi-Phikwe tailings dam based on modelling, mineralogical analysis and a humidity cell test (Schwartz et al., 2006). According to these lower rates, all pyrrhotite would be oxidized within 120–140 years.

The time period found in this study coincidences with that given in the literature for tailings. For tailings impoundments with low to moderate sulfide contents and shallow water tables, the duration of sulfide oxidation can be relatively brief, with peak oxidation occurring over the first 20 to 30 years. In sulfide-rich tailings with a deep water table, sulfide oxidation is predicted to continue for centuries in the absence of remedial actions (Blowes et al., 2003).

5. Conclusions

The time period of the complete oxidation of the pyrrhotite in the tailings dam is between 80 and 140 years (constant rates assumed). The time period of AMD production is critical for the evaluation of remediation measures. The time period estimated in this study coincidences with that given in the literature for tailings. To reduce AMD formation during mining operations in Selebi-Phikwe, pyrrhotite (and other metal sulfides) should be separated from the tailings material (gravimetric or magnetic separation) and separately deposited to prevent oxidation (e.g. under a dry or water cover).

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4.2.2 Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage

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Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage

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Abstract

The impact of microbiological metal sulfide oxidation on acid mine drainage generation was studied for two different mine tailings. Microorganisms were quantified using different methods: (1) SYBR Green II direct counting, (2) TaqMan quantitative, real-time PCR (Q-PCR), (3) catalyzed reporter deposition–fluorescence in situ hybridization (CARD-FISH) and (4) most probable number (MPN) cultivation of acidophilic Fe(II) oxidizers. Potential pyrite or pyrrhotite oxidation rates were measured by microcalorimetry.

In the uncovered, pyrrhotite-containing tailings near Selebi-Phikwe, Botswana, acidophilic Fe(II)-oxidizing microorganisms were present in high numbers (MPN) of up to 10^7 cells g^{-1} dw (mean value 3×10^6 cells g^{-1} dw) throughout the entire water unsaturated, oxidized zone of about 25 m (at the tailings dam periphery) with a paste pH in the range of 3–4. Mean numbers of living Bacteria (CARD-FISH) and total microorganisms (SYBR Green II) were 1×10^7 cells g^{-1} dw and 8×10^7 cells g^{-1} dw, respectively. Cell numbers obtained by Q-PCR analysis were in the same range. The average potential pyrrhotite oxidation rate measured by microcalorimetry was 3.4×10^{-4} mol pyrrhotite m^{-3} tailings s^{-1} at 25 °C. About half of the pyrrhotite oxidation activity was biologically catalyzed.

By contrast, in the covered pyrite-containing tailings in Impoundment 1 in Kristineberg, northern Sweden, acidophilic Fe(II)-oxidizing microorganisms (mean value 5×10^5 cells g^{-1} dw) were only detected in a distinct zone of oxidized tailings between the cover and the unoxidized tailings where low pH values down to 3 prevailed. Bacterial numbers obtained by Q-PCR analysis were much higher (mean value 3×10^8 cells g^{-1} dw). The proportion of biological pyrite oxidation was up to 100% for the oxidized zone. The average potential pyrite oxidation rate was 1.6×10^{-5} mol pyrite m^{-3} tailings s^{-1} at 10 °C, an order of magnitude lower than that for the pyrrhotite-containing tailings.

Keywords: Acid mine drainage; Mine tailings; *Acidithiobacillus ferrooxidans*; Quantitative real-time PCR; CARD-FISH; SYBR Green II

1. Introduction

Acid mine drainage (AMD) is a strongly acidic solution containing high amounts of heavy metals and sulfate threatening groundwater quality. AMD is generated by chemical and biological oxidation of pyrite, pyrrhotite and other metal sulfides in mine waste heaps or in tailings from sulfidic ore processing [1–3]. The pyrite oxidation rate depends on temperature, pH, humidity and the availability of oxygen in the tailings, which is mainly controlled by diffusion. In addition, the oxidation rate strongly depends on the abundance of acidophilic Fe(II)- and metal sulfide-oxidizing microorganisms, which accelerate the kinetics of pyrite

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oxidation 30–300-fold [4,5]. Geomicrobiological and geochemical studies of pyrite and pyrrhotite-containing mine tailings pointed out the important role of acidophilic Fe(II)-oxidizing microorganisms, such as *Acidithiobacillus ferrooxidans* for the generation of AMD [6–11]. Only a few studies included the measurement of pyrite or pyrrhotite oxidation rates [9,12,13].

In mine waste, pyrite is oxidized according to Eq. (1):

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1)$$

Pyrrhotite oxidation occurs according to Eq. (2) [14]:

$$Fe_{1-x}S + (2-x/2)O_2 + xH_2O \rightarrow (1-x)Fe^{2+} + SO_4^{2-} + 2xH^+ \quad (2)$$

Based on the mineralogical composition of pyrrhotite, x ranging between 0 and 0.125 Fe(II) may be further oxidized to Fe(III) hydroxide according to Eq. (3):

$$Fe^{2+} + 0.25O_2 + 2.5H_2O \rightarrow Fe(OH)_3 + 2H^+ \quad (3)$$

In the present study, the uncovered pyrrhotite-containing tailings dam near Selebi-Phikwe, Botswana [15], and the covered pyrite-containing tailings in Impoundment 1 in Kristineberg, northern Sweden [16–21], were geomicrobiologically analyzed including the microcalorimetric measurement of potential pyrite oxidation rates [9,10,22,23]. The abundance of bacteria in the tailings has been quantified using four different techniques: (a) SYBR Green II staining, (b) TaqMan quantitative, real-time PCR (Q-PCR), (c) catalyzed reporter deposition–fluorescence in situ hybridization (CARD-FISH) and (d) most probable number (MPN) cultivation of acidophilic Fe(II)-oxidizers.

2. Materials and methods

2.1. Site description and sampling

2.1.1. Covered pyrite-containing tailings in Impoundment 1 in Kristineberg, northern Sweden

The annual precipitation in the humid Kristineberg area, northern Sweden, varies between 400 and 800 mm year ^{1} and the annual mean temperature is 0.7 °C. The design, the mineralogy and the chemistry of the pyrite-containing tailings in Impoundment 1 have been described in detail [16–21]. Briefly, the tailings in Impoundment 1 cover an area of 0.1 km^2 and have a thickness of up to approximately 11 m, with an average thickness of 6–8 m. They were covered in 1996 with a soil cover consisting of 0.3-m compacted till and 1.5-m unspecified till. From the 1940s until 1996, the impoundment was unremediated and sulfide oxidation occurred in distinct depth layers (oxidized tailings). Based on the chemical composition, the sulfide mineral content of the unoxidized tailings ranges from 10% to 30%, totally dominated by pyrite. In the oxidized tailings, the sulfide content is generally lower [16]. Three boreholes at different locations (cores K, O and Q) were drilled using a drill-rig. The drill cores were split into 30 subsamples from the oxidized and the unoxidized tailings for laboratory analysis. Brown precipitates of iron (hydrox)oxides due to pyrite oxidation were found in the oxidized tailings at all three locations.

2.1.2. Uncovered pyrrhotite-containing tailings dam near Selebi-Phikwe, Botswana

The climate in Selebi-Phikwe, Botswana, is semiarid with an average annual temperature of 21 °C. The mine waste tailings dam near Selebi-Phikwe generates high amounts of AMD, which are collected in a drainage ditch surrounding the dam. To prevent contamination of surface water, AMD is purified in a plant by addition of limestone to increase the pH and to precipitate metals. The geochemistry of the tailings dam has been previously described [15]. Briefly, the tailings dam consists of waste from about 32 years of Ni-, Cu-, Zn- and Co-sulfidic ore processing. The original material from the flotation plant consists of about 35% solid material with an average grain size diameter of about 0.1 mm. The solid material contains about 11% pyrrhotite, about 1.5% other metal sulfides and hornblende and feldspar as major gangue minerals. The approximately 40-m high dam (2003) covers an area of ca. 1 km^2. The final height shall be 50 m in the year 2014. Currently, the surface of the central part is water covered, whereas the periphery of the dam surface is dry. Here, three holes (B-H1=core 1, B-H2=core 2, B-H3=core 3) were drilled through the water unsaturated down to the saturated zone at about 25 m depth. Brown precipitates of iron (hydrox)oxides due to pyrrhotite oxidation were found throughout the entire unsaturated zone. A high proportion of the originally deposited pyrrhotite has been already oxidized within the first years of tailings deposition. Altogether, 65 solid samples were taken in 1-m intervals.

2.2. Geochemical analysis

The paste pH was measured with an electrode after shaking of 5-g sample in 12.5 mL 1 M KCl for 1 h. Humidity was determined as weight difference after

drying. The elemental composition of the solid material was determined by XRF analysis. The total amount of sulfur (S_{total}) was measured with the instrument LECO CS 200. Total reduced inorganic sulfur (TRIS) was determined as chromium reducible sulfur following a previously described procedure [15,24].

2.3. Potential pyrite or pyrrhotite oxidation rate

The potential pyrite or pyrrhotite oxidation rate at atmospheric oxygen partial pressure was determined by microcalorimetry [9,10,22] because the reaction rate correlates with the heat output. A Thermal Activity Monitor Thermostat type 2277 (Thermometric; Järfälla, Sweden) equipped with 20 mL Ampoule Micro Calorimetric Units (type 2230–000) was used to measure the heat output (μW) due to the exothermal pyrite or pyrrhotite oxidation in 10-g sample each. The heat output caused by chemical pyrite or pyrrhotite oxidation only was measured in a second run after inactivation of microorganisms at 65 °C for 6 h. The difference between the values of the two measurements per sample is the heat output due to potential biological pyrite or pyrrhotite oxidation in μW.

Measurements for the pyrite-containing tailings were done at 10 °C close to the average summer temperature in Kristineberg, northern Sweden. A complete oxidation of FeS_2 to Fe(III) and sulfate produces a reaction energy $\Delta_f H^0$ of −1546 kJ mol^{-1}. Using this value, the molecular mass of FeS_2 of 0.12 kg mol^{-1}, the measured heat output a (μW) and the sample weight w (g), the pyrite oxidation rate r was calculated according to the following equation:

$$r\ (\mu g\ kg^{-1}\ s^{-1}) = -1.546^{-1}\ (mmol\ kJ^{-1})0.12 \times (kg\ mol^{-1})a\ (\mu W)w^{-1}\ (g^{-1})$$

The rate was converted to mol m^{-3} s^{-1} using a tailings density of 2186 kg m^{-3} calculated from values given for water content, grain density and porosity for 10 tailings samples [20].

Measurements for the pyrrhotite-containing tailings dam near Selebi-Phikwe, Botswana were done at 25 °C close to the average annual temperature of 21 °C. A complete oxidation of FeS to Fe(III) and sulfate produces a reaction energy $\Delta_f H^0$ of −940 kJ mol^{-1}. The molecular mass of FeS was 0.088 kg mol^{-1} and the estimated tailings density was 1700 kg m^{-3}.

Since the microcalorimetric measurements were done at atmospheric and not at in situ oxygen partial pressure, the obtained rates are considered as potential oxidation rates.

2.4. Quantification of microorganisms

The total number of microorganisms (including living and dead cells) was determined by counting of SYBR Green II stained cells in tailings material on filters under a fluorescence microscope as described elsewhere [25]. The tailings material had been previously fixed in formaldehyde after sampling following a published procedure [26]. To quantify the abundance of Bacteria and Archaea by Q-PCR analysis [27], high-molecular-weight DNA was extracted from 3 g of frozen sample following a modified FastDNA Spin Kit for Soil (Bio101) protocol [28], and the 16S rDNA copy numbers of prokaryotes, Archaea and Bacteria were determined using TaqMan assays [29,30]. 16S rDNA gene copy numbers were converted to cell numbers using a conversion factor of 3.6 [31]. The number of living Bacteria and Archaea in formaldehyde-fixed material was determined by catalyzed reporter deposition–fluorescence in situ hybridization (CARD-FISH) as previously described [27,32]. The number of metal sulfide-oxidizing bacteria of the type *A. ferrooxidans* was quantified by the "most probable number technique" using a medium for enrichment of acidophilic Fe (II)-oxidizers [33].

3. Results

The geomicrobiological analyses of the unsaturated, oxidized zone of the uncovered pyrrhotite-containing tailings dam at Selebi-Phikwe, Botswana, and the oxidized zone of the covered tailings in Impoundment 1 in Kristineberg, northern Sweden showed significant differences for both tailings. Mean values of all parameters from analyses of the three different cores for each tailings are given in Table 1. Depth profiles of selected parameters are shown for one core of each tailings in Figs. 1 and 2.

In the covered, pyrite-containing tailings iron (hydro) oxides were visible only in a distinct zone of oxidized tailings between the cover and the unoxidized tailings. Significant differences between the about 0.5-m-thick zone of oxidized tailings and the unoxidized tailings in core O are shown in Fig. 1. In the oxidized tailings, the amounts of S_{total} and the pH are lower, and the number of Bacteria determined by Q-PCR is much higher than in the unoxidized tailings. The total cell number (SYBR Green II) in the oxidized tailings is identical to the number of Bacteria (Q-PCR). Acidophilic Fe(II)-oxidizing microorganisms of the type *A. ferrooxidans* (MPN) were only detected in the oxidized tailings, which correlates with a high proportion of up to 100% of

Table 1
Mean values of geochemical and geomicrobiological parameters for the unsaturated, oxidized zone of the uncovered pyrrhotite-containing tailings at Selebi-Phikwe, Botswana, and the oxidized zone of the covered tailings in Impoundment 1 in Kristineberg, northern Sweden

	Oxidized zone of tailings at Selebi-Phikwe, Botswana	Oxidized zone of tailings in Kristineberg, Sweden
Main metal sulfide	Pyrrhotite	Pyrite
Soil cover	No	Yes
In operation (2003)	Yes	No
Annual mean temperature	21 °C	0.7 °C
S_{total}	4.4%	5.3%
Total reduced inorganic sulfur (TRIS)	3.8%	nm
Fe_{total}	14%	9%
Humidity	12%	24%
pH	3.8	4.8
Total number of microorganisms (SYBR Green II)	8×10^{7} cells g^{-1} dw	3×10^{8} cells g^{-1} dw
Number of prokaryotes (Q-PCR)	6×10^{7} cells g^{-1} dw	nm
Number of bacteria (Q-PCR)	3×10^{7} cells g^{-1} dw	3×10^{8} cells g^{-1} dw
Number of living bacteria (CARD-FISH)	1×10^{7} cells g^{-1} dw	nm
Number of *Acidithiobacillus ferrooxidans*-like bacteria (MPN)	3×10^{6} cells g^{-1} dw	5×10^{5} cells g^{-1} dw
Potential pyrite or pyrrhotite oxidation rate at atmospheric oxygen pressure (weight per weight)	17.5 μg pyrrhotite kg^{-1} tailings s^{-1} at 25 °C	0.9 μg pyrite kg^{-1} tailings s^{-1} at 10 °C
Potential pyrite or pyrrhotite oxidation rate at atmospheric oxygen pressure (moles per volume)	3.4×10^{-4} mol pyrrhotite m^{-3} tailings s^{-1} at 25 °C	1.6×10^{-5} mol pyrite m^{-3} tailings s^{-1} at 10 °C
Proportion of biological pyrite or pyrrhotite oxidation	52%	80%

nm=not measured.

biological pyrite oxidation. The average potential pyrite oxidation rate measured by microcalorimetry was 1.6×10^{-5} mol pyrite m^{-3} tailings s^{-1} at 10 °C.

In the uncovered, pyrrhotite-containing tailings brown precipitates of iron (hydro)oxides were discernible and microorganisms were present in high numbers throughout the entire water unsaturated, oxidized zone of about 25 m (at the tailings dam periphery). The paste pH shows low variation in the range of 3–4. The amounts of S_{total} and total reduced inorganic sulfur (TRIS) are almost identical, which means that pyrrhotite is the dominant sulfur-containing mineral. The quantification of microorganisms by the different methods exhibits comparable results. SYBR Green II total counts comprise living and dead cells and give the highest mean value of 8×10^{7} cells g^{-1} dw. The Q-PCR values for total prokaryotes and total Bacteria are somewhat lower but in the same order of magnitude. The mean number of living Bacteria detected by CARD-FISH was 1×10^{7} cells g^{-1} dw. Archaea could be quantified neither by Q-PCR nor by CARD-FISH, which exhibits the dominance of Bacteria in the tailings. A high proportion of the CARD-FISH detectable, living Bacteria (30%) could be detected via MPN cultivation of acidophilic Fe(II)-oxidizing microorganisms of the type *A. ferrooxidans*. Their numbers were significantly lower at the tailings surface than at deeper layers, but a depth trend could not be observed. These organisms are able to oxidize pyrrhotite throughout the entire unsaturated zone. The average potential pyrrhotite oxidation rate measured by microcalorimetry was 3.4×10^{-4} mol pyrrhotite m^{-3} tailings s^{-1} at 25 °C. The proportion of biological to chemical pyrrhotite oxidation was highly variable, did not show a depth trend and was found to be 52% in average.

4. Discussion

For the first time, the microbial community in sulfidic mine tailings has been quantified using four different methods. The fluorochrome SYBR Green II (as well as acridine orange or DAPI) binds unspecifically to nucleic acids and thus does not provide information on the viability of the cells. Potentially, a major part of the counted cells could be dormant or even dead and yet retain stainable DNA [34,35]. Consequently, for the pyrrhotite-containing tailings, the highest mean cell number was obtained with SYBR Green II direct counting. A slightly lower mean number produced the Q-PCR method, which targeted high molecular weight DNA. The different mean numbers for these two methods may be explained by the fact that SYBR Green II also binds to degenerated DNA which is not detectable by Q-PCR. However, both methods gave identical results for the pyrite-containing tailings.

RNA, in contrast, is much more labile and is readily degraded in cells that become inactive due to starvation [35]. Cell death in pure cultures accelerates when less than half of the RNA remains. Starved cells may still maintain an intact cell membrane and nucleic acids such

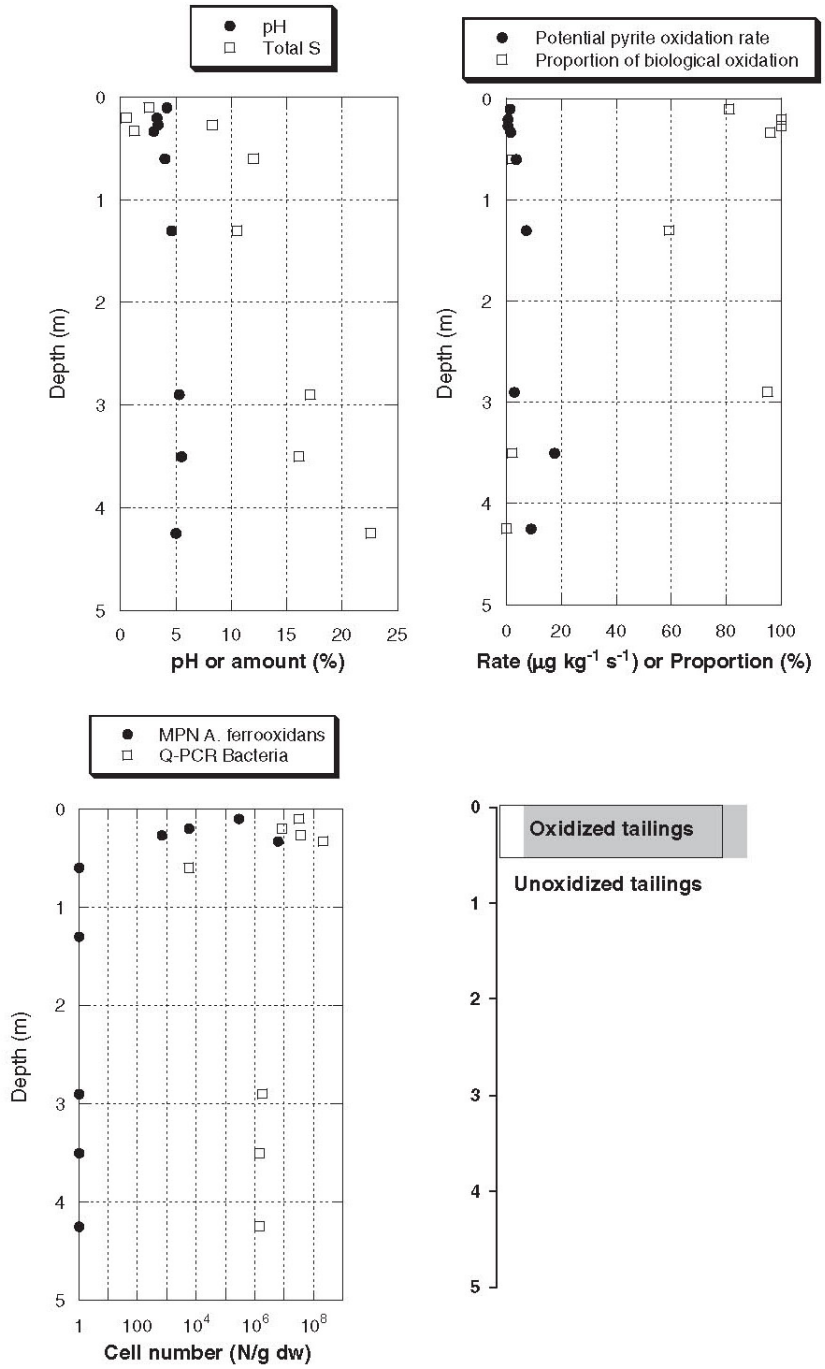

Fig. 1. Geomicrobial and geochemical data for core O of the covered, pyrite-containing tailings in Impoundment 1 in Kristineberg, northern Sweden.

as DNA or tRNA, but they rapidly loose their ribosomes [36]. The experience from pure culture studies is that cells with a significant ribosome content are living and metabolically active. Therefore, ribosomal RNA is an indicator of living cells, which could be quantified by CARD-FISH, as has recently done for deeply buried marine sediments [27]. The CARD-FISH analysis of the pyrrhotite-containing tailings gave a mean number of living Bacteria, which is eight times lower than the number of the SYBR Green II direct counts. However,

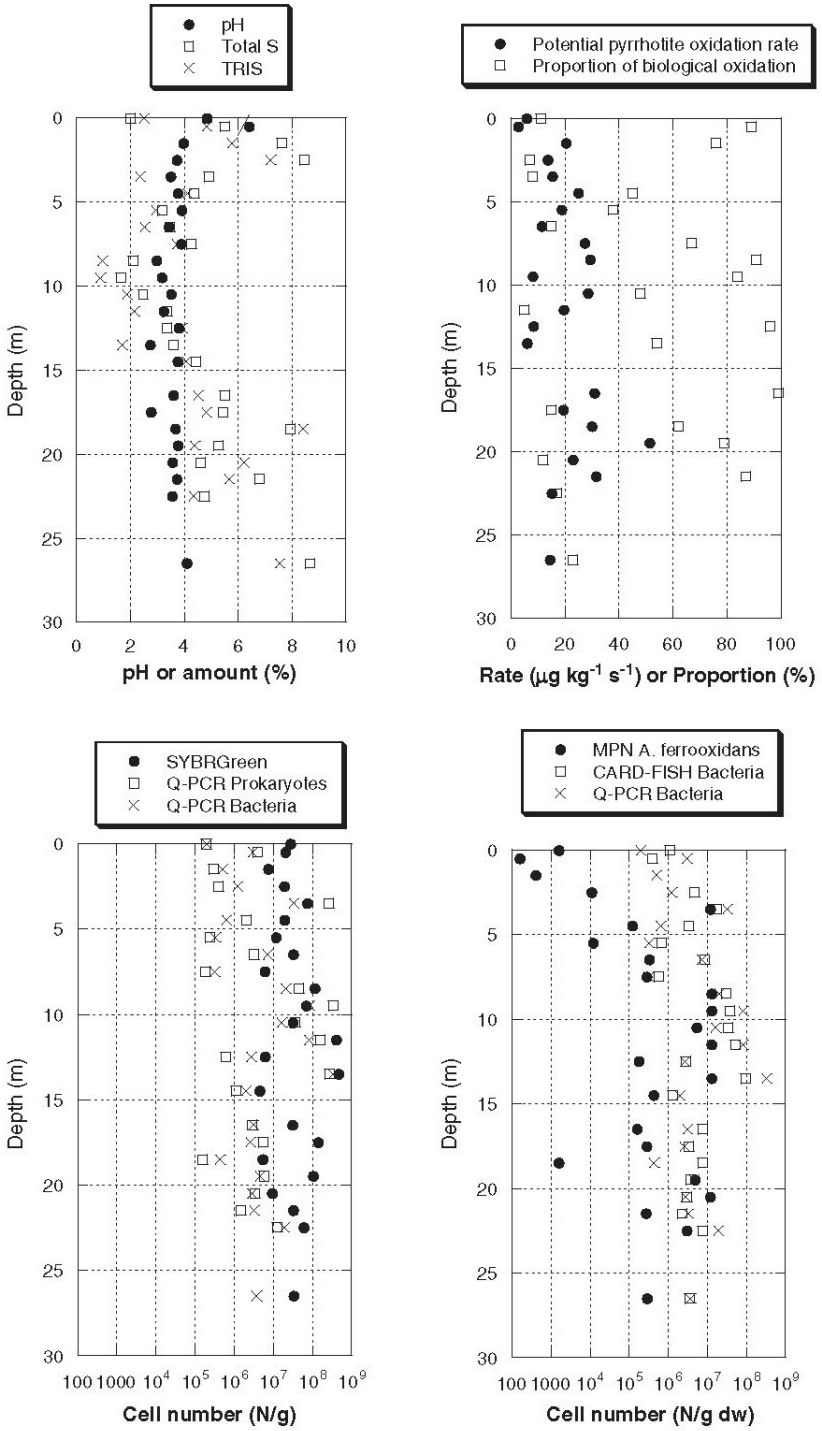

Fig. 2. Geomicrobial and geochemical data for core 1 of the uncovered, pyrrhotite-containing tailings dam near Selebi-Phikwe, Botswana.

the mean cell numbers of the SYBR Green II direct counts as well as those of the molecular methods Q-PCR and CARD-FISH were in the same order of magnitude, which shows that a high proportion of the detectable microorganisms are alive. Both molecular methods, Q-PCR and CARD-FISH, documented the dominant role

of Bacteria in the tailings since Archaea could not be quantified due to their low abundance.

A high proportion of 30% of the living Bacteria could be detected as acidophilic Fe(II)-oxidizers of the type *A. ferrooxidans* by the cultivation based MPN technique. Thus, acidophilic Fe(II)-oxidizers were a dominant group of the microbial community in the pyrrhotite-containing tailings. In contrast, only a minor proportion of less than 1% of the Bacteria was detected as acidophilic Fe(II)-oxidizers by MPN for the pyrite-containing tailings. Other not determined species of the Bacteria may also contribute to the overall metal sulfide oxidation activity. Further work is in progress to quantify *A. ferrooxidans* and other acidophilic Fe(II)-oxidizers by CARD-FISH. Numbers of *A. ferrooxidans*-like bacteria determined by MPN in this study are comparable or higher than those in other tailings [6–10].

FISH was previously used to analyze the microbial populations in an acid mine drainage outflow of the extreme environment Iron Mountain, California, USA [37–39]. In the mine tailings studied here, less cells were quantified in selected samples using conventional FISH with Cy3-monolabeled probes in contrast to the much more sensitive CARD-FISH (data not shown). Presumably, the amount of ribosomes, which determines the intensity of the fluorescence signal in conventional FISH, was lower in the cells in the mine tailings than in the cell from Iron Mountain. Since a high amount of ribosomes indicates a high activity status of a cell [35], the bioleaching activity at Iron Mountain is most likely much higher than that in the mine tailings. Unfortunately, pyrite oxidation rates for Iron Mountain had not been measured to be able to verify this assumption.

The microcalorimetric data of this study clearly show that pyrite and pyrrhotite are biologically and chemically oxidized in the tailings. The microcalorimetrically determined rates are potential rates, because the microcalorimetric measurements were done at atmospheric and not at the in situ oxygen content in the tailings dam, which varies with depth. The in situ pyrite or pyrrhotite oxidation rate in the tailings is limited by oxygen diffusion, which also determines the thickness of the oxidation zone in the tailings. For other tailings, depletion of molecular oxygen within the top 0.2 to 0.6 m was measured, strongly depending on the sulfide content and the humidity of the tailings material [8,9,12,40]. For the covered pyrite-containing tailings in northern Sweden, a 0.3-m-thick oxidation zone was assumed which gives an areal potential average pyrite oxidation rate of 4.7×10^{-6} mol m^{-2} dam surface s^{-1} (18 kg FeS_2 m^{-2} year^{-1}). For the pyrrhotite-containing tailings in Botswana, a thickness of the oxidation zone of only 0.1 m was assumed because of the high oxygen consumption due to the high pyrrhotite oxidation rate. In this case, the areal potential average pyrrhotite oxidation rate would be 3.4×10^{-5} mol m^{-2} dam surface s^{-1} (94 kg FeS m^{-2} year^{-1}) [15]. This rate is about 10 times higher than the pyrite oxidation rate for the tailings in Sweden, 10 times higher than the maximum pyrrhotite oxidation rate (determined as in situ O_2 consumption rate) for a pyrrhotite-rich tailings pond in the Sudbury area, Ontario, Canada [12], and about 30 times higher than a previously measured pyrite oxidation rate in an Arctic tailings impoundment in northern Canada [9]. The latter study has shown that in situ O_2 consumption rates and microcalorimetrically determined rates are almost identical.

The different rates determined for different tailings may be explained by differences in molecular oxygen diffusion, temperature, metal sulfide reactivity and abundance of metal sulfide-oxidizing bacteria. The diffusion of molecular oxygen is mainly controlled by the water content. Consequently, variations in pyrite oxidation rates by a factor of 100 depending on the water content at different sampling sites of an Arctic tailings impoundment were found [9]. Temperature differences explained variations in pyrite oxidation rates by a factor of 2 in case of a temperature shift from 10 °C to 20 °C [9]. The reactivity of different metal sulfides varies over a wide range. The chemical pyrrhotite oxidation rate was found to be on the order of 20–100 times those measured for pyrite [41]. Metal sulfide-oxidizing bacteria such as *A. ferrooxidans* have shown to increase the chemical pyrite oxidation rate by a factor of 30 to 300 [4,5].

However, according to the microcalorimetrically determined potential average pyrrhotite oxidation rate of 94 kg FeS m^{-2} dump surface year^{-1}, an annual sulfate production of approximately 100 kg m^{-2} dump surface year^{-1} occurs, which means an annual sulfate production of 100,000 t for the whole tailings dam in Botswana. Based on the mean TRIS value, a total sulfate formation potential of 8×10^{6} t could be calculated. Assuming a constant pyrrhotite oxidation rate over time, all pyrrhotite would be oxidized within 80 years [15].

Somewhat lower annual sulfate production rates of 56–66 kg m^{-2} dump surface year^{-1} were determined for the tailings dam in Botswana based on modelling, mineralogical analysis and a humidity cell test [42]. According to these lower rates, all pyrrhotite would be oxidized within 120–140 years (constant rates assumed). The time period of AMD production is critical for the evaluation of remediation measures to be applied

after mine closure. The time period found in this study coincidences with that given in the literature for tailings. For tailings impoundments with low to moderate sulfide contents and shallow water-table positions, the duration of sulfide oxidation can be relatively short, with peak oxidation occurring over the first 20 to 30 years. In sulfide-rich tailings with a deep water table, sulfide oxidation is predicted to continue for centuries in the absence of remedial actions [43].

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4.2.3 Quantitative microbial community analysis of three different sulfidic mine tailings dumps generating acid mine drainage

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Quantitative Microbial Community Analysis of Three Different Sulfidic Mine Tailing Dumps Generating Acid Mine Drainage[▽]

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The microbial communities of three different sulfidic and acidic mine waste tailing dumps located in Botswana, Germany, and Sweden were quantitatively analyzed using quantitative real-time PCR (Q-PCR), fluorescence in situ hybridization (FISH), catalyzed reporter deposition-FISH (CARD-FISH), Sybr green II direct counting, and the most probable number (MPN) cultivation technique. Depth profiles of cell numbers showed that the compositions of the microbial communities are greatly different at the three sites and also strongly varied between zones of oxidized and unoxidized tailings. Maximum cell numbers of up to 10^9 cells g^{-1} dry weight were determined in the pyrite or pyrrhotite oxidation zones, whereas cell numbers in unoxidized tailings were significantly lower. *Bacteria* dominated over *Archaea* and *Eukarya* at all tailing sites. The acidophilic Fe(II)- and/or sulfur-oxidizing *Acidithiobacillus* spp. dominated over the acidophilic Fe(II)-oxidizing *Leptospirillum* spp. among the *Bacteria* at two sites. The two genera were equally abundant at the third site. The acidophilic Fe(II)- and sulfur-oxidizing *Sulfobacillus* spp. were generally less abundant. The acidophilic Fe(III)-reducing *Acidiphilium* spp. could be found at only one site. The neutrophilic Fe(III)-reducing *Geobacteraceae* as well as the *dsrA* gene of sulfate reducers were quantifiable at all three sites. FISH analysis provided reliable data only for tailing zones with high microbial activity, whereas CARD-FISH, Q-PCR, Sybr green II staining, and MPN were suitable methods for a quantitative microbial community analysis of tailings in general.

Mining residues from mining activities are dumped as waste rock or as tailings, which are metal-degraded materials from ore processing. Both kinds of dumps often contain sulfide minerals such as pyrite (FeS_2) or pyrrhotite ($Fe_{1-x}S$, $x = 0$ to 0.125) and release acidic metal-rich waters known as acid mine drainage (AMD)/acid rock drainage (ARD) because of chemical and microbial sulfide oxidation processes, e.g.,

$$FeS_2 + 3.5\ O_2 + H_2O \rightarrow Fe^{2+} + 2\ H^+ + 2\ SO_4^{2-}$$

Over a period of several years, an oxidized zone with depleted sulfide content, low pH, and enrichment of secondary minerals develops above an unoxidized zone with unaltered material in the waste dump (e.g., references 4, 14, 21, 40, and 49).

Several geomicrobiological investigations of sulfidic mine waste dumps located in different climate zones have been undertaken to gather information about microbial processes and diversity in these extreme environments. At such sites, aerobic, acidophilic, chemolithotrophic *Bacteria* or *Archaea* dissolve metal sulfides by oxidizing Fe(II) and sulfur compounds and generate AMD/ARD. Products resulting from these oxidation processes can be used by Fe(III)- and sulfate-reducing prokaryotes. When Fe(III) (hydr)oxides are dissolved, adsorbed or precipitated metals are released. Sulfate-reducing *Bacteria* or *Archaea* may also precipitate metals as metal sulfides (22, 49, 50).

Primarily cultivation techniques have been used to enumerate prokaryotes involved in oxidation and reduction processes in sulfidic mine waste dumps (3, 17, 52, 57, 64). Cultivation techniques yield cell numbers merely according to physiological properties; therefore, only a subset of the whole microbial community is detected. Up to this point, only qualitative molecular biological tests were applied to sulfidic mine dumps such as cloning and subsequent sequencing (7), denaturing gradient gel electrophoresis (12, 38), and terminal restriction fragment length polymorphism (7, 13). In addition, protein and lipid analysis (37, 58) were performed with tailing samples. These investigations provided valuable information about the microbial diversity in mine dumps but little information about the quantities of the different microbial groups or species. The molecular biological quantification technique fluorescence in situ hybridization (FISH) and metagenomic and proteomic techniques have been previously applied to quantify acidophiles in water samples from AMD sites (2, 6, 15, 20, 23, 46, 56, 61) but not solid samples from mine dumps.

In this study, the microbial communities in three mine tailing dumps, each with a different mineralogy and located in different climate zones, were quantified by cultivation and molecular biological methods. Depth profiles of cell numbers of different microorganisms were created using Sybr green II direct counting, quantitative real-time PCR (Q-PCR), FISH, catalyzed reporter deposition-FISH (CARD-FISH), and the most probable number (MPN) cultivation technique. Some of the data from MPN and total number determination of *Bacteria* and *Archaea* were previously published in connection with geochemical and mineralogical studies, as were data from determinations of metal sulfide oxidation rates determined by calorimetry (21, 25, 30, 31, 55). Here, a detailed Q-PCR and

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FISH analysis of microorganisms relevant for Fe and S oxidation and reduction processes is presented for the first time.

MATERIALS AND METHODS

Site description and sampling. (i) Uncovered pyrrhotite-containing tailing dump, Selebi-Phikwe, Botswana. The tailing dump in Botswana exists in a semiarid climate with an average annual temperature of 21°C. At the time of sampling in 2003, it was still in operation and uncovered. It was approximately 40 m high and spread over an area of approximately 1 km^2. It contained Ni, Cu, Zn, and Co sulfidic ore processing waste accumulated over a period of about 32 years. The solid material of the original tailings consisted of 11% pyrrhotite, about 1.5% other metal sulfides, and hornblende and feldspar as gangue minerals. A set of 24 drilling samples was taken to a depth of 26 m. The content of total reduced inorganic sulfur was 4.1% on average in the investigated samples, and total organic carbon (TOC) was below 0.1%. The geochemistry of the tailing dump and the sampling procedure have been previously described (55).

(ii) Covered pyrite-containing tailings in impoundment 1, Kristineberg, northern Sweden. The climate in Kristineberg, northern Sweden, is humid with an annual precipitation between 400 and 800 mm per year and an annual mean temperature of 0.7°C. The tailing dump had been in operation for about 10 years and was left unremediated for approximately 40 years before it was covered in 1996 with a soil cover consisting of 0.3 m compacted till and 1.5 m unspecific till. Before covering, sulfide oxidation occurred in distinct depth layers (oxidized tailings). The tailing dump consists of waste from Zn and Cu sulfidic ore processing, covers an area of 0.1 km^2, and is 6 to 7 m high. In the unoxidized tailings, the content of sulfide minerals ranges from 10 to 30% and is completely dominated by pyrite, while in the oxidized tailings the sulfide content is generally around 1 to 2%. In 2003, nine samples were taken by drilling to a depth of 6.5 m. The TOC was below 0.8%. The geochemistry and mineralogy of the tailing dump and the sampling procedure have been described by others in detail (8, 9, 25–27, 30, 39, 41).

(iii) Uncovered pyrite- and arsenopyrite-containing tailing dump, Freiberg, Germany. The tailing dump in Freiberg, Germany, is located in a temperate zone with an annual mean precipitation of 763 mm per year and an annual mean temperature of 7.7°C. Its height is 30 m, and it is spread over an area of 0.06 km^2. It is unremediated and partly covered by a layer of soil less than 0.2 m in thickness. The tailing dump consists of waste from Pb and Zn sulfidic ore processing with the main metal sulfides being pyrite, arsenopyrite, sphalerite, and galena. Three thin, cemented layers occur in a depth range of 0.6 to 0.63 m. They separate an upper zone (metal sulfide content of ≤0.1%), which underwent oxidation, from a zone of active oxidation (metal sulfide content of ≤1%) beneath it. An unoxidized zone with a metal sulfide content of 1% occurs at a depth below 0.85 m. In 2005, 21 samples were taken down to a depth of 1.2 m in an outcrop profile from the center of the tailing dump. TOC was below 0.1%. The geochemistry and mineralogy of the tailing dump and the sampling procedure have been previously described (21).

Quantification of microorganisms. (i) Sybr green II direct counting and MPN. Total cell numbers were determined in formaldehyde-fixed samples (36) by staining with Sybr green II as described previously (63). The MPN technique was used to enumerate acidophilic chemolithoautotrophic Fe(II) oxidizers and sulfur oxidizers. For quantifying Fe(II) oxidizers, the medium described by Leathen et al. (34) at pH 3.5 was used: $(NH_4)_2SO_4$, 0.15 g; KCl, 0.05 g; $MgSO_4 \cdot 7H_2O$, 0.50 g; K_2HPO_4, 0.05 g; $Ca(NO_3)_2$, 0.01 g; $FeSO_4 \cdot 7H_2O$, 1.00 g in 1,000 ml of distilled water. The following medium described by Starkey (60) at pH 3.5 was used for quantifying sulfur oxidizers: $(NH_4)_2SO_4$, 0.30 g; KH_2PO_4, 3.50 g; $CaCl_2$, 0.25 g; $MgSO_4$, 0.50 g; $Fe(SO_4)_3$, 0.01 g; elemental sulfur, 10 g in 1,000 ml of distilled water.

(ii) FISH and CARD-FISH. Living *Bacteria* (probes EUB338 I to EUB338 III) and *Archaea* (ARCH915) were quantified by CARD-FISH and by FISH in formaldehyde-fixed samples on filters as previously published (11, 43, 44, 54, 59). FISH was also applied for quantifying specific Fe- and S-oxidizing and/or -reducing prokaryotes by using the following probes: LF655 (specific for *Leptospirillum* species of groups I, II, and III), ACM732 (specific for the genus *Acidimicrobium* and relatives), SUL228 (specific for the genus *Sulfobacillus*) (5), Acdp821 (specific for the genus *Acidiphilium*), Thio820 (specific for *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*) (42), TF539 (specific for *A. ferrooxidans*) (56), and LGC0355 (specific for gram-positive bacteria with low G+C content [23]). The detection limit for the microscopic methods Sybr green II, FISH, and CARD-FISH was 10^5 cells g^{-1} dry weight each. The specificity for selected probes was tested with different pure cultures.

(iii) Real-time PCR. The DNA was extracted from 1 to 5 g of a frozen tailing sample following a modified FastDNA Spin Kit for Soil (Bio 101) protocol (62). Q-PCR was used to quantify *Bacteria*, *Archaea*, *Geobacteraceae*, *Leptospirillum* spp., *Acidithiobacillus caldus*, and *Sulfobacillus* spp. by targeting their 16S rRNA genes; *Eukarya* by targeting their 18S rRNA genes; and sulfate-reducing prokaryotes by targeting their specific functional gene *dsrA* as described previously (35, 51). Furthermore, the 16S rRNA gene of *Acidithiobacillus* spp. was quantified using a modified protocol published by Zammit et al. (65). Mastermix (Eurogentec) with Sybr green I (12.5 μl) was used with 1 μl of each primer (250 nM; 27F [33] and At.f384R [5′-CATTGCTTCGTCAGGGTTG-3′] [65]) and 1.25 μl bovine serum albumin in a total reaction volume of 25 μl. The cycling parameters were as follows: one cycle of 94°C for 10 min and 35 cycles of 94°C for 45 s, 61°C for 1 min, and 72°C for 45 s.

Each DNA extract was measured in triplicate and in at least two dilutions to check for PCR inhibition. After each Q-PCR run, melting curves were measured for Sybr green I assays. The primer specificity for the specific Q-PCR assays was confirmed by sequence alignment in databases (Blast, Ribosomal Database Project) and tested using DNA of *A. ferrooxidans*^{T}, *A. caldus*^{T}, *A. thiooxidans*^{T}, *Leptospirillum ferrooxidans*^{T}, *Leptospirillum ferriphilum*^{T}, and *Sulfobacillus acidophilus*^{T}. To convert DNA copy numbers to cell numbers, the following conversion factors were used: 4.1 for *Bacteria* and *Geobacteraceae*, 1.5 for *Archaea* (29), 1 for the *dsrA* gene (51), 12 for *Acidithiobacillus* spp. and *A. caldus*, 6 for *Sulfobacillus* spp., and 10 for *Leptospirillum* spp. (65). The detection limits for Q-PCR analyses were 10^3 DNA copies g^{-1} dry weight for the assays specific for *Bacteria* and *Acidithiobacillus* spp.; 10^2 DNA copies g^{-1} dry weight for the assays specific for *Geobacteraceae*, *Eukarya*, *Leptospirillum* spp., and the *dsrA* gene; and 10^1 DNA copies g^{-1} dry weight for the assays specific for *Archaea*, *Sulfobacillus* spp., and *A. caldus*.

RESULTS

Oxidized and unoxidized tailing zones. We observed significant differences in the location and thickness of oxidized zones with reduced sulfide content due to sulfide weathering and unoxidized zones in the three sulfidic mine tailing dumps. In the active tailing dump in Selebi-Phikwe, Botswana, the entire investigated depth of 26 m comprised an oxidized zone defined by the occurrence of brownish Fe(III) (hydr)oxides and a low pH of 3 to 4 measured in the paste of a sample (Fig. 1B). In contrast, the remediated tailing dump in Kristineberg, Sweden, was divided into an approximately half-meter-thick oxidized zone with an average pH of 3.5 measured in the paste of a sample below a 2-m-thick cover of soil and above the unoxidized tailings (pH 4.9) (Fig. 1D). A special situation was found in the inactive tailing dump in Freiberg, Germany, where a zone of cemented layers in 0.6- to 0.63-m depth (pH 3 to 4) divided the previously oxidized tailings above (pH 4 to 5) from the zone of active oxidation below (pH ~3). The zone of unoxidized tailings (pH 5 to 7) was located below an 0.85-m depth (Fig. 1F). The TOC was generally low in all tailing dumps; thus, metal sulfides must provide the main energy source for microbial activity.

Quantification of total cells, *Bacteria*, *Archaea*, and cultivable Fe(II) oxidizers. The quantitative microbial community analysis showed significantly different depth profiles of cell numbers for the various microbial groups (Fig. 1 and 2). Sybr green II total cell counts comprised the highest cell numbers in all three tailings over the whole depth profiles (10^6 to 10^9 cells g^{-1} dry weight) (Fig. 1A, C, and E). Bacterial cell numbers determined via Q-PCR were somewhat lower but of the same order of magnitude as the total cell counts at all three sites. Furthermore, *Archaea* were detected via Q-PCR in the oxidized zone of the tailing dump in Sweden and above the zone of cemented layers in the tailing dump in Germany but not in Botswana. By the use of CARD-FISH, *Bacteria* were detected in different proportions of total cell numbers at the different

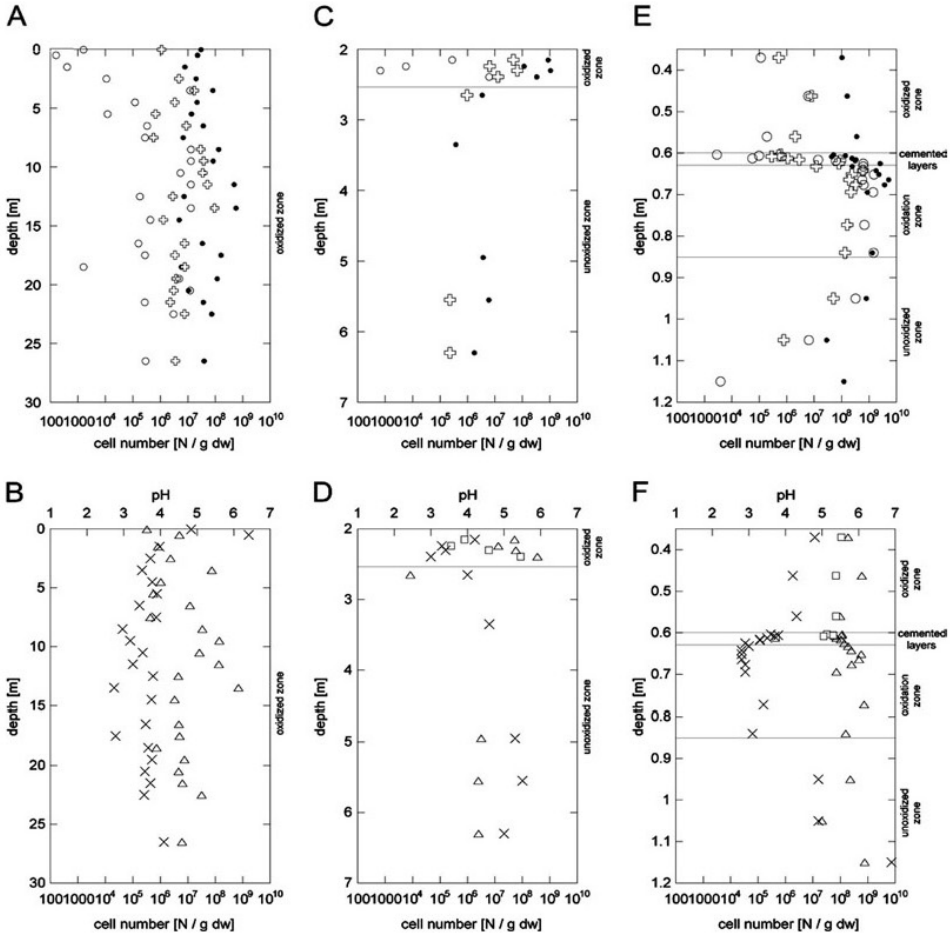

FIG. 1. The pH values (×) and cell numbers of samples from mine tailing dumps located in Selebi-Phikwe, Botswana (A and B); Kristineberg, Sweden (C and D); and Freiberg, Germany (E and F). The cell numbers were detected by Sybr green II direct counting (●), CARD-FISH for *Bacteria* (✣), the MPN technique for detection of Fe(II) oxidizers (○), Q-PCR for *Bacteria* (△), and Q-PCR for *Archaea* (□). The number of *Archaea* detected by CARD-FISH was below the detection limit of 10^5 cells g^{-1} dry weight (dw) at all three sites. Note different zones and depth scales. (Panels A and B are both adapted from references 30 and 55 with permission from Elsevier. Panel C is adapted from reference 25 with permission of the publisher [copyright 2008 American Chemical Society] and reference 30 with permission from Elsevier. Panel D is adapted from reference 30 with permission from Elsevier. Panels E and F are both adapted from reference 31 with permission and reference 21 with permission from Elsevier.)

sites, while *Archaea* remained below the detection limit of 10^5 cells g^{-1} dry weight at all three sites.

The highest cell numbers (10^8 to 10^9 cells g^{-1} dry weight) were detected with all methods in the active oxidation zone (0.63- to 0.85-m depth) in the tailing dump in Germany. Above and below this zone, the cell numbers of living and cultivable cells detected by CARD-FISH and MPN, respectively, were significantly lower than cell numbers detected by Sybr green and Q-PCR (Fig. 1E and F). In the tailing dump in Botswana, cell numbers generally did not show a depth trend except for the MPN numbers of Fe(II) oxidizers, which decreased toward the tailing surface correlating with the water content (data not shown). CARD-FISH and MPN detectable cell numbers generally exhibited a high proportion of Sybr green and Q-PCR detectable cell numbers (Fig. 1A and B). A pronounced depth trend was observed in Sweden, where the cell numbers detected in the oxidized zone of the tailing dump (10^7 to 10^8 cells g^{-1} dry weight) were approximately 1 order of magnitude higher than those in the unoxidized zone (Fig. 1C and D).

Quantification of microorganisms of specific groups detected via Q-PCR analysis. Since the Q-PCR data showed high bacterial cell numbers, this method was chosen for a detailed microbial community analysis by the quantification of genes of particular microbial phylogenetic groups, genera, or species relevant for mine tailings (Fig. 2). In the tailing dump in Botswana, the DNA copy numbers of all specific genes were between 10^4 and 10^6 copies g^{-1} dry weight (Fig. 2A and B). In many samples of the investigated depth profile, the acidophilic Fe(II) and/or sulfur oxidizers *Acidithiobacillus* spp., the Fe(III) reducers *Geobacteraceae*, and sulfate reducers (*dsr*) were detected. The 16S rRNA genes specific for the acidophilic Fe(II) oxidizers *Leptospirillum* spp. and the acidophilic sulfur oxidizer

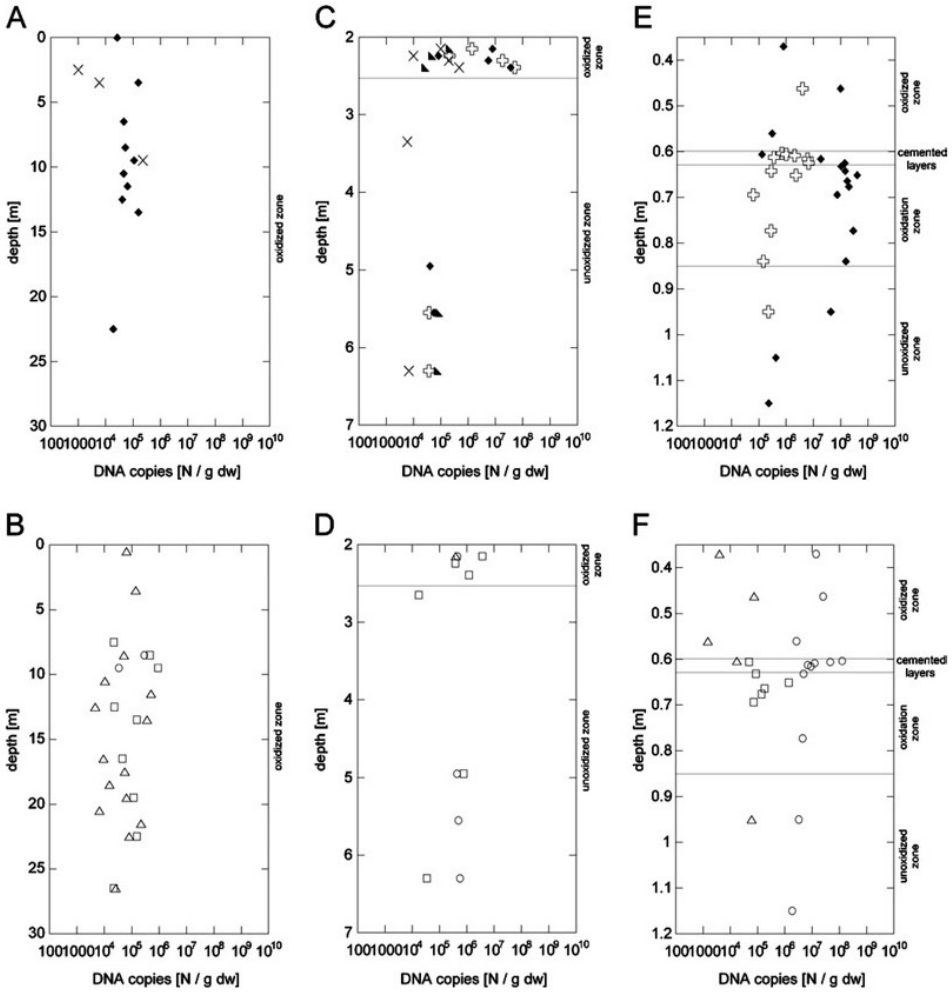

FIG. 2. DNA copy numbers of the 16S rRNA genes of *Acidithiobacillus* spp. (◆), *Acidithiobacillus caldus* (◣), *Leptospirillum* spp. (✣), *Sulfobacillus* spp. (×), *Geobacteraceae* (△), the *dsrA* gene of sulfate reducers (□), and the 18S rRNA gene of *Eukarya* (○) in samples from mine waste tailings located in Selebi-Phikwe, Botswana (A and B); Kristineberg, Sweden (C and D); and Freiberg, Germany (E and F). Note different zones and depth scales. dw, dry weight.

A. caldus were below the detection limit of 10^3 copies g^{-1} dry weight. The acidophilic Fe(II) and sulfur oxidizer *Sulfobacillus* spp. and eukaryotic 18S rRNA genes were detected in only a few samples.

In the tailing dump in Sweden, *Leptospirillum* spp. and *Acidithiobacillus* spp. were most abundant in the oxidized zone with the highest DNA copy numbers of up to 10^7 copies g^{-1} dry weight (Fig. 2C and D). DNA gene copy numbers specific for *Eukarya*, *Geobacteraceae*, sulfate reducers, *Sulfobacillus* spp., and *A. caldus* were between 10^4 and 10^6 copies g^{-1} dry weight in both zones.

In the tailing dump in Germany, the microbial community was dominated by *Acidithiobacillus* spp., which were most abundant in the oxidation zone below the cemented layers with the maximum DNA gene copy number of 10^8 copies g^{-1} dry weight and exceeded the abundance of *Leptospirillum* spp. by 3 orders of magnitude in higher DNA gene copy numbers (Fig. 2E and F). While the DNA gene copy numbers of *Acidithiobacillus* spp. were most abundant, *A. caldus* was not detected by Q-PCR. Also, *Sulfobacillus* spp. were below the detection limit of 10^3 copies g^{-1} dry weight. DNA gene copy numbers between 10^3 and 10^6 copies g^{-1} dry weight were obtained for the 16S rRNA gene of *Geobacteraceae* and for *dsr*. The latter gene was detected only in the zone of active oxidation. Eukaryotic 18S rRNA genes could be found throughout the entire investigated depth in high amounts (10^6 to 10^8 copies g^{-1} dry weight).

Quantification of microorganisms of specific groups detected via FISH analysis. In addition to the detailed quantitative microbial community analysis using Q-PCR, FISH analyses of five selected samples from each tailing dump were performed with different specific probes. For comparison, the cell numbers obtained by FISH as well as CARD-FISH, Q-PCR, and MPN for the selected samples are shown in Table 1.

TABLE 1. Cell numbers of five selected samples from each mine tailing dump located in Botswana, Sweden, and Germany determined by different quantification methods[a]

Organism type	Method	Cell no. (log *n*/g dry wt) for location, zone, and depth (m)													
		Selebi-Phikwe, Botswana (oxidized zone)					Kristineberg, Sweden					Freiberg, Germany (oxidation zone)			
							Oxidized zone				Unoxidized zone (2.65 m)				
		6.5	8.5	9.5	11.5	17.5	2.15	2.24	2.3	2.39		0.63	0.64	0.65	0.68	0.77
Total	Sybr green	7.6	8.1	7.9	8.7	8.2	8.9	8.6	9.0	8.5	6.5	9.4	9.3	9.4	9.6	8.3
Bacteria	Q-PCR	7.1	7.5	8.1	8.1	6.7	7.7	7.1	7.8	3.8	3.9	8.1	8.4	8.8	8.4	8.9
	CARD-FISH	6.9	7.5	7.6	7.7	6.5	7.7	6.8	7.8	7.1	6.0	7.9	8.8	8.4	8.5	8.2
	FISH	5.8	6.4	ND	6.3	5.1	ND	6.3	ND	ND	ND	8.1	8.9	8.4	8.6	8.4
Archaea	Q-PCR	ND	ND	ND	ND	ND	5.9	5.4	6.8	7.9	ND	ND	ND	ND	ND	ND
Fe(II) oxidizers	MPN	5.5	7.1	7.1	7.1	5.4	5.5	3.8	2.8	6.8	ND	8.8	8.8	9.2	8.8	8.8
S oxidizers	MPN	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	6.8	6.2	6.5	7.8	7.8
Acidithiobacillus spp.	Q-PCR	3.6	3.6	4.0	3.7	ND	5.8	3.8	5.7	6.5	ND	7.1	7.1	7.5	7.2	7.4
	FISH	ND	5.8	ND	5.8	ND	ND	ND	ND	ND	ND	8.0	8.8	8.1	8.6	7.7
A. ferrooxidans	FISH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.7	8.8	8.4	8.5	7.4
A. caldus	Q-PCR	ND	ND	ND	ND	ND	4.2	3.6	ND	3.3	ND	ND	ND	ND	ND	ND
Leptospirillum spp.	Q-PCR	ND	ND	ND	ND	ND	5.1	4.3	6.3	6.7	ND	5.8	4.5	5.4	ND	4.4
L. ferrooxidans	FISH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	5.9	ND	5.8	ND	ND
Sulfobacillus spp.	Q-PCR	ND	ND	4.6	ND	ND	4.2	3.2	4.5	4.9	ND	ND	ND	ND	ND	ND
Acidiphilium spp.	FISH	ND	5.3	ND	5.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Geobacteraceae	Q-PCR	ND	4.1	ND	5.1	4.1	5.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfate reducers (*dsr*)	Q-PCR	ND	5.7	6.0	ND	ND	6.6	5.6	ND	6.1	4.2	ND	ND	6.1	5.1	ND

[a] Cell numbers were below detection limits for FISH and CARD-FISH for *Archaea*, FISH for *Acidimicrobium* and relatives, FISH for gram-positive *Bacteria* with low G+C contents, and FISH for *Sulfobacillus* spp. in the investigated samples of all three sites. Conversion factors for calculating cell numbers from DNA copy numbers and detection limits of the methods are given in Materials and Methods. NM, not measured; ND, not detected.

By the use of CARD-FISH and Q-PCR, *Bacteria* were detected in all samples in similar numbers and usually slightly below the total cell counts. By the use of FISH, *Bacteria* could not be quantified for all samples and the bacterial cell numbers were significantly lower than those determined by CARD-FISH in samples from Botswana and Sweden. In contrast, for the five samples from the zone of active oxidation processes in the tailing dump in Germany, similar bacterial numbers were obtained for FISH and CARD-FISH, even though the brightness of the cell signals was considerably different for the two methods (Fig. 3). *Archaea* were quantified via Q-PCR in samples from Sweden but not by CARD-FISH or FISH. No other specific groups were detected via FISH in the latter tailing dump.

In the tailing dump in Botswana, *Acidithiobacillus* spp. were detected via FISH only in two of five of the investigated samples and in significantly lower cell numbers than those detected via MPN for Fe(II) oxidizers. The cell numbers were somewhat higher than those detected with Q-PCR for *Acidithiobacillus* spp. FISH probes specific for the acidophilic Fe(II) oxidizers *A. ferrooxidans* and *L. ferrooxidans* did not show quantifiable positive signals. *Acidiphilium* species-specific FISH probes showed cell numbers in the same samples and of the same order of magnitude as those for the *Acidithiobacillus* species-specific FISH probes.

In contrast to samples from the tailing dumps in Botswana and Sweden, the five samples from the zone of active oxidation in the tailing dump in Germany revealed cell numbers detected by FISH for *Acidithiobacillus* spp. and *A. ferrooxidans* that were of the same order of magnitude as those of the cell numbers revealed by FISH, CARD-FISH, and Q-PCR for *Bacteria* as well as the cell numbers obtained by MPN for Fe(II) oxidizers. *L. ferrooxidans* was the only other microorganism which was detected by FISH, and it was significantly less abundant, in agreement with the Q-PCR data for this site.

DISCUSSION

Sulfide weathering in tailings. The microbial communities in sulfidic mine waste tailings were comprehensively quantified by molecular biological methods for the first time in this study. All three investigated tailing dumps showed an intense colonization by microorganisms. Depth profiles of cell numbers showed that the compositions of the microbial communities are significantly different at the three sites and also varied greatly between zones of oxidized and unoxidized tailings.

Physical, geochemical, and mineralogical parameters of the tailings, e.g., temperature, pH, oxygen diffusion, or mineral reactivity, determine the composition of the microbial communities. Metal sulfide oxidation is the main energy-delivering process for microorganisms in sulfidic mine tailings (4, 49); thus, cell numbers are generally higher in the oxidized than in the unoxidized tailings in this study. Previous studies of sulfidic mine waste dumps showed that the microbial metal sulfide oxidation activity correlates with cell numbers of acidophilic Fe(II)-oxidizing microorganisms (16, 52, 53, 55). Microcalorimetric measurements of the tailing samples used in this study exhibited distinct differences in the potential pyrrhotite and pyrite oxidation rates between the oxidized zones in Botswana (17.5 μg pyrrhotite kg^{-1} tailings s^{-1}) and in Sweden (0.9 μg pyrite kg^{-1} tailings s^{-1}). The distinctly lower oxidation rates in Sweden were presumably caused by a lower temperature and, based on the till cover, by a lower oxygen diffusion rate and less water infiltration combined with a 20- to 100-times-less-reactive main metal sulfide (pyrite) than that in Botswana (pyrrhotite) (26, 30, 55).

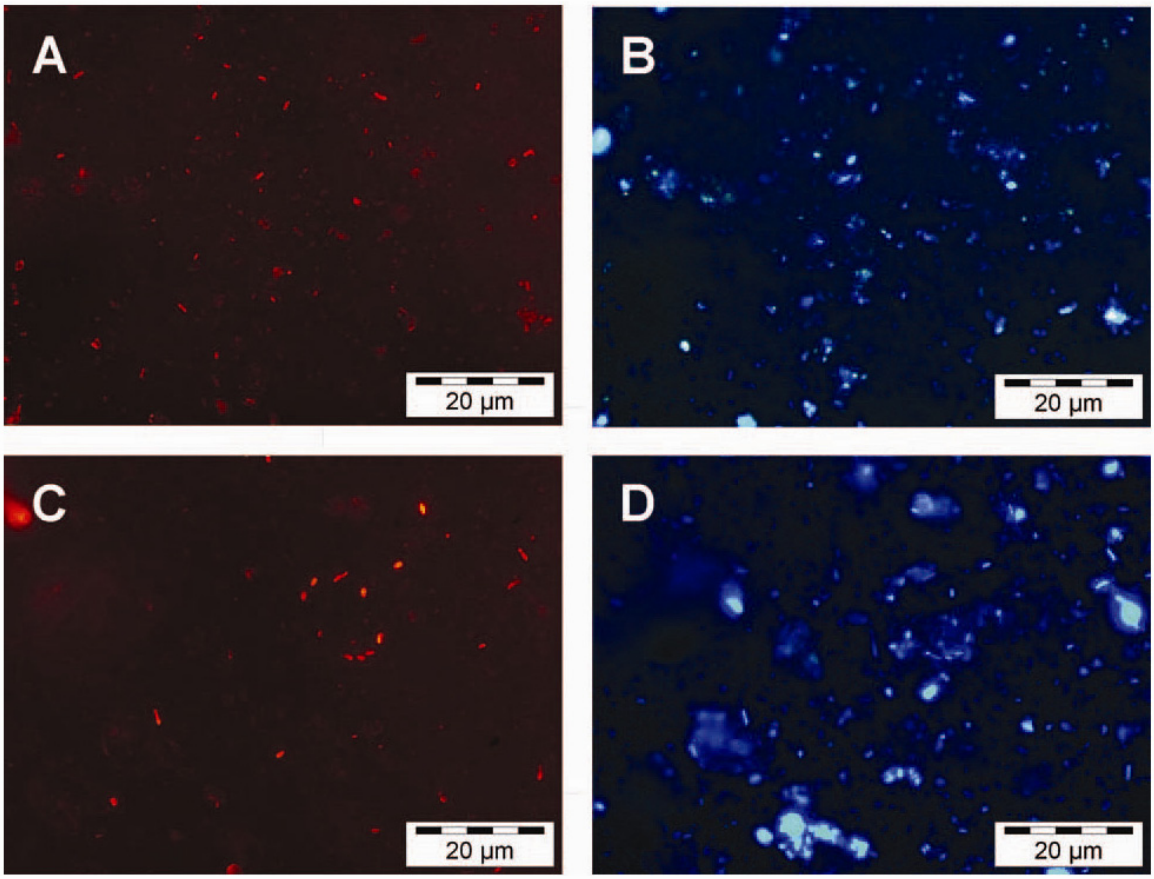

FIG. 3. FISH, CARD-FISH, and DAPI (4′,6′-diamidino-2-phenylindole) fluorescence images for a selected sample from the oxidation zone of the sulfidic mine waste dump in Freiberg, Germany. (A and C) Weak FISH (A) and bright CARD-FISH (C) signals of cells detected with probes EUB338 I to III specific for *Bacteria*. (B and D) Same microscopic fields as panels A and C, respectively, with UV excitation (DAPI staining).

In the tailing dump in Germany, microbial growth and the formation of cemented layers were probably favored by the temperate climate, the unremediated state of the dump, and the relatively high content in the tailings of arsenopyrite, sphalerite and galena, which are more reactive than pyrite (21, 31).

Comparison of results for cultivation and different molecular biological methods. It was proven that the quantification methods used here are applicable to mine tailings. The fluorescent dye Sybr green II binds to all DNA of living and dead cells and therefore provided the highest cell numbers (total cell counts). The Q-PCR method quantifies amplifiable DNA presumably of living, and to some extent dead, cells and mainly yielded somewhat lower cell numbers than the total cell counts. Variation of Q-PCR cell numbers may be explained either by DNA preservation and/or degradation or by variable 16S rRNA operon numbers and/or variable genome copy numbers of distinct species (51). CARD-FISH and FISH probes target quickly degradable rRNA and, therefore, detect only living cells (44, 54). The bacterial numbers determined by Q-PCR and CARD-FISH were of the same order of magnitude for most of the samples, which means that a very high proportion of the total cell numbers in the tailings were alive.

In agreement with this interpretation, high MPNs of living acidophilic, chemolithoautotrophic Fe(II) oxidizers were found. The C-free medium used here at pH 3.5 provides cell numbers comparable with those of the molecular methods, which also detected the chemolithoautotrophic Fe(II) oxidizers *Acidithiobacillus* spp. and *Leptospirillum* spp. In the active oxidation zone (0.63- to 0.85-m depth) in the tailing dump in Germany, the MPNs were as high as the bacterial numbers determined by Q-PCR, CARD-FISH, and FISH, which argues for a high microbial activity and growing cells with a high ribosome content. In the other two tailings, the bacterial FISH numbers were lower (or below the detection limit) than the bacterial CARD-FISH numbers; thus, the microbial activity and the ribosome content of the *Bacteria* were lower, and only the more-sensitive method CARD-FISH enabled quantification of most cells. As a consequence, reliable FISH data for tailings can be obtained only for zones of high microbial activity, whereas CARD-FISH, Q-PCR, and MPN are suitable for tailings in general.

Quantification of microbial communities. At all the sites investigated in this study, *Bacteria* dominated the microbial community. *Archaea* and *Eukarya* were less abundant. The autotrophic (or facultatively autotrophic) and acidophilic Fe(II)- and S-oxidizing bacterial genera *Acidithiobacillus*, *Leptospirillum*, and *Sulfobacillus* were detected by Q-PCR and FISH analysis in different numbers in the three tailings. *Acidithiobacillus* was detected at all three sites and overall dominated over *Leptospirillum* and *Sulfobacillus*. *Leptospirillum* spp. occurred in numbers similar to those of *Acidithiobacillus* spp. only in Sweden, occurred in lower numbers in Germany, and occurred not at all in Botswana. *Sulfobacillus* occurred in even lower numbers than *Leptospirillum* in Sweden, occurred in only a few samples in Botswana, and occurred not at all in Germany. In contrast to these findings, Diaby et al. (13) reported that *Leptospirillum* and *Sulfobacillus* relatives dominated over *Acidithiobacillus* in a pyrite-containing porphyry copper tailing impoundment in Chile based on terminal restriction fragment length polymorphism analysis. This technique allows calculation of a relative abundance of species in a clone library but cannot provide cell numbers of particular species, as was possible in this study. However, the results of this study and those of Diaby et al. (13) clearly demonstrate that the composition of the Fe(II)- and S-oxidizing bacterial community varies greatly at the different tailing sites. It is not yet understood which physical, chemical, and mineralogical parameters favor particular genera and species based on their physiological properties in mine tailings.

Previous bioleaching and AMD studies revealed that pH, redox potential, and Fe concentration control the composition of the acidophilic Fe(II)- and S-oxidizing community. In bioleaching processes which operate at a pH of <2, a high redox potential, and a high Fe(III) concentration, the Fe(II) oxidizers *Leptospirillum* spp. but not *A. ferrooxidans* has been shown to be dominant (47, 48). At the Iron Mountain AMD site, the dominance of *Leptospirillum* spp. over *Acidithiobacillus* spp. was shown by FISH analysis of extremely acidic water of pH ~0.5 to 1.5 (6, 15, 56). Both genera occurred on average in similar numbers in the Tinto River at a pH of ~2.0 to 2.5 (20). In less-acidic AMD with a pH of 2.7 to 3.7 from a mine in Norway, *A. ferrooxidans* dominated over *Leptospirillum* spp. (28), in agreement with this study of oxidized tailings with a similar pH range of ~3 to 4. As shown by culture studies, the pH optimum for *Leptospirillum* spp. (1.3 to 3.0) is below that of *Acidithiobacillus* spp. (2.0 to 4.0) (50), supporting the findings of the ecological studies. Besides pH, redox potential, and Fe concentration, the availability of oxygen and inorganic sulfur compounds should be relevant. While *Leptospirillum* spp. are obligate aerobic Fe(II) oxidizers, *A. ferrooxidans* and *Sulfobacillus* spp. are more versatile and are able to oxidize Fe(II) and sulfur compounds aerobically as well as to reduce Fe(III) anaerobically (22, 50).

Quantifiable heterotrophic, facultative anaerobic Fe(III)-reducing *Bacteria* belonging to the acidophilic genus *Acidiphilium* and the neutrophilic Fe(III)-reducing family *Geobacteraceae* were detected in this study. *Acidiphilium* spp. were detected in the tailing dump in Botswana and previously in other sulfidic mine waste dumps (13, 52). The heterotrophic genus is widely distributed in bioleaching and AMD environments and in the case of the species *Acidiphilium acidophilum* is also able to oxidize inorganic sulfur compounds (22, 28, 50). Different studies implicate synergistic interactions between autotrophic and heterotrophic microorganisms by removing metabolic by-products of the autotrophs within the community (1, 24, 45, 52). *Geobacteraceae* were present at all three sites of this study. In other studies of sulfidic mine waste *Geobacteraceae* were detected by 16S rRNA gene sequence analysis (10) and neutrophilic Fe(III)-reducing microorganisms were found by cultivation techniques (52, 64). Microenvironments may protect the anaerobic and presumably neutrophilic *Bacteria* from acidity and oxygen (17, 52).

Sulfate-reducing *Bacteria* were quantified via their functional gene *dsr* coding for dissimilatory sulfite reductase. So far, other attempts to amplify the *dsr* gene in AMD sites with extremely low pH and high iron concentrations have been unsuccessful (1). Sulfate-reducing *Bacteria*, e.g., *Desulfosarcina* spp. and *Desulfotomaculum* spp., were regularly detected in sulfidic mine tailings (3, 7, 13, 17, 18, 64). These anaerobic and presumably neutrophilic *Bacteria* might be protected by microenvironments from acidity and oxygen, or they are able to remove oxygen by active respiration (32).

The high abundance of Fe(II)- and S-oxidizing, as well as of Fe(III)- and sulfate-reducing, *Bacteria* in the tailing dumps studied here and elsewhere shows that biogeochemical Fe and S cycling are predominant processes mediated by the activity of *Bacteria*.

In contrast to the dominant *Bacteria*, *Archaea* were detected only in low numbers in the oxidized zones of two tailings in this study. *Archaea* were not detected at all in the tailing deposit in Chile (13). The reason that *Archaea* do not predominate in mine tailings is most likely that the described species relevant for Fe and S cycling are extremophiles. *Ferroplasma* spp. are often found in AMD and bioleaching operations at very acidic conditions (pH of <2) and very high iron and sulfate concentrations (1, 12, 20, 50), conditions which usually do not prevail in mine tailings. Extremely thermophilic *Archaea* (e.g., *Sulfolobus* and *Metallosphaera*) have been found only at sites of high temperatures in sulfidic waste rock dumps because of high rates of pyrite oxidation (19, 52), but not yet in mine tailings.

For the first time, it is reported that the eukaryotic 18S rRNA gene was quantified in sulfidic tailing dumps in this study. The 18S rRNA gene occurred at all sites in lower copy numbers than the 16S rRNA gene of *Acidithiobacillus* spp., except for the unoxidized tailings in Sweden. Up to this point, only AMD sites such as the Rio Tinto and Iron Mountain sites were investigated in this regard and *Eukarya* such as algae, ciliates, flagellates, amoebae, and fungi were detected (1, 2, 66). To determine the impact of *Eukarya* on the microbial community in sulfidic mine waste, further studies will be necessary.

In conclusion, microorganisms occur in significantly different numbers at different sulfidic mine waste tailing zones and sites. Bacteria which are relevant for biogeochemical Fe and S cycling dominate the microbial community. The molecular methods applied here have proven to be useful for the quantification of microbial communities in sulfidic tailing dumps. These methods should also be useful for the monitoring of bioleaching processes such as heap or tank leaching.

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4.2.4 Quantification of microorganisms involved in cemented layer formation in sulfidic mine waste tailings (Freiberg, Saxony, Germany)

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Quantification of microorganisms involved in cemented layer formation in sulfidic mine waste tailings (Freiberg, Saxony, Germany)

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Abstract. Cemented layers predominantly consisting of gels/poorly crystalline mineral phases have been formed as a consequence of mineral weathering in sulfidic tailings near Freiberg, Saxony, Germany. These layers function as natural attenuation barrier for toxic compounds and reduce oxidation and erosion processes of tailings surfaces. Quantitative molecular biological and cultivation methods were applied to investigate the role of microorganisms for mineral weathering and cemented layer formation. High resolution depth profiles of numbers of microorganisms showed maximal cell numbers in the oxidation zone where cemented layers had been formed. Highest total cell numbers of $>10^9$ cells g^{-1} dry weight (dw) were detected by SybrGreen direct counting. Using quantitative real-time PCR (Q-PCR) between 10^7 and 10^9 Bacteria g^{-1} dw and up to 10^8 Archaea g^{-1} dw were determined. As well high numbers of cultivable and living Bacteria could be detected by MPN (most probable number) for Fe(II)- and S-oxidizers and CARD-FISH (catalyzed reporter deposition - fluorescence in situ hybridization). Overall, the high numbers of microorganisms determined with various quantification techniques argue for a significant role of microorganisms in cemented layer formation due to microbial mineral weathering. It is hypothesized that EPS (extracellular polymeric substances) mediate the formation of secondary mineral phases.

Introduction

Chemical and biological oxidation of metal sulfides in mine waste heaps or in tailings from sulfidic ore processing produces acidic heavy metal enriched waters (acid mine drainage, AMD) which threatens the ground water quality. This process is influenced by oxygen availability and humidity. Furthermore, Fe(II)- and metal sulfide-oxidizing microorganisms play a pivotal role for the metal sulfide oxidation by accelerating e.g. the pyrite oxidation rate 30-300 times in comparison to the chemical rate [1]. A few studies were carried out to quantify microorganisms and to investigate the microbial diversity in sulfidic mine waste deposits by combining different cultivation and molecular biological methods [2-5]. These investigations yield information about the importance of microorganisms in the biogeochemical process of AMD generation. Additionally, understanding the biogeochemistry of natural attenuation processes in mine tailings is of great importance to develop low-cost remediation measures. The mineral weathering can form hard layers cemented by gels/ poorly crystalline mineral phases. These layers are called cemented layers or hardpans and have been mineralogically, chemically and physically investigated in sulfide-bearing mine tailings [6-10]. The few cm to up to ~4 m thick cemented layers limit the diffusion of oxygen and dissolved pore water constituents. Heavy metals were enriched in high concentrations directly above and within a cemented layer. Therefore the presence of cemented layers has significant environmental

implications by restricting the movement of dissolved metals through the tailings (natural attenuation), decreasing the rate of oxidation and reducing the wind and water erosion of the tailings surface. Microbial extracellular polymeric substances (EPS) with complexed metals [11] may serve as nucleation sites for mineral formation [12, 13] and contribute to the formation of cemented layers.
The aim of this work was to investigate the contribution of microorganisms to mineral weathering and cemented layer formation in mine tailings by quantification of microorganisms combining cultivation and molecular biological methods.

Material and Methods

Site description and sampling. The tailings dam is located in the polymetallic sulfide mine district of Freiberg, Saxony, Germany. The low-sulfide and low-carbonate-bearing tailings dam covers an area of ~60,000 m^2 and consists of waste from ~13 years of Pb and Zn sulfidic ore processing. After termination of tailings deposition in 1968, the tailings dam was partly covered with a soil cover consisting of $\leq$ 0.2 m coarse sand and topsoil. The dominant metal sulfides in the tailings material are pyrite, arsenopyrite, sphalerite and galena. Oxidized tailings occur down to 85 cm depth at the sampling site. An oxidation zone could be defined from ~60 cm to 85 cm depth. At the top of this zone at the depth of ~60-63 cm, three distinct 0.2-0.5 cm thick cemented layers occur, underlain by altered silt layers. In this narrow zone reactive mineral phases such as sulfides and alumosilicates are enriched in layers. As and Pb are enriched in the poorly crystalline secondary mineral phases in the cemented layers. The sulfide content in solids was determined to be up to 0.01% within the oxidized zone and increases to 1% in the zone of cemented layers. Down to the unoxidized zone (below 85 cm depth) an approximately constant sulfide content of 1% was detected [14]. At the sampling site 21 solid samples were taken down to a depth of 1.1 m, while 13 of these samples were taken from the interval at ~60-70 cm depth.

Geomicrobiological analysis. The geochemical data were detected as previously described [3]. The quantification of microorganisms by SybrGreen direct counting and CARD-FISH were performed as described elsewhere [3]. To quantify the abundance of Bacteria and Archaea by Q-PCR analysis published protocols were applied [3]. To convert the 16S rDNA gene copy numbers to cell numbers, conversion factors of 1.5 for Archaea and 4.1 for Bacteria were used [15]. The number of Fe(II)- and elemental sulfur-oxidizing microorganisms was determined by the "most probable number (MPN)" technique as previously published [14].

Results and Discussion

Depth profiles of cell numbers determined by molecular and cultivation methods, and of pH and amount of total sulfur are shown in Fig. 1. All methods for the quantification of microorganisms consistently yielded very high cell numbers (Fig. 1B, C). The high resolution depth profiles showed highest cell numbers for the oxidation zone (~60-85 cm) including the zone of cemented layers at ~60-63 cm depth. There, the correlation between the shift of cell numbers (Fig. 1B, C) and pH (Fig. 1A) indicated microbial metal sulfide oxidation with accompanied sulfuric acid production. Above the oxidation zone (<60 cm) lower cell numbers, a low total sulfur content and a higher pH defined the former zone of metal sulfide oxidation. Below the oxidation zone (>85 cm) the high pH and the high total sulfur content characterized the unoxidized zone where considerably lower cell numbers prevailed.

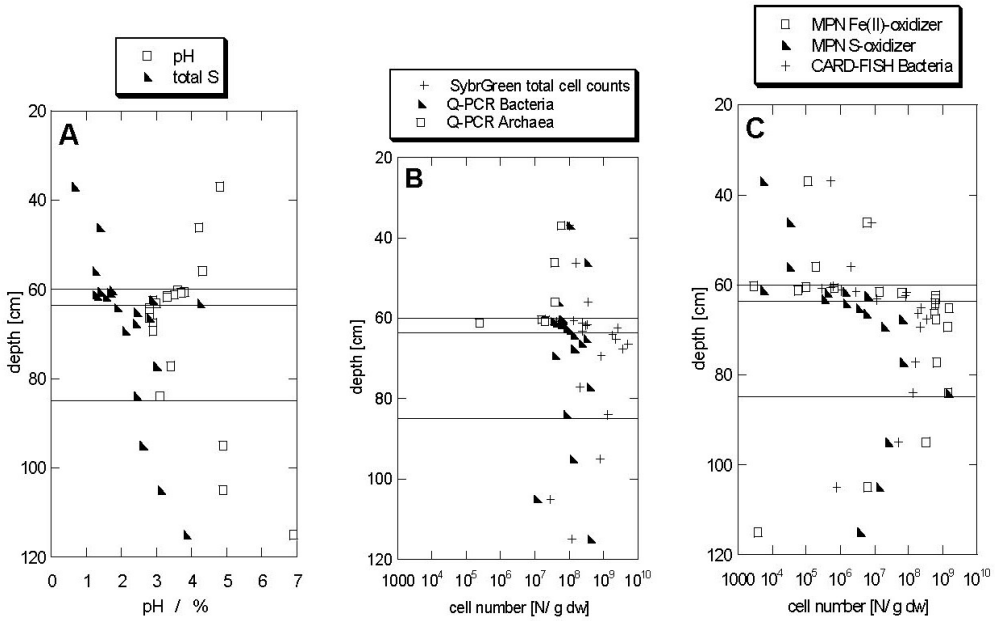

Figure 1. Depth profiles of pH and amount of total sulfur (A), and cell numbers determined by SybrGreen counting and Q-PCR (B) or by MPN and CARD-FISH (C) for the tailings dam near Freiberg, Saxony, Germany. The lines define the zone of cemented layers at ~60-63 cm and the oxidation zone at ~60-85 cm depth.

A comparison of the microbial quantification methods showed that highest cell numbers were detected by SybrGreen direct counting (>10^9 cells g^{-1} dw) for determination of living and dead cells (total cell numbers, Fig. 1B). In average one order of magnitude fewer cells were detected by Q-PCR for Bacteria (Fig. 1B). This difference might be caused by the fact that SybrGreen stains also degraded DNA in contrast to Q-PCR which targets only high molecular weight, amplifiable DNA. Archaea were determined only above the oxidation zone via Q-PCR (up to 10^8 cells g^{-1} dw). High cell numbers were also detected with quantification methods for cultivable and living microorganisms (MPN and CARD-FISH, Fig. 1C). In the oxidation zone, the numbers of living Bacteria detected by CARD-FISH were in the same order of magnitude than those detected by Q-PCR (10^7-10^9 cells g^{-1} dw). In contrast to Q-PCR, Archaea could not be detected by CARD-FISH. In the oxidation zone, highest cell numbers for living, cultivable Fe(II)-oxidizing microorganisms (MPN) reached values (10^9 cells g^{-1} dw) of total cell numbers (SybrGreen), which demonstrates the high microbial activity in these samples and the suitability of the enrichment medium for samples from this environment. For several samples the MPN cell numbers for Fe(II)-oxidizing microorganisms even exceeded the CARD-FISH cell numbers for Bacteria which indicated the dominance of Fe(II)-oxidizing microorganisms in the tailings. A similar depth profile showed the S-oxidizing microorganisms (MPN) but their numbers were considerably lower than those of the Fe(II)-oxidizing microorganisms. Especially the high numbers of living, cultivable Fe(II)-oxidizing microorganisms in the zone of the cemented layers reflect an active microbial metal sulfide oxidation. The oxidation products form secondary mineral phases and most likely microbial EPS with complexed metals [11] may serve as nucleation sites for mineral formation [11-13].

Conclusions

The very high numbers of microorganisms in the oxidation zone of the tailings argue for a significant role of microorganisms in cemented layer formation by microbial mineral weathering and formation of secondary mineral phases. The here applied cultivation (MPN) and molecular methods (Q-PCR, CARD-FISH) for the quantification of microorganisms in mine waste samples gave consistent results. These methods should also be suitable to quantify microorganisms in bioheaps and other bioleaching operations.

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4.2.5 Formation of sequences of cemented layers and hardpans within sulfide-bearing mine tailings (mine district Freiberg, Germany)

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Formation of sequences of cemented layers and hardpans within sulfide-bearing mine tailings (mine district Freiberg, Germany)

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Abstract

The roles of mineral dissolution, precipitation, transformation and mass transport processes related to formation of characteristic cemented layer–hardpan sequences were studied in low sulfide and low carbonate Freiberg polymetallic mine tailings. Using high resolution profiling, combined geochemical, geomicrobiological and geophysical methods allowed description of the process of weathering of reactive mineral phases and the position of the oxidation front in detail, as well as revealing the mechanisms of cementation of tailings predominantly by the formation of gels/poorly crystalline phases. Autochthonous and allochthonous gels reduced the porosity of cemented layers to values $\leqslant 1\%$, whereas secondary crystalline phases were less efficient in filling the pore space. Electron microprobe analysis of cemented tailings showed that common jarosite-group minerals contained up to about 8 wt.% PbO and 0.2–1.9 wt.% As_2O_5. Iron–As–Si gels reached contents of up to ~44 wt.% As_2O_5 in gel-rich cemented layers. Zinc was below the detection limit in the studied secondary phases. Sequential extraction of cemented and related oxidized brown silt layers confirmed that the bulk of As was bound to amorphous/poorly crystalline hydrous oxides of Fe, whereas Pb was often bound to jarosite. Zinc was found preferentially in the water-soluble and the exchangeable fractions. In the grey silt and the sand directly underlying the oxidized layers, As, Pb and Zn occurred as sulfide minerals. The main effects of the cemented layer–hardpan sequences at the studied site are (1) a temporary natural attenuation of the toxic compounds, (2) a restriction of the downward movement of the oxidation front, and (3) a reduction of the extent of the erosion of the surface of the tailings impoundment by wind and water. The potential of a heap to form cemented layers and hardpans is greatly increased by a heterogeneous distribution of grain sizes and reactive materials in the topmost zone, as well as by the occurrence of sulfide-rich tailings on top of layers with low permeability.

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1. Introduction

The study of natural attenuation (NA) processes on Acid Mine Drainage (AMD) formation in mine waste deposits is of great importance, because NA may counteract serious pollution threats to the water systems. Mobilisation of As, Zn and Pb from metallic mine tailings is known to be induced by oxidative weathering of metal sulfides. For example, oxidation of arsenopyrite (FeAsS) and Fe^{2+}-bearing sphalerite ((Zn,Fe)S) results in AMD containing H_2SO_4, Fe(III) as amorphous ferric oxyhydroxide precipitates, Fe(II) as soluble Fe species, As(III) as arsenite (AsO_3^{3-}), As(V) as arsenate (AsO_4^{3-}), and Zn(II). Microorganisms are known to catalyze sulfide oxidation (e.g. *Acidithiobacillus ferrooxidans*, *Thiomonas* sp.; e.g. Tuovinen et al., 1994; Blowes et al., 1998; Bruneel et al., 2003; Schippers, 2004; Morin and Calas, 2006). Several contaminant attenuation processes in mine waste deposits must be considered, like precipitation, sorption, and ion substitution. For example, arsenate may precipitate as scorodite, $FeAsO_4 \cdot 2H_2O$ (Ehrlich, 2002) or more commonly as ferric arsenate (Paktunc et al., 2004), sorb to amorphous ferric oxyhydroxide phases and clay minerals (e.g. Korte and Fernando, 1991), or substitute for sulfate in jarosite, $KFe_3(SO_4)_2(OH)_6$ (e.g. Paktunc and Dutrizac, 2003; Waychunas et al., 1995). Because hardpans and cemented layers may consist of reactive secondary minerals, they are expected to play a crucial role in contaminant attenuation.

Cemented, indurated layers in sulfide-bearing mine tailings (so-called hardpans) have been studied for their physical, chemical and mineralogical properties (Blowes and Jambor, 1990; Tassé et al., 1997; Coggans et al., 1999; Johnson et al., 2000; McGregor and Blowes, 2002; Courtin-Nomade et al., 2003; Giere et al., 2003; Gilbert et al., 2003; Moncur et al., 2005; Gunsinger et al., 2006). Field studies have reported on hardpans in e.g. Canadian tailings impoundments (McGregor and Blowes, 2002; Moncur et al., 2005; Gunsinger et al., 2006). The thickness of the hardpans varied from a few cm up to ~4 m. They consisted of ferrihydrite ($Fe_2O_3 \cdot 0.5\ H_2O$), gypsum ($CaSO_4 \cdot 2H_2O$), jarosite, lepidocrocite (γ-FeOOH), melanterite ($FeSO_4 \cdot 7H_2O$), and rozenite ($FeSO_4 \cdot 4H_2O$). The highest concentrations of originally dissolved metals were observed directly above and within a hardpan layer, thus the hardpans may have restricted the movement of dissolved metals through the tailings and may have acted as a zone of metal accumulation. In addition, the permeability of the hardpans was lower than that of uncemented tailings. Column experiments verified that hardpans that are situated between reactive tailings and cover material, improved leachate water quality and reduced the rate of sulfide oxidation (Gilbert et al., 2003).

The terms hardpan and cemented layer are widely used, but these terms are not exactly defined in the literature. In the authors' understanding, hardpans are zones at the capillary fringes where agglutination of particles is basically due to processes driven by capillary transport within an O_2 dominated environment. Supersaturation results in the precipitation of secondary phases and gels, which may coat particles, agglutinate them, and reduce the porosity. Cemented layers on the other hand, can be observed at the transition between oxidized and reduced zones, which often occurs at the transition between saturated and unsaturated zones. At these transitions, variations in master geochemical variables, such as Eh and pH, occur.

Despite that hardpan layers probably play a pivotal role in the NA of unwanted metals that may originate from sulfide-bearing mine tailings, their formation processes have not been thoroughly studied. A combination of processes such as dissolution of primary mineral phases, transport processes, and precipitation of secondary phases seems to be responsible for hardpan/cemented layer formation in mine tailings. The formation of amorphous phases (gels) and/or poorly crystalline phases plays a key role in this process. Gel formation has been documented for a wide range of geochemical conditions; they occur in alkaline (pH 9–14; Wan et al., 2004; Rammlmair et al., 2005) and also in neutral to acidic, sulfate-rich environments (pH < 1–6; e.g. in AMD; Rousel et al., 1999). However, the mechanisms of formation of gel-rich layers in tailings, their distribution characteristics and stability, as well as possible genetic relationships between gel-rich layers and sulfate-rich hardpans are controversially discussed or lacking in the literature.

A multi-disciplinary approach based on geochemical, mineralogical, geophysical and geomicrobiological methods was applied to study (i) the mechanism of formation of gel-rich and gel-poor cemented layers and hardpans in an AMD environment, and (ii) the NA capacity of the cemented layers and hardpans for As, Pb and Zn. The low-sulfide and low-carbonate tailings deposited at the studied site represent a major type of tailings impoundment of great international importance (e.g. Diaby et al., 2007).

2. Material and methods

2.1. Site description

The Muenzbachtal tailings impoundment (polymetallic sulfide mine district Freiberg, Saxony, Germany) is ~320 m wide, has a maximum height of ~30 m and an almost flat plateau which covers an area of ~60,000 m^2 (Fig. 1a). It was used to deposit sulfide-bearing tailings from 1955 to 1968. The volume of the deposited material was estimated at ~835,000 m^3 (1.3 Mt; ACD report, 1993). It almost exclusively derived from the processing plant of the "Grube Beihilfe" mine in Halsbruecke, which produced Pb–Zn concentrate using ore from the hydrothermal "Halsbruecker"-, "Ludwig"-, and "Drei Prinzen-Spat" veins. The metal content of the original heap material was determined at ~0.1 wt.% Pb, ~0.2 wt.% Zn, ~1.4 wt.% S, 0.02 wt.% Cu and ~0.1 wt.% As (ACD report, 1993). After termination of deposition, the tailings impoundment was partly covered with coarse sand and topsoil (thickness of cover: ⩽0.2 m).

2.2. Profiling methods

The depth of the oxidation zone was investigated in a SE-NW profile using a drilling stock (Fig. 1a). For each location, two samples of unaltered material were collected from a depth of ~1.6–1.8 m. These samples were analysed for particle-size distribution using an automatic particle-size analyser (<63 μm fraction; Micrometrics SediGraph 5100) which is based on X-ray scattering.

2.3. Geophysical methods

The apparent resistivity was measured along three profiles parallel to and two profiles across the Muenzbach stream in a Wenner configuration

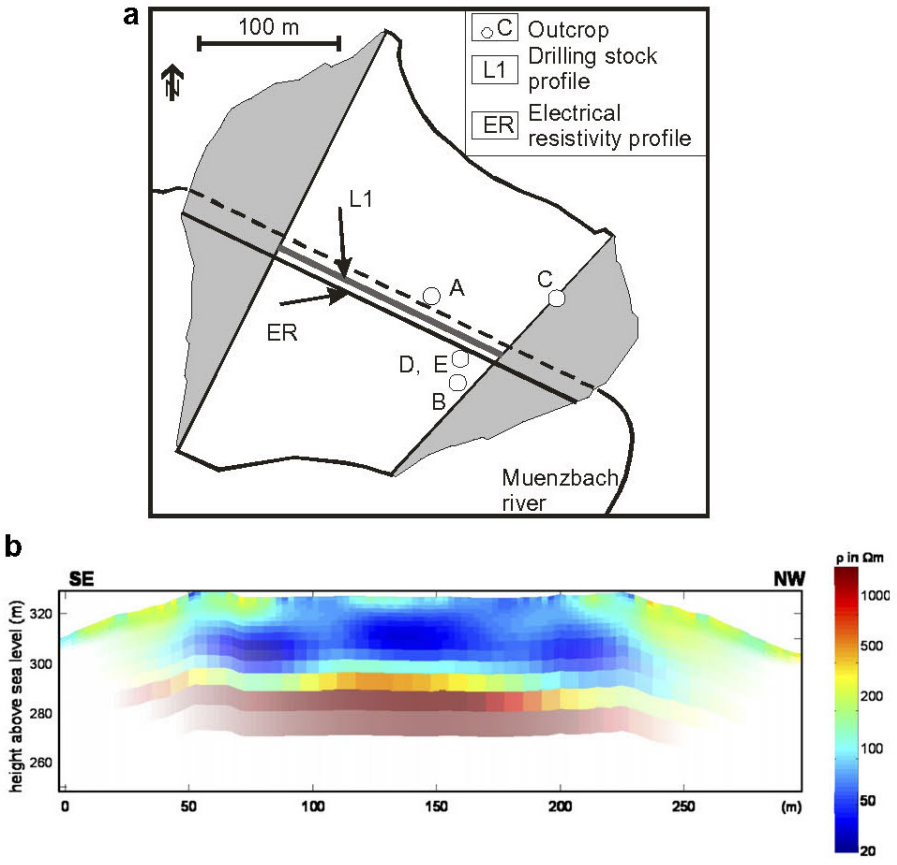

Fig. 1. (a) Schematic map of the Muenzbachtal tailings impoundment showing the locations of the profiles discussed here. (b) 2D cross section of electrical resistivity parallel to the Muenzbach stream from SE to NW.

ranging over the whole dump body. These data were used to compute 2D-models of resistivity distribution using a finite difference (FD) inversion technique. Since resistivity is a function of porosity, water conductivity, and water content of sediments, the resistivity models give a qualitative impression of grain size distribution and water content of the tailings assuming a constant conductivity of water (Archie, 1942).

The measurement of spectral induced polarisation (SIP) records the amplitude of resistivity and, additionally, the time delay between voltage signal and the injected current for different frequencies. In the frequency domain, this time delay corresponds to a negative phase angle between current and voltage. Phase shifts are mainly caused by polarizable materials (e.g. sulfides; Pelton et al., 1976).

2.4. Geochemical and mineralogical methods

Sequential extractions were used for determination of pollutant content and speciation within the dump pore waters and mineral phases (cf. Dold, 2003; Gault et al., 2005). Selective dissolution of mineral phases was achieved applying a sequential extraction method modified from that of Zeien (1995). Table 1 contains the extraction sequence including the expected phases that would dissolve in each extraction step. Sequential extractions were performed at 2.5–5.0 g of sediment samples. Samples were placed in 100 mL borosilicate glass centrifuge tubes, reacted with 50 mL of solvent solution and centrifuged after each extraction step (steps I–VIII). For aqua regia dissolution (step IX), samples were transferred to teflon vessels and reacted in a microwave extraction unit. Multi-element chemical analysis was performed on supernatant liquids using ICP-OES (Spectro Ciros^{CCD}). Merck multi element standards and an As standard were used for calibration. Detection limits were 0.01 mg/L for As, Pb and Zn, 0.02 mg/L for Fe and Mg analysis, 0.05 mg/L for Al, Ca and Na, 0.1 mg/L for K and Si, and 0.3 mg/L for S. Inorganic C analysis by conversion to CO_2 and subsequent IR detection (Shimadzu TOC-$V_{CPH/CPN}$) had a detection limit of 0.5 mg/L.

Frequency distributions were estimated for mineral and amorphous phases as well as for open or partially filled pore spaces within cemented layers, hardpans and unconsolidated sediments. A 4-step procedure was applied: (i) Mapping of element distribution within a profile of adjacent areas of $\sim 3 \times \sim 3$ mm in polished thin sections that were not coated with C. Mapping was carried out with the EDAX module (C Kα, Si Kα, Al Kα, Ca Kα, S Kα, Ba Lα, Fe Kα, As Kα) of a Quanta 600 FEG system (FEI Company) using a maps resolution of 512×512 (dwell time: 400 μs; 64 frames). (ii) Based on microscopic studies, rectangular regions of interest (ROI) were defined for (a) cemented and (b) loose parts of the mapped areas. These ROIs were representative of the studied zones. (iii) Each mineral phase as well as the open or partially filled pore space was described by a special element signature (Table 2). For some phases, two or more elements were combined, or the signal of other phases was subtracted to separate them clearly (e.g. combination of Ca and S for gypsum). (iv) For processing of false colored images the Analysis© system was used. The averaging filter was applied to erase artefacts. Combined images of

Table 1
Extraction protocol (modified from Zeien, 1995)

Extraction step	Solvent	Extracted pollutant fractions
I	Aqua dest.; 2 h	Pore water, sulfate minerals
II	1 M $SrCl_2$; 30 min; twice	Cation exchange complex
III	1 M NH_4OAc; pH 6; 12 h; repeat until $TIC_{extract} = 0$	Carbonates
IV	1 M NH_4OAc + 0.1 M NH_2OH; pH 6; 30 min	Easily reducible phases (e.g. Mn oxides)
V	0.025 M NH_4 EDTA; pH 4.7; 90 min	Organic matter, hydrated Si gels
VI	0.2 M NH_4-oxalate; pH 3.3; 4 h (in darkness)	Poorly crystalline Fe oxyhydroxides (e.g. ferrihydrite) and jarosite-group minerals, dehydrated Si gels
VII	0.2 NH_4-oxalate + 0.1 M ascorbic acid; 30 min; boiling (water bath); pH 3	Crystalline Fe oxides (e.g. goethite, hematite) and jarosite, Al oxyhydroxides, aluminosilicates (minor)
VIII	8.8 M H_2O_2; pH 2; at room temperature until end of degassing (7 14 d)	Sulfides (e.g. ZnS, PbS, FeS_2), aluminosilicates
IX	Aqua regia; 180 °C; 20 bar; 15 min	Sulfides (e.g. PbS, ZnS) enclosed in silicates, aluminosilicates

Table 2
Processing of data for estimation of frequency distributions of mineral phases and pore spaces

Phase	Filters	Operations	Images
Pore space	N × N		C Kα
Barite ($BaSO_4$)	Ave		Ba L
Sulfide pyrite (FeS_2)	Sigma, Ave	Fe Kα × S Kα	S Kα, Fe Kα
Gypsum ($CaSO_4 \cdot 2H_2O$)	Ave	Ca Kα × S Kα	Ca Kα, S Kα
Fluorite (CaF_2)	DCE, Ave	Gypsum (inverted) × Ca Kα	Ca Kα, gypsum
Quartz (SiO_2)	DCE, Ave	Invert Fe Kα, K Kα, ...; multiply each with Si Kα	Si Kα, Fe Kα, K Kα, Ca Kα, Al Kα
Silicates (excl. quartz)	N × N, Ave	Quartz (inverted) × Si Kα	Si Kα, quartz
Fe phases (excl. sulfides)	Ave	Sulfide (inverted) × Fe Kα = X; Si Kα (inverted) × X	Si Kα, Fe Kα, sulfide (pyrite)
As-bearing phases (MIA)	N × N	Coordinate the images without correlation	As Kα

Filters: Ave averaging filter; DCE differential contrast enhancement; N × N definition of parameters for averaging filter with optional square matrix; Sigma filtering of the shot noise. MIA multiple image alignment.

two or more elements were edited using filters to optimize the fitting threshold. Table 2 specifies the elements used for each studied phase, as well as the filters and operations applied.

For XRD analysis, a Philips PW 3710 series automated powder diffractometer was employed which uses Cu radiation (Kα line with a mean wavelength of 1.542 Å) operated at 40 kV and 30 mA, and glancing angles 2θ between 2° and 65°. A secondary graphite crystal monochromator was used. For evaluation of the data the software package Galaxy linked with a PDF2 data base was employed.

Electron microprobe (EM; CAMECA SX-100 microprobe) analysis was applied to study amorphous and poorly crystalline Fe(III) phases, jarosite-group minerals and gypsum from cemented layers as well as from unconsolidated oxidized sediment for their compositions and NA capability concerning the contaminants. Operating conditions were a 15 kV accelerating voltage and a 20 nA beam current. Mineral standards were used and data were obtained using a TAP crystal for Si Kα, Al Kα, Mg Kα, Na Kα and As Lα analysis, a PET crystal for K Kα, Ca Kα, Pb Mα and S Kα, and a LiF crystal for Fe Kα and Zn Kα. Detection limits under the applied measurement conditions were 0.15 wt.% for As and Fe, 0.10 wt.% for Pb and Zn, and 0.05 wt.% for S, Si, Ca, Na, Al, K and Mg.

2.5. Microbiological methods

The number of metal sulfide oxidizing microorganisms was determined by the "most probable number" technique (MPN) as previously described by Schippers and Bosecker (2005). Two different media were used for enrichment of acidophilic Fe(II) oxidizers of the type *A. ferrooxidans* (Leathen et al., 1951) and S-oxidizers of the type *Acidithiobacillus thiooxidans* (Starkey, 1925). The concept of the MPN is described elsewhere (Cochran, 1950). Two grams of tailings material was suspended in 20 mL of medium for Fe(II) oxidizers without substrate (i.e. without Fe(II) sulfate). The suspension was incubated for 2 h on a rotary shaker at 130 rpm to detach cells from the substratum. The suspension was diluted in 10-fold steps to 10^{-8} and used to inoculate the MPN tubes. Cultures were incubated on a rotary shaker (130 rpm) in the dark at 30 °C for 3 weeks. The tubes were counted as positive if the pH had dropped below a value of 2 (S oxidizers) or if Fe(III) was formed (Fe(II) oxidizers).

3. Results and discussion

3.1. Internal structure of the tailings impoundment

The 2D cross section of electrical resistivity (Fig. 1b) shows four zones for the tailings impoundment: (i) the basement as indicated by resistivities of >1000 Ωm, (ii) the slimes area of the tailings impoundment as characterized by resistivities of <50 Ωm, (iii) the sandy tailings near the slopes with intermediate resistivities, and (iv) the sandy slopes with intermediate resistivity values as well. The different resistivities measured for the central part (slimes area) and the edges and slopes of the dam are explained by a particle-size induced difference in water content and due probably to elevated ion contents of the solution in the capillary fringe (Furche et al., 2007). The grain size distribution (Fig. 2b) indicates that the central part mainly consists of silt and fine sand, and that

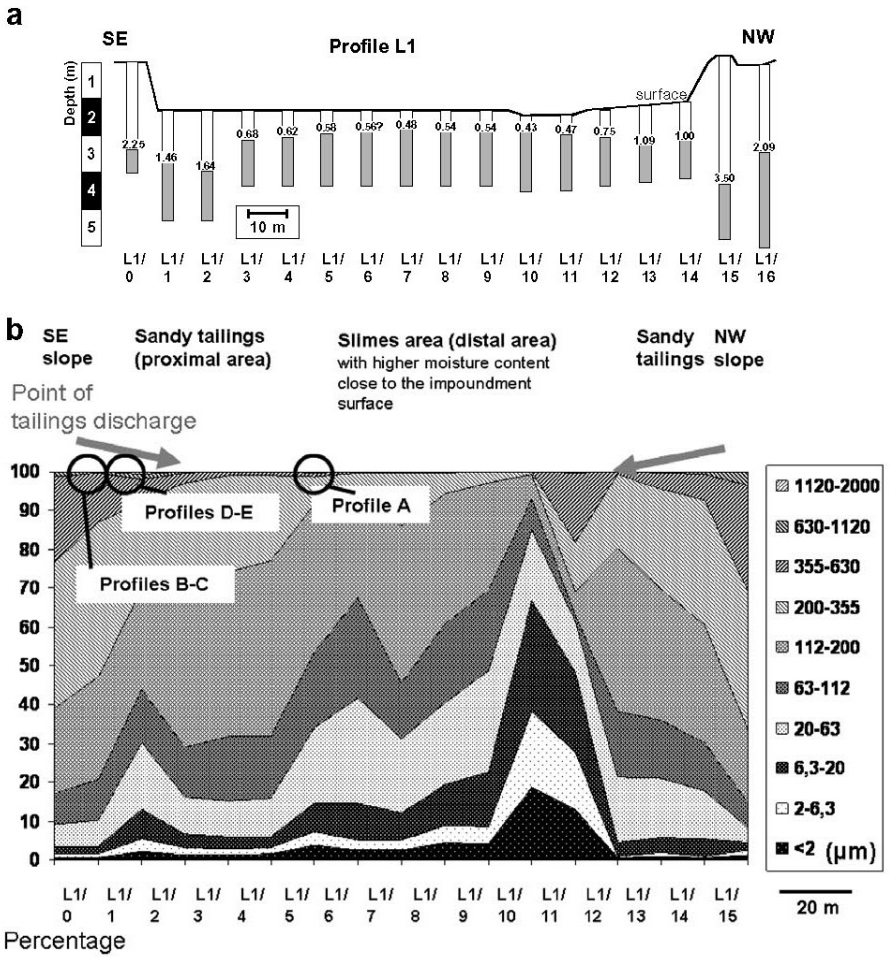

Fig. 2. (a) and (b) SE-NW drilling stock profile across the Muenzbachtal tailings impoundment. (a) The depth of the transition between the unaltered material (grey part of column) and the oxidized zone (unfilled upper part of column) is added for each location. (b) Grain size distribution of unaltered material at each location (depth for samples L1/1 14: ~1.6 1.8 m); average of duplicate measurements is plotted.

the edges and slopes of the dam mainly consist of fine to medium graded sand.

Numerous delta systems overlie each other due to the spill displacement along the rims of the dam. Mineral segregation resulting from this process favoured the development of heavy mineral rich (e.g. sulfides) layers due to sedimentary enrichment processes. The loamy parts of the tailings impoundment are characterized by graded bedding on cm-scale. The sandy tailings locally show a strong variability caused by complex depositional patterns. This variability is characterized by (i) interlayered packages with different alteration behaviour, (ii) the presence of (locally multiple) thin silt layers, and (iii) the occurrence of thin sulfide-enriched layers or lenses, which may, in the case of extremely sulfide-enriched layers, result from malfunctions during processing of the ore.

The slimes area and sandy tailings show differences concerning the depth of the oxidation front and the development and extent of the cemented layers and hardpans formed. The depth of the oxidation front was <0.4 to ~0.5 m for the silty slimes. It increased to values between 1.6 and >3.0 m for the sandy tailings at the slopes of the dam (Fig. 2a). The transition between oxidized and unaltered zones is commonly marked by one or more thin greyish (weakly altered) silt layers. The specific surface areas of the material show an increase from values of ~2 to ~4 m^2/g in the unaltered material to values up to ~9 m^2/g in the oxidized zone (Jung, 2003).

3.2. Distribution of cemented layers and hardpans

At the edges of the slimes area, cemented layers were ⩽1 cm thick and their occurrence was essentially limited to a narrow zone between the unaltered and oxidized zones (e.g. profile A). Fig. 3 gives a depth profile for outcrop A (for position see Figs. 1a and 2b). The topmost part of profile A consists of ~0.10 m top soil as covering material, followed by a ~0.50 m thick sequence of oxidized sands with small lenses of weathered silt. Multiple, 2–5 mm thin, but more or less continuously developed cemented layers occur in the depth range between 0.57 and 0.65 m; they were always directly underlain by altered silt layers. At greater depth, this sequence was followed by several thin and essentially unaltered silt layers, with color changes from brown to grey in associated fine sand layers.

In the sandy tailings, multiple cemented layers and hardpans were observed. Fig. 4 gives examples of depth profiles which were situated in the sandy tailings at the edge of the tailings impoundment. Cemented layers occurred in the lower parts of the oxidized zone only. Hardpans were mostly formed in the upper part of the oxidized zone. Cemented layers and hardpans were present (i) as horizontal layers or (ii) as lens-shaped bodies (Fig. 4). Hardpan layers often were interbedded with <0.1 cm layers of loose material, forming packages ranging in thickness from <1.0 cm to ⩾20 cm. Lens-shaped hardpans may reach a thickness of ⩾30 cm as shown in Fig. 4 for profile B. Silt layers, or systems of fine

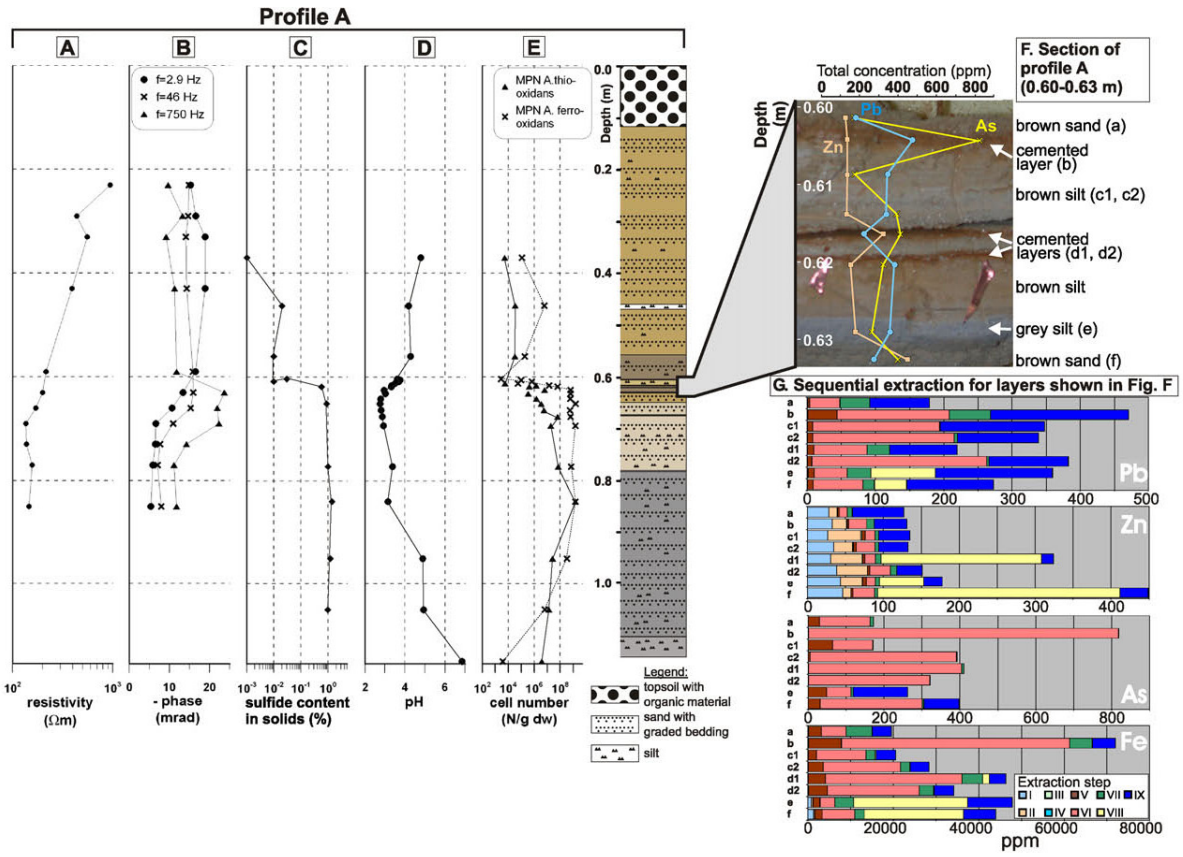

Fig. 3. (A) to (G) Geophysical, geochemical and geomicrobiological profiles through the oxidized zone in the marginal part of the slimes area (profile A). (A) and (B) Results of SIP measurements at different depth (resistivity; phase values for three frequencies). (C) Sulfide content in solids. (D) pH values. (E) MPN values for acidophilic Fe(II) oxidizers (*Acidithiobacillus ferrooxidans*) and sulfur oxidizers (*Acidithiobacillus thiooxidans*). (F) Section of profile A showing the zone where the oxidation front is located. (G) Release of Pb, Zn, As and Fe during sequential extraction for layers a to f as shown in the detailed profile (F). Extraction steps: I water soluble fraction; II exchangeable fraction; III carbonates; IV Mn oxides; V organic matter, hydrated Si gels; VI poorly crystalline Fe oxyhydroxides, jarosite-group minerals; VII crystalline Fe oxyhydroxides, jarosite-group minerals; VIII sulfides, alumosilicates; IX alumosilicates with sulfide inclusions; for more details see Table 1 and text.

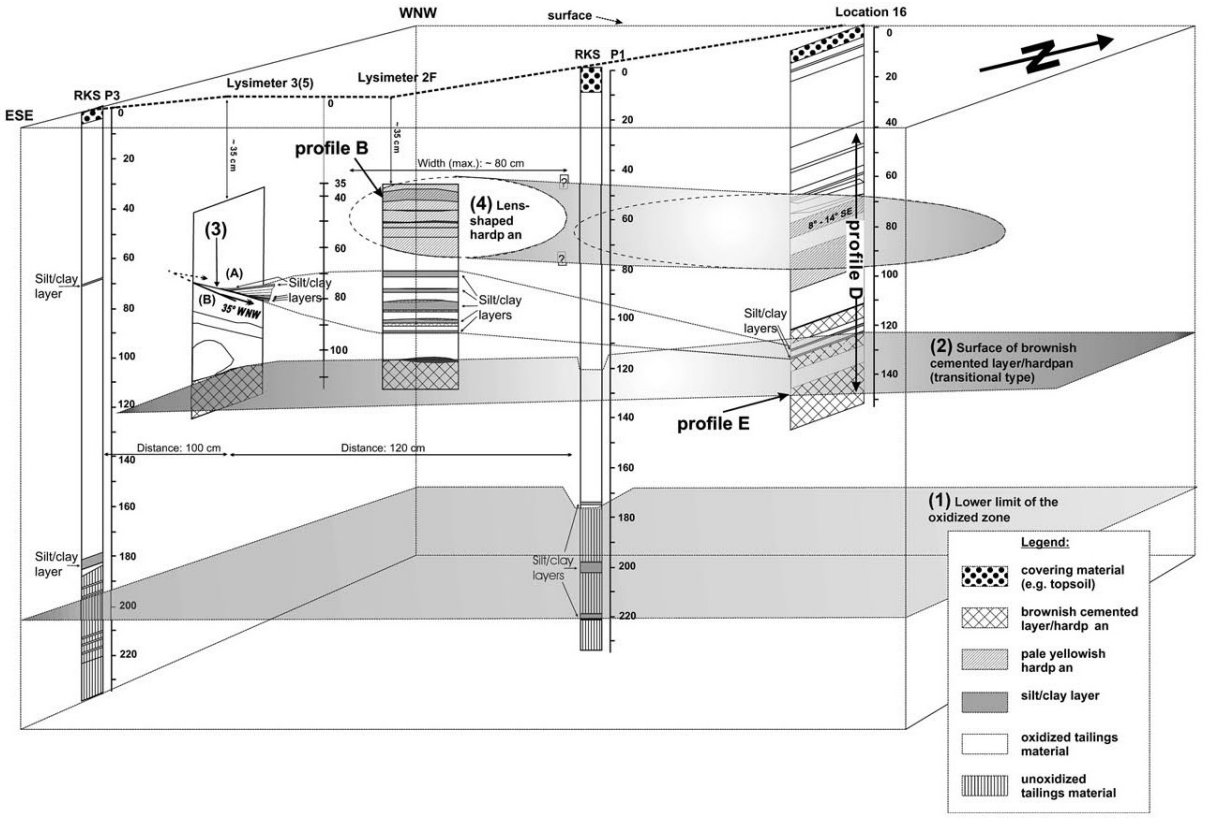

Fig. 4. Internal structure of the SE margin of the sandy tailings (Muenzbachtal tailings impoundment). Highlighted depositional and alteration structures are (from bottom to top of profile): (1) The lower limit of the oxidized zone; (2) The surface of a brownish cemented layer/hardpan as found in all outcrops; (3) The WNW dipping margin of the SE slope of the impoundment separating (A) the sandy tailings and (B) the slope; (4) A lens-shaped pale-yellowish hardpan overlying a system of altered silt/clay layers with brownish cemented layers. Depth is given in centimeters if not otherwise stated.

sand/silt layers, were usually present below cemented layer–hardpan sequences.

3.3. Mineralogical composition of the heaped material

3.3.1. Primary phases

Primary phases at the tailings impoundment include: fragments of the Freiberg gneiss (for modal mineralogy of the rock see e.g. Tichomirowa, 2001), hydrothermal vein quartz, barite, fluorite, pyrite, arsenopyrite, sphalerite and galena, minerals probably formed in the oxidation zone of the hydrothermal ore veins (e.g. anglesite, $PbSO_4$; cuprite, Cu_2O) and also subordinate technical products.

Unaltered silt layers were enriched in sulfides and strongly enriched in micas compared to unaltered sandy tailings. Heavy mineral-rich layers deposited directly on top of the silt were enriched in barite, sulfides and accessory minerals (e.g. zircon), especially in the outer (less fine-grained) zones of the slimes area. Unaltered sandy tailings were mainly composed of quartz, feldspar and fluorite, and contained only low concentrations of sulfides.

In the oxidized zone, arsenopyrite and sphalerite were completely replaced in all tailings materials, whereas pyrite and galena were often partly replaced only in heavy mineral-rich layers (arsenopyrite is more reactive than pyrite; e.g. Blowes et al., 2005). The only sulfide occasionally found in sandy materials is galena, commonly with rims of anglesite. Oxidation of galena is probably retarded due to the covering of their surfaces with oxidation products (e.g. Haubrich et al., 2000; Tichomirowa et al., 2002). Carbonates were scarce in the unaltered material and absent in the oxidized tailings materials. Chemical decomposition of mica and plagioclase was widespread and provided a source of Si, Al, K and Ca especially in the silt layers.

3.3.2. Secondary phases

Secondary phases most frequently formed in the oxidized zone of the tailings impoundment were gypsum, Fe(III) oxyhydroxides, and Fe(III) oxysulfates (i.e. jarosite). Additionally, Si-, Si–Fe-, or Fe–As-rich gels, alunite, native S, a ferric arsenate precipitate which shows a composition similar to scorodite, and clay minerals occurred. In the <2 μm fraction of a sample from profile A (Fig. 3), the following clay minerals were identified by XRD: kaolinite, an irregular illite-dominated illite(0.7)-smectite(0.3) mixed-layer clay, and possibly chlorite.

Thin layers of autochthonous gels encrusted reactive sulfides shortly after the first contact with rain water. These gels generally consisted of Fe(III)-rich, relatively S-poor and commonly Si-bearing phases. They outlined the contours (skins) of the replaced sulfide fragments (Rammlmair, 1996) or highlighted micro-cracks starting from the surface of the altered mineral grain (formation of "honeycomb-like" textures at originally sulfide-rich locations; Fig. 5b). The degree of fill of the space inside the "honeycombs" varies considerably (see below). Multiple thin layers of allochthonous gels agglutinated mineral fragments and dust in pore spaces or covered fluid channels with cemented material (Fig. 5a).

3.3.2.1. Gel-rich environments. In extremely reactive mineral-rich mine tailings layers, the pores of the material were almost completely filled with allochthonous gel (poorly crystalline) phases as a result of oxidative dissolution of reactive sulfide minerals (profile C; Fig. 5a). Water circulation, and also mobilisation and transport of ions were essentially restricted to a small number of remaining fluid channels (Fig. 5a).

Cemented tailings in such environments showed multiple layers of poorly crystalline Fe(III)–As–S

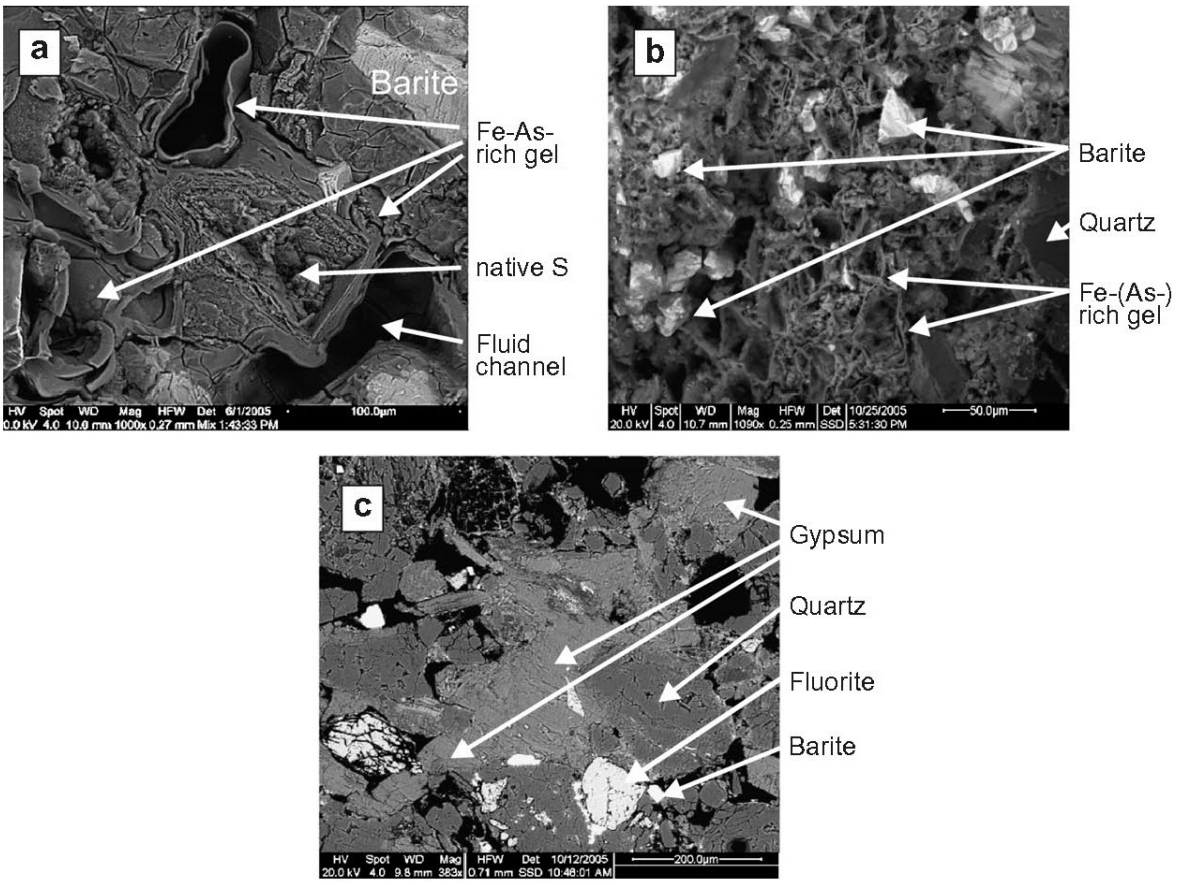

Fig. 5. (a) (c): BSE images of weathered tailings material from the Muenzbachtal tailings impoundment: (a) Fe As Si gel-rich cemented layer. Note the occurrence of a high percentage of allochthonous gel and a limited number of coated fluid channels (profile C; ESEM-mixed image). (b) "Honeycomb-like" texture of autochthonous Fe As Si gels in a moderately gel-bearing cemented heavy mineral-rich layer overlying an altered silt layer (profile A). (c) Sediment cemented with gypsum (profile C).

Table 3
Results of electron microprobe analyses of jarosite-group minerals and poorly crystalline phases from cemented mine tailings materials (Muenzbachtal tailings impoundment, profiles C and D)

	Jarosite								Embedding material		Poorly crystalline phases (Fe As Si gels)						Arsenopyrite
	In sulfate-rich hardpans[a]				In packages with inter-laminated mica and heavy mineral-rich layers and lenses[b]						In sulfate-rich hardpans[a]		In a gel-rich cemented layer[c]				
	J19	J20	J22	J24	J34	J36	J39	J40			X13	X16	X1	X15	X17	X18	
Al_2O_3, wt.%	b.d.	b.d.	0.06	b.d.	b.d.	b.d.	0.05	b.d.	b.d.	Al_2O_3, wt.%	0.36	0.58	b.d.	0.13	0.06	b.d.	
Fe_2O_3	42.45	45.90[d]	42.36	42.18	39.26	38.34	39.88	37.51	b.d.	Fe_2O_3	29.34	48.17	39.70	35.84	39.71	40.34	
MgO	0.10	b.d.	0.10	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	MgO	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	
CaO	0.11	0.07	0.08	0.09	0.24	0.11	0.23	0.14	b.d.	CaO	0.12	0.24	0.16	0.14	0.14	0.28	
Na_2O	1.69	1.53	1.87	2.21	0.19	0.15	0.15	0.21	b.d.	Na_2O	0.16	0.23	b.d.	0.06	b.d.	b.d.	
K_2O	3.81	4.00	3.72	3.90	4.24	4.13	4.81	4.46	b.d.	K_2O	0.26	0.51	b.d.	b.d.	b.d.	b.d.	
PbO	1.74	0.86	0.69	0.52	8.09	7.53	7.91	6.64	b.d.	PbO	b.d.	0.87	1.66	1.43	1.69	1.97	
SO_3	28.18	30.55	28.50	28.00	27.41	26.39	27.48	27.19	0.23	ZnO	b.d.	b.d.	b.d.	b.d.	b.d.	b.d.	
As_2O_5	0.18	0.16	0.15	0.25	1.47	1.08	1.91	1.25	b.d.	SO_3	3.94	8.57	5.79	5.97	6.72	7.42	
H_2O	9.82	10.81	10.12	9.69	10.92	10.10	10.99	11.23		As_2O_5	2.40	3.73	40.33	41.72	42.11	44.23	
										SiO_2	4.29	10.67[e]	2.72	4.62	0.34	0.50	
Total	88.08	93.89	87.70	86.92	91.85	87.88	93.45	88.68		Total	40.91	73.58	90.43	89.94	90.84	94.85	
Fe^{3+}	3.00	3.00	2.97	3.00	2.77	2.83	2.78	2.68		Fe	34.3	34.3	34.3	34.3	34.3	34.3	34.3
Mg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		As[f]	2.6	2.5	32.5	37.2	33.9	35.1	46.0
Ca	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		As loss[g] (%)	*94*	*95*	*29*	19	26	*24*	
Na	0.31	0.26	0.34	0.40	0.03	0.03	0.03	0.04		S[f]	0.9	3.4	1.9	1.7	2.2	2.4	19.7
K	0.46	0.44	0.44	0.47	0.51	0.52	0.56	0.54		S loss[g] (%)	*95*	*83*	*90*	*91*	*89*	*88*	
Pb^{2+}	0.04	0.02	0.02	0.01	0.20	0.20	0.20	0.17									
H_3O^+	0.15	0.26	0.18	0.11	0.06	0.05	0.01	0.08									
$SO_4 + AsO_4$	2	2	2	2	2	2	2	2									

b.d. below LOD.
[a] Sample FB(05)16-KP11.
[b] Sample FB(05)16W.
[c] Sample FB(05)14-KP2.
[d] Value corrected for intergrown Fe oxyhydroxide.
[e] Si value probably contaminated from underlying quartz grain.
[f] As and S concentrations normalized to stoichiometric FeAsS mineral composition.
[g] Estimated maximum loss of As and S during oxidative weathering of sulfides assuming ideal FeAsS composition of original sulfides (not arsenical pyrite).

phases and aggregates of native S inside the "honeycombs"; jarosite crystals were absent here. Up to several wt.% of Si, which was distributed heterogeneously, occurred in the outermost autochthonous gel rims (skins) only (analyses X1, X15 and X18 in Table 3), whereas the internal layers with low crystallinity were generally low in Si (e.g. analysis X17 in Table 3). This observation points to a development of Si networks with polyvalent cations in gel layers accessible for Si-bearing pore solutions (cf. formation of stable silica rock coating in deserts; Kolb et al., 2004; Perry et al., 2006), in contrast to the gels formed somewhat later in the interior of the replaced sulfide grains. Such a formation of "Si glues" may have contributed to the good stability of first water contact gels (also in sulfate-rich, gel-poor environments; e.g. analysis X13 in Table 3) during subsequent mineral transformation reactions.

3.3.2.2. Gel-poor environments. Water circulation took place almost unhindered in generally coarser, less reactive material-rich and, subsequent to oxidative weathering, less gel-rich tailings using the remaining high active porosity. In contrast to above, the "honeycombs" were commonly partially filled only with jarosite-group minerals here, with or without crystalline Fe(III) oxyhydroxides present in addition. The jarosite covered surfaces of silicates, barite and fluorite also; it formed overgrowths on thin rims of Fe- or Si-rich allochthonous gels. The jarosite may have formed via precipitation from a supersaturated pore solution in decreasing pH conditions (e.g. Nordstrom, 1977; Dutrizac and Jambor, 2000), or by mixing of Fe–S-rich solutions from channels in the gel with K-rich pore solutions resulting from a somewhat later dissolution of aluminosilicates. A transformation of unstable (Si-poor; Fe-rich) S-poor gel into jarosite with a supply of K and S also can not be excluded.

3.4. Detailed characterization of cemented layers and hardpans

3.4.1. Types, mineralogical compositions and reduction of pore space by cementation

Cemented layers were gel-rich (bluish-grey colored) or moderately gel-bearing (mostly reddish-brown colored). Pale yellowish hardpans were not enriched in gels. A transitional type between cemented layers and hardpans, possibly cemented layers overprinted by precipitation of phases at the capillary fringe, was also reddish-brown colored. Interestingly, gel-rich cemented layers and hardpans were commonly found to occur in close spatial relationship (see discussion further down).

An example of an extremely gel-rich *cemented layer* was found in profile C (Fig. 6Aa), located in the sandy tailings close to the SE slope of the dam. Within an ~2 mm thin heavy mineral-rich layer, ~45% of Fe-rich secondary minerals including >30% of Fe–As–Si gels, were formed (Fig. 5a; Table 4). This gel-rich cemented layer is directly overlain by an essentially sulfide- and gel-free, gypsum-rich, jarosite-poor and ~2 mm thick hardpan with 16–27% of gypsum (Fig. 5c; Table 4).

The most important constraint on the remaining open pore space in the cemented layers is given by the amount of gel phases present; gels reduce the proportion of (connected) macro-pores much more efficiently than crystalline mineral phases in the studied samples. In the extreme case of profile C, the filled area was estimated to be ~99% in the gel-rich cemented layer (Table 4; Figs. 5a and 6Aa).

Hardpans were characterized by an enrichment in secondary gypsum and jarosite-group minerals making up from ~5 to sometimes >25% of the ROIs in studied thin sections (Tables 4 and 5; jarosite was attributed to the secondary Fe-rich phases). Gypsum occurred in anhedral aggregates or as crystals, whereas the jarosite-group minerals were microcrystalline, granular or formed micro-crusts.

The elevated concentrations of hydronium $(H_3O)^+$ in the jarosite-group minerals in sulfate-rich hardpans (Table 3; Fig. 7), as inferred from the analyzed low alkali contents in the minerals, indicate a generally low availability of Na and K during jarosite precipitation or may result from an extremely rapid weathering of the sulfides (e.g. Dutrizac and Jambor, 2000). In contrast, jarosite-group minerals, formed in interlaminated (a) mica-rich and (b) heavy mineral-rich layers and lenses (profile D – sequence (iii); Fig. 8d) are characterized by higher K and insignificant $(H_3O)^+$ concentrations (Table 3; Fig. 7). Small Fe(III) excess in the uncorrected jarosite formula probably results from an intergrowth of tiny grains of Fe(III) oxyhydroxides with the jarosite (corrected for analysis J20 in Table 3).

In contrast to gel-rich cemented layers, significant portions of partially filled pore areas (~12–>15%; edge agglutinated fragments/grains) and open pore areas (~3 to ~10%) were found for sulfate-rich hardpans without significant gel formation (e.g. profile E; Table 5; Fig. 6B).

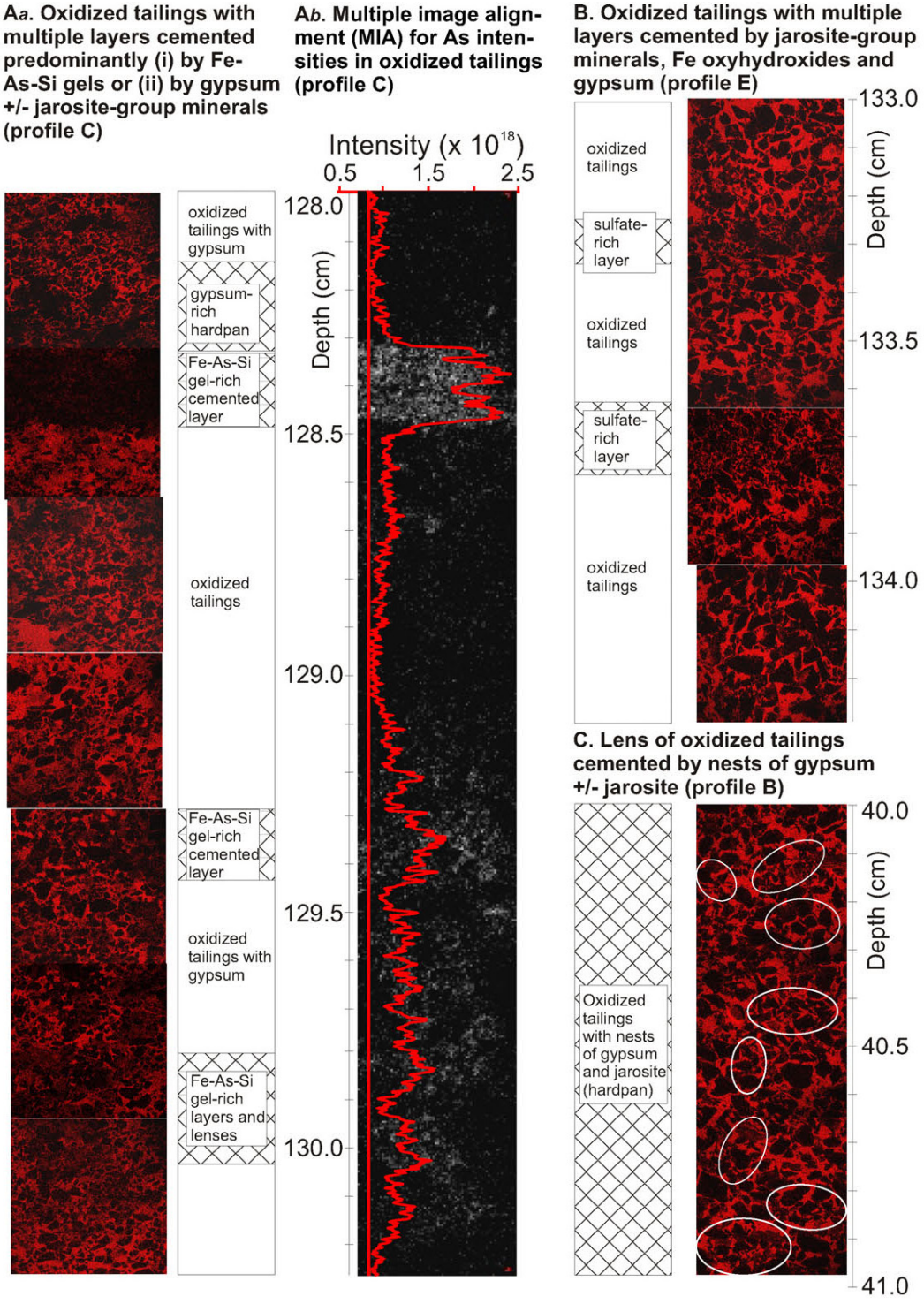

Fig. 6. (A) (C) Results of the image processing for cemented layer/hardpan sequences from the sandy tailings within the Muenzbachtal tailings impoundment. Figures Aa, B and C show results of mapping for carbon (red), which is present in significant amounts in the embedding medium only (excluding small scale organic material). Therefore, it gives a good constraint on the distribution of filled, partially filled and open pores in the sediments. See text for details.

Table 4
Frequency distribution of primary and secondary mineral phases as well as of open and partially filled pores for profile C (depth: 128 130 cm) containing oxidized tailings, cemented layers and hardpans

Section/image	Filled area	Pore area		Primary mineral phases					Secondary mineral phases		Total[a]
		Partially filled	Open	Barite	Quartz	Silicates (excl. quartz)	Sulfides (pyrite)	Fluorite	Gypsum	Fe-rich phases (excl. sulfides)	
Oxidized tailings with gypsum/I-2	58.4	18.6	14.5	21.7	12.3	13.3	0.1	8.3	15.6	4.3	90.1
Gypsum-rich hardpan/I-2	80.6	12.1	3.3	18.7	12.7	15.3	0.2	2.6	27.0	4.1	83.9
Gel-rich cemented layer/I-1	99.2	0.6	<0.2	29.3	1.4	4.5	6.4	2.0	<0.1	46.2	89.8
Oxidized tailings/I-1	34.6	19.7	32.0	17.4	6.3	6.8	0.1	0.5	5.3	0.9	69.3
Oxidized tailings/I-3	55.8	17.6	17.0	21.9	13.7	9.5	1.2	1.2	7.1	5.7	77.3
Oxidized tailings/I-4	44.0	17.1	28.3	22.5	10.5	9.1	<0.1	0.7	0.7	8.5	80.3
Gel-rich cemented layer/I-5	72.6	11.9	10.2	26.3	5.8	15.2	1.1	2.0	0.7	27.8	89.1
Partialy cemented oxidized tailings/I-5	62.3	16.6	12.6	23.9	11.1	12.7	1.9	0.5	5.6	2.6	70.9
Gel-rich layers and lenses/I-6 and I-7	69.8	18.5	5.4	29.5	8.6	7.9	0.8	0.7	5.3	13.1	71.3
Oxidized tailings/I-7	41.5	21.3	25.0	32.1	8.4	6.7	0.1	3.0	0.7	5.5	81.5

Data from element distribution mapping image processing technique; data in area%.
[a] Total was calculated from the area% of all minerals and of the open pore areas.

Table 5
Frequency distribution of primary and secondary mineral phases as well as of open and partially filled pores for profile E (depth: 133 134 cm) containing oxidized tailings and sulfate-rich hardpan layers

Section/image	Filled area	Pore area		Primary mineral phases				Secondary mineral phases		Total[a]
		Partially filled	Open	Barite	Quartz	Silicates (excl. quartz)	Fluorite	Gypsum	Fe-rich phases (excl. sulfides)	
Oxidized tailings/I-1	52.3	11.6	29.6	0.3	25.4	18.4	1.2	2.2	1.4	78.5
Sulfate-rich hardpan/I-1	75.0	12.2	7.0	2.3	15.8	38.8	4.8	4.7	17.3	90.7
Oxidized tailings/I-2	53.1	11.1	29.3	0.5	23.9	22.4	1.4	0.8	4.1	82.4
Sulfate-rich hardpan/I-3	68.6	15.2	9.9	2.7	13.8	24.7	3.2	4.4	20.1	78.8
Oxidized tailings/I-3	57.2	12.4	23.7	0.1	14.6	25.3	1.7	2.9	12.3	80.6
Oxidized tailings/I-4	65.5	10.5	18.3	1.8	23.8	26.1	1.2	4.3	4.7	80.2

Data from element distribution mapping image processing technique; data in area%.
[a] Total was calculated from the area% of all minerals and of the open pore areas.

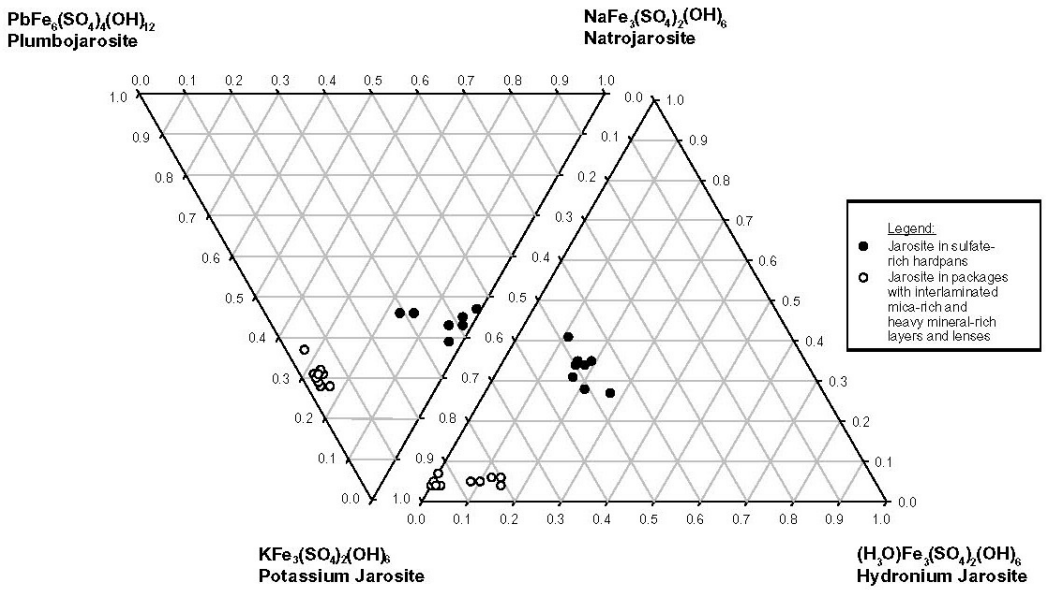

Fig. 7. Composition of jarosite-group minerals in different types of cemented layers/hardpans.

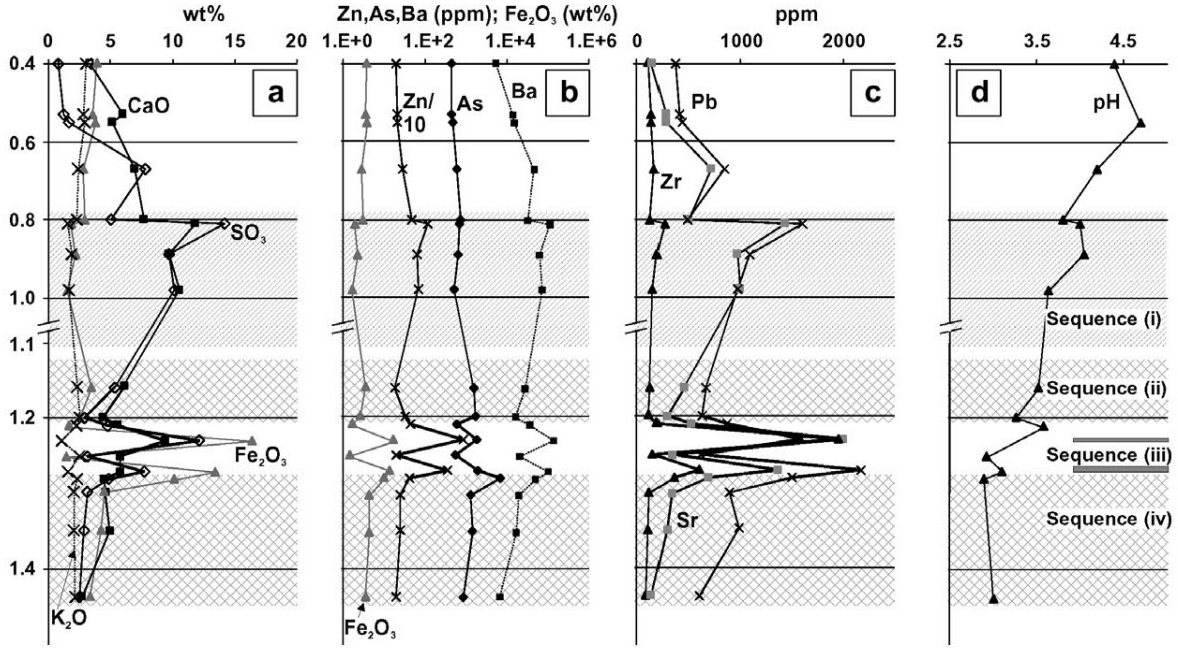

Fig. 8. (a) (d) Geochemical profile (profile D) through a sequence of oxidized tailings with and without cementation by secondary phases. The measured pH values (paste pH) for the sediments are shown in (d).

3.4.2. Geochemical characterization

Fig. 8 shows the chemical composition of sandy tailings from profile D as a function of depth (Figs. 1a and 4). Fig. 8d illustrates that the paste pH values gradually decrease from top (pH ~4.5) to bottom (pH ~2.9) with their minimum in the reddish-brown sequences. The pH of the unaltered material at greater depth was ~6.4 to ~7.5 (this study and Jung, 2003). In profile D, four sequences are of special importance:

Sequence (i) is a pale-yellowish sequence of medium grained sand layers located at depths between ~0.8 and ~1.1 m. It consists of layers and lenses of variable thickness, which are either consolidated to hardpans or are unconsolidated. Sequence (i) is characterized by a strong enrichment in CaO, SO_3, Sr, Pb and Ba compared to the overlying, unconsolidated material. Microscopic analysis of thin sections indicated ~3 to ~5 area% of gypsum and ~3 to ~11 area% of secondary Fe-rich phases in the ROIs. Sulfur is predominantly bound in gypsum and barite. The high S values for this sequence cannot be generated by in situ oxidative weathering of Fe(II) sulfides only due to low Fe_2O_3 values; S was probably transported from oxidized sulfide-rich tailings (e.g. the underlying sequence (iii)).

Sequence (ii) is a strongly consolidated dark reddish-brown sequence (transitional type of consolidated layer; see Section 3.4.1) of predominantly medium grained sands at depths between ~1.1 and ~1.2 m. It is characterized by higher Fe_2O_3 and As concentrations, but significantly lower CaO, SO_3, Sr and Pb values compared to sequence (i). These data suggest a lower abundance of gypsum and a higher abundance of secondary Fe(III)-rich phases compared to sequence (i). This sequence thins out at locations where the underlying sequence (iii) is missing.

Sequence (iii) consists of interlaminated sand/silt layers of dark grey sand and pale-yellowish silt at depths between 1.21 and 1.27 m. The silt layers were $\leqslant$1 mm thick. These layers were weakly agglutinated in the field, however, drying resulted in their consolidation, which is explained by precipitation of euhedral gypsum. Sequence (iii) is characterized either by strong enrichment in Fe_2O_3, SO_3, Zn, As, Ba, Zr, Pb and Sr in the thin greyish layers, or by increased K_2O and CaO, but relatively low Fe_2O_3 values, in the pale yellowish silt layers. These data are consistent with petrographic and XRD results, which indicate heavy mineral enrichment (e.g. barite, zircon, pyrite) in the sand layers, and increased concentrations of aluminosilicates (micas $\pm$ Ca-bearing plagioclase) in the silt layers. Sequence (iii) shows a low occurrence of Fe–As–Si-rich gels and a high enrichment in Fe hydroxysulfates. Large aggregates of jarosite-group minerals occurred in bended layers outlining the weathered sheet structure of the replaced micas. They also occurred in interstitials between mineral grains or inside scarce "honeycombs" in these layers.

Sequence (iv) is another strongly consolidated dark reddish-brown sequence (transitional type), which occurs at depths between ~1.3 and at least 1.5 m. This sequence is very similar in appearance to sequence (ii).

3.4.3. Metal sulfide oxidizing microorganisms

Cell numbers of Fe-oxidizing and S-oxidizing microorganisms were investigated at different depths in profile A (Fig. 3E). A pronounced change in cell numbers of metal sulfide oxidizing bacteria in accordance with lithology was observed. Cell numbers were between 10^4 and 10^7 cells/g dry weight (dw) in the zone of oxidized tailings, while they increased to 10^9 cells/g dw below the upper cemented layer in the sulfide-bearing zone. The relatively low cell numbers for the upper cemented layer may be explained by the remaining relatively low sulfide content of this layer (Fig. 3C), and probably indicates a downward movement of the oxidation front with time. Cell numbers remained at high levels down to ~1 m, and decreased in the deepest grey silt layer. Cell numbers of acidophilic Fe(II)-oxidizing microorganisms (*A. ferrooxidans*-like bacteria) were generally higher than those of acidophilic S-oxidizing microorganisms (*Acidithiobacillus thiooxidans*-like bacteria), except for depths below ~1 m. The maximum numbers of Fe-oxidizing microorganisms are among the highest detected in sulfidic tailings (Southam and Beveridge, 1992; Blowes et al., 1998; Diaby et al., 2007).

In the oxidized zone of the sandy tailings, cell numbers for Fe(II)-oxidizing microorganisms (maximum value: ~10^7 cells /g dw) were distinctly lower than measured for profile A in the slimes area. The only location with significant numbers of living cells up to 10^7 cells/g dw (*A. ferrooxidans*) was a grey silt layer underlying a cemented package of sandy material (data not shown).

3.4.4. Geophysical characterization

Profile A was studied by 11 SIP measurements at different depths (Fig. 3A and B). The amplitude of resistivity shows a continuous decrease due to increasing water contents, which is intensified by decreasing grain sizes. The shapes of the phase spectra at 2.9 and 750 Hz showed a remarkable change at the position of the cemented layers. This corresponds to a change in sulfide content in the same depth range (Fig. 3C). The spectral electrical data indicate another change at a depth of ~0.7 m, which cannot be explained by the similar sulfide contents

over the depth range ~0.65 to ~1.0 m; however, it could be related to increased surface areas of the sulfides in the narrow zone from ~0.65 to ~0.70 m as a result of strong oxidative weathering.

The outcrops in the sandy tailings (e.g. profile D; Fig. 4), did not show any change in the phase spectra patterns. All measured phase angles are lower than 10 mrad over the total measured frequency range (results not shown). This finding is consistent with the fact that these outcrops are located completely in the strongly weathered zone (essentially no sulfides present).

3.5. Simplified model for cemented layer and hardpan formation in the Muenzbachtal tailings impoundment

The extent of cemented layer and hardpan formation in mine tailings primarily depends on (i) the amount of reactive materials present and their accessible surface areas (selective dissolution of phases or particles during alteration might enhance the reactive surface area up to a factor of 10,000; Rammlmair, 2002), (ii) the presence of relatively impervious layers in direct proximity of the reactive phases, and (iii) mass transport processes that allow selective leaching of reactive phases from one layer and agglutination of mineral grains in another layer. The evolution of micro-porosity and micro-channels due to selective leaching of reactive phases (e.g. Rammlmair, 2002) and agglutination of mineral grains plays a key role in mass transport and precipitation processes.

Cemented layers occurred in originally reactive material-rich heavy mineral layers overlying fine-grained weathered silt layers or related to interlaminated reactive material-rich sequences, suggesting a genetic relationship between these layers during weathering. A simplified model for the formation of the sequence of cemented layers and hardpans as typically found in the Muenzbachtal tailings impoundment is outlined in Fig. 9 and discussed in detail below.

Fig. 9(1) shows a typical sequence of layers before weathering. From top to bottom can be recognised: fine to medium grained sands, a heavy mineral-rich layer, a silt layer, followed by multiple interlaminated sand and silt layers. The major primary reactive phases within the heavy mineral-rich layer are sulfides, which were the main sources of S, Fe, As, Zn and Pb. Within the silt layers, the main reactive mineral in addition to the sulfides is biotite, which is a major source of K, Fe, and Si. The main Ca source is Ca-bearing plagioclase (cf. Bhatti et al., 1994), which occurs in the sand layers, and in the silt layers as well, but in lower concentrations. Mineral processing solutions contained Ca hydroxide and minor Ca hypochlorite, which may, to a small degree, have also been deposited in the tailings impoundment. A re-dissolution of highly soluble secondary phases could be considered as a source of elements as well.

The most significant weathering process is the oxidation of metal sulfides, which produces large amounts of sulfuric acid and mobile Fe(II). Eqs.

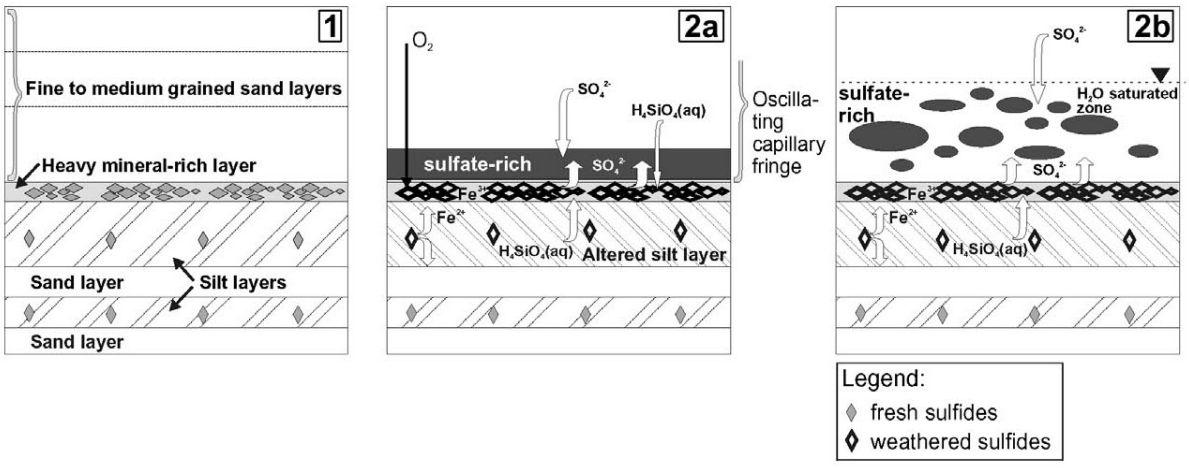

Fig. 9. Simplified model for formation of hardpan sequences within the tailings impoundment Muenzbachtal. (1) Schematic profile before weathering of tailings material. (2a and b). Profiles after weathering of reactive phases and formation of gel-rich (light grey) and gel-poor (dark grey) cemented layers and hardpans. See text for further details.

(1)–(4) may characterize the oxidation of arsenopyrite, which is a very complicated process involving many individual reaction steps. Reactions (1), (2) and (4) are usually bacterially mediated (e.g. Tuovinen et al., 1994; Morin and Calas, 2006).

$$FeAsS_{(s)} + 11/4O_2 + 3/2H_2O \Longleftrightarrow Fe^{2+} + SO_4^{2-} + H_3AsO_3 \quad (1)$$

$$Fe^{2+} + 1/4O_2 + H^+ \Rightarrow Fe^{3+} + 1/2\ H_2O \quad (2)$$

$$Fe^{3+} + 3H_2O \Longleftrightarrow Fe(OH)_{3(s)} + 3H^+ \quad (3)$$

$$H_3AsO_3 + 1/2O_2 \Longleftrightarrow H_2AsO_4^- + H^+ \quad (4)$$

Iron- and S-oxidizing microorganisms have been shown to play a significant role in catalyzing these reactions (Fig. 3). Field eluates prepared from cemented heavy mineral-rich layers and underlying silt layers (profile A) yielded pH values between 2.5 and 4.0 and dissolved Fe(II) concentrations up to ~250 mg/L. These conditions allow transport of Fe and SO_4 towards the redox interface between the reduced silt on the one hand, and the water-unsaturated sand layers on the other hand (Fig. 9(2a)). Subsequent oxidation of Fe(II) to Fe(III) leads to the precipitation of amorphous Fe-oxyhydroxides at this interface (Eqs. (2 and 3)). Increased oxidation of As(III), formed by arsenopyrite oxidation (Eq. (1)), to As(V), was observed in experiments with increased Fe(III) concentrations (Yunmei et al., 2004). However, As(III) oxidation (Eq. (4)) may also be catalyzed by microorgansims, e.g. *Thiomonas* sp. (Bruneel et al., 2003; Morin and Calas, 2006). The low As(III)/As(V) ratios (0.03 to mostly $\leqslant$0.11; only one value of 0.19) found for the oxidized or partly oxidized tailings materials in profile A (Fig. 3) may, therefore, indicate an increased activity of the aforementioned bacteria for arsenite oxidation at this location.

In micro-domains, the released acid (Eqs. (3) and (4)) may result in lower pH values than measured for the bulk material (pH ~ 2.5). In sulphide-rich micro-domains, pH values <1 were measured in biofilms (Vlasceanu et al., 2000). Under these conditions aluminosilicates like mica and plagioclase would become increasingly unstable, causing a stronger release of Si, Al, K, Ca and F into the pore solutions (e.g. Blum and Stillings, 1995), but also resulting in an effective buffering of the pH. Precipitation of Fe(III) phases would take place in adjacent sand or silt layers in the case that the sulphide-rich layers are poor in aluminosilicates.

The initial precipitates after oxidative weathering processes, which cover the surfaces of the weathered minerals and of adjacent sand grains, are usually poorly crystalline (gel-like) because such phases have lower interfacial free energies than more crystalline products (Steefel and van Cappellen, 1990). These covers may also include portions of extra-cellular polymeric substances (EPS) produced by the Fe-oxidizing bacteria (e.g. Schultze-Lam et al., 1996; Gehrke et al., 1998; Sand et al., 2001; Harneit et al., 2006) with accumulated metals (e.g. Fe(III) complexes). The amounts of EPS as produced by growth of one strain of *A. ferrooxidans* on pyrite was 2.76 mg per 10^{10} cells (Gehrke et al., 1998). Assuming that the Fe-oxidizing bacteria produce an equal amount of EPS in nature, the concentration of EPS would be considerable (~0.3 mg/g dw) at the oxidation front of the tailings material in profile A (where 10^9 cells/g dw were detected; Fig. 3E). The initial precipitates are expected to be composed of Fe(III) oxyhydroxides and hydroxysulfates with significant H_2O, adsorbed As and Ca, as well as Si at strongly varying concentrations. Subsequent drying would result in the formation of stable Fe–Si cemented layers by loss of water molecules. Furthermore, Si-dominated gels were observed as thin coatings on mineral grains in oxidized mine tailings.

Fig. 9(2a) shows the generation of a sulfate-rich hardpan overlying the gel-rich cemented layer. This hardpan mainly consists of gypsum and jarosite-group minerals, and is essentially gel-poor. Under influence of oscillating capillary fringes on top of the semi-impermeable cemented layer, transport of significant portions of SO_4 from the layers with intense sulfide oxidation into the hanging sulfide-poor, intermediate-grained sand layers, has to be assumed. Additional sources of SO_4 are the overlying oxidized sand layers. From mineralogical observations it is inferred that the gypsum-rich hardpans were mainly formed somewhat later than the gel-rich cemented layers (in a continuous process). This inference is in agreement with the experimental data of Bhatti et al. (1994), who explained a similar observation by a retarded release of Ca from the plagioclase feldspar in his experiments. Under special conditions, i.e. the formation of basins with widely oscillating capillary fringes and the formation of preferential solution pathways in the sandy matrix on top of semi-impermeable silt/clay layers, gypsum will not precipitate in continuous layers, but in irregularly distributed nests over a much wider cemented zone (Fig. 9(2b)).

After completion of the weathering processes, the topmost greyish, sulfide-bearing silt/clay layer has been transformed into a bleached, sulfide-free and clay-mineral enriched silt layer, overlain by a cemented heavy mineral-rich layer with agglutinating Fe(III) oxyhydroxide-silicate coatings. The interface between this cemented layer and the coarser-grained, water-unsaturated sand layer acts as a contact surface for dissolved O_2. A sulfate-rich hardpan that has formed somewhat higher in the profile, completes the typical sequence.

3.6. Contaminant attenuation adherence in oxidized mine tailings

Contaminants that may pose serious pollution threats to the water systems at the Muenzbachtal site are mainly As, Zn and Pb. Table 3 displays the concentrations of contaminants and major elements in jarosite-group minerals and poorly crystalline phases collected from hardpans and cemented layers from the Muenzbachtal site. Contaminant concentrations in gypsum were below the limit of detection (LOD) for both gypsum-rich hardpans and cemented layers.

Zinc concentrations were below the LOD in analysed secondary minerals and gels. Zinc was not fixed effectively in the precipitated secondary mineral phases and was lost in large parts to the groundwater. Data of Martin et al. (1994) confirm a similar scenario for the oxidative weathering of sulfides from the Freiberg mine workings and estimates its contribution to the Zn load of the Elbe river to be ~30%.

Table 3 shows that the analyzed crystals of jarosite-group minerals always contained As and Pb. Jarosite-group minerals may act as a sink for trace elements by adsorption/substitution/co-precipitation processes (e.g. Scott, 1987; McGregor and Blowes, 2002). Lead probably substitutes for K (Dutrizac and Jambor, 2000) and AsO_4^{3-} for SO_4^{2-} in the jarosite structure (Paktunc and Dutrizac, 2003). All analysed jarosite-group mineral grains from packages with mica- and heavy mineral-rich layers (profile D – sequence (iii); Fig. 8; analyses J34–J40 in Table 3) contained significantly higher Pb and As concentrations than the studied grains (analyses J19–J24 in Table 3) derived from sulfate-rich hardpans.

The highest As concentrations were found in gel phases (Table 3). The As and Pb concentrations in gels from the extremely gel-rich cemented layer in profile C were always significantly higher (by a factor of >10 for As) than in gels from sulfate-rich and gel-poor hardpans. The high As/Fe mole ratios (>0.15) support formation of FeOHAs (amorphous/poorly crystalline Fe(III) hydroxy arsenate) minerals (e.g. Carlson et al., 2002; Morin et al., 2003). These poorly crystalline Fe–As-rich phases contain significantly lower S concentrations than the jarosite-group minerals and would have chemical formulas that are rather variable (commonly with SiO_2 contents; Table 3) and different from that of schwertmannite ($Fe_8O_8(OH)_{6-}SO_4 \cdot nH_2O$; e.g. Murad et al., 1994). However, the presence of portions of schwertmannite in the gel rims would not contradict the measured pH conditions in the zones with hardpan formation (pH: ~3 or slightly lower), which fit well with the experimental determined formation conditions for schwertmannite (pH: ~3; e.g. Bigham and Nordstrom, 2000). Furthermore, XRD or EM analysis did not indicate a high abundance of scorodite or of similar Fe(III) arsenate precipitates to be intergrown with the gel at the Muenzbachtal site, despite pH conditions which fit the range for precipitation of poorly crystalline scorodite as found by Langmuir et al. (2006; pH 2–3). Goethite could also not be observed using XRD.

In Table 3, the As and S concentrations of the gels were normalized to the stoichiometric composition of arsenopyrite. This procedure is justified by the shapes of the measured gel aggregates in the gel-rich cemented layer; these clearly confirm arsenopyrite as the main sulfide replaced here. The results of the calculation show that the As loss from the solids to the migrating pore water during oxidative weathering of the sulfides was between <20% and 30%, whereas the loss of S seems to be much higher (~90%). However, portions of the S are present as accumulated native S in the central parts of the replaced grains. In sulfate-rich hardpans, the estimated As loss from the oxidized sulfides to the migrating pore water could have been higher (up to 95%) than at gel-rich locations, and maybe similar to the estimated values for the S loss (data for first contact gels from "honeycomb-like" textures – see Table 3).

The contents of Zn, As and Pb in the dam pore waters and sediment components were determined by sequential extractions. Fig. 3G shows the contaminant and Fe amounts extracted from profile A (Fig. 3F) sediment samples. The bars indicate the amounts as extracted in the individual extraction

steps (Table 1) as well as the total sediment contents (compare also the lines in Fig. 3F).

Zinc is the only pollutant with significant dissolved and cation exchangeable fractions (fractions I and II), while Zn amounts bound to Fe oxyhydroxides are low when compared to As and Pb (fractions VI and VII). In layers where readily oxidizable Zn sulfides are still present (step VIII; samples d1, e and f), the total Zn contents of the sediments rise by factors of >2. Additionally, the presence of inclusions of Zn phases (probably sphalerite) in e.g. silicates is indicated for all profile A samples by the step IX data (Fig. 3G).

Lead was extracted from organic bonds (step V), poorly crystalline Fe oxyhydroxides, jarosite-group minerals and gels (step VI), crystalline Fe oxides and jarosites (step VII), readily oxidizable Pb sulfides (step VIII; samples e and f) and from oxidation products of galena or Pb phases (probably galena) enclosed in silicates (step IX). In contrast to Zn, the Pb released during extraction step IX accounts for almost half of the total Pb content of the sediments. Except for samples e and f (presence of readily oxidizable Pb sulfides), poorly crystalline Fe phases contain about half of the total sediment Pb.

Arsenic sediment contents show their highest values in the cemented layers (samples b, d1, d2), where poorly crystalline Fe phases (predominantly gels; see Table 3) are the only As-bearing fraction (step VI). In all other samples, minor As amounts were additionally extracted in step V (probably deriving from hydrated gels). Samples e and f contain As bound to sulfide inclusions in silicates (arsenopyrite).

Contaminant attenuation in the oxidized zones is related to their retention at the cation exchange complex (1–3% Zn saturation) and to their adsorption/incorporation on/in poorly-crystalline Fe oxyhydroxides (Zn occupation of high affinity cation sorption sites), jarosites (Pb–K and AsO_4–SO_4 substitutions) and gels (AsO_4 and Pb incorporation) as well as on crystalline Fe oxides (As sorption at non-specific sorption sites) and jarosites (Pb–K substitution). Reverse calculations from secondary mineral phase compositions (Table 3) and elemental concentrations of the extraction solutions VI and VII (cf. Table 1) reveal dehydrated gels to be the predominant As carriers, while jarosites contain most of the Pb. Zinc retention in Fe oxyhydroxides relies on sorption in high affinity cation sorption sites and is limited by the amount of those sites (Dzombak and Morel, 1990).

4. Summary and environmental implications

The studied tailings impoundment is characterized by an intense mineralogical and textural reorganization of the heaped material in its uppermost oxidized zone. The oxidation of sulfide-rich and underlying silt layers catalyzed by bacterial activity has resulted in the formation of characteristic sequences of gel-rich and gel-poor cemented layers and overlying hardpans in the depths range 0.3 to ~1.5 m. Thick gel-poor hardpans characterized by edge agglutinated fragments (cf. Rammlmair, 2002) are frequent in the oxidized zone of the sandy tailings.

The occurrence and abundance of hardpans in oxidized tailings depends primarily on the amount of available reactive materials. It is concluded from the data that a heterogeneous distribution of the reactive minerals with strong enrichment in sulfides in heavy mineral-rich layers, and in aluminosilicates in adjacent silt layers, as well as their location with respect to the water table, may be a very important constraint for effective cemented layer and hardpan formation at a given site. Micro-domains with development of extreme pH and pe conditions and formation of Redox interfaces triggered by the heterogeneity of the heaped material result in an effective formation of sequences with gel- and sulfate-rich cemented layers and hardpans with a high potential for natural attenuation of As, Pb and other contaminants.

High resolution profiling carried out in this study shows that the dramatic change in cell numbers of microorganisms (i.e. *A. ferrooxidans*) at the recent position of the oxidation front, which is also defined by the strong changes in the geophysical and geochemical data, clearly coincides with the location of the cemented layer – hardpan sequences (Fig. 3). This highlights the importance of biogeochemical processes not only for the mediation of metal sulfide oxidation, but also for cemented layer formation. The surface of the cells itself or their EPS consisting of organic C with complexed metals (Sand et al., 2001) may have served as nucleation sites for secondary mineral formation (e.g. Ferris et al., 1989; Southam and Beveridge, 1992).

Hardpans in mine tailings have been suggested to have a number of important effects especially in minimizing the discharge of toxic substances from the heaped material (e.g. McGregor and Blowes, 2002; Rammlmair, 2002; Gilbert et al., 2003). Formation of dense hardpans at the surface of the heap

by agglutination of sediment with gels in a multi-step process (cf. Rammlmair, 2002), which would primarily result in an encapsulation of significant cells of the mine tailings materials, and thus reducing infiltration of rain water and lessen the rate of sulfide oxidation, did not occur at the studied location. The lateral transport at the slightly inclined layers towards the centre of the heap might play a role for the immediate vicinity, but is not significant for the whole dam system at Freiberg. However, the gel-rich cemented layers with remaining low porosities in conjunction with associated silt/clay layers seem to have been rather effective locally in slowing down the downward movement of the oxidation front in the studied parts of the tailings impoundment.

The main effect of the cemented layer–hardpan sequences at the Muenzbachtal site is the, at least temporary, natural attenuation of the toxic As and Pb species in the formed secondary phases. The data show that (i) there is an extreme decrease in the occurrence of As-bearing phases at the margins of Fe–As–Si gel-rich cemented layers (Fig. 6Ab; MIA data from image processing). This indicates only small As losses from these originally highly As sulfide-bearing layers subsequent to sulfide oxidation and is reflected in a limited incorporation in secondary As phases within the under- or overlying sediments. This interpretation is consistent with (ii) the microchemistry of the Fe–As–Si-gel phases in gel-rich hardpans (Table 3 and discussion) and the calculated relatively small As losses during sulfide weathering and subsequent gel formation in these layers. Arsenic enrichment in cemented layers is caused by its incorporation in dehydrated gels, which act as cementing agent and show distinct enrichment in cemented layers. In neutral and basic environments, Fe(III) arsenate compounds having Fe/As molar ratios <4 are less thermodynamically stable than those with higher ratios (Robins, 1987; Krause and Ettel, 1989; Paktunc et al., 2004). However, it is assumed that the Fe–As–Si gels are stabilized by the developed Si networks with polyvalent cations in these phases.

Jarosite-group minerals cementing sediments close to heavy mineral-rich layers were also capable of incorporating significant amounts of As and particularly Pb (see Table 3 and discussion). The stability of jarosite-group minerals is good in the acidic environment; however, they become unstable at pH >5 (replacement by goethite; McGregor and Blowes, 2002) with loss of K^+, SO_4^{2-}, H^+ and, at least, some of the AsO_4^{3-} and Pb^{2+} to the solution. On the other hand, the freshly formed Fe oxyhydroxides would provide reactive sites for sorption of both anions (e.g. AsO_4^{3-}) and cations (e.g. Pb^{2+}). Their points of zero charge range from pH 6 to 10 (Cornell and Schwertmann, 2003), suggesting both negative and positive surface charges at pH > 5. Additionally, Fe oxyhydroxides possess high affinity cation sorption sites, which preferentially bind Zn^{2+} (formation of inner sphere complexes). Thus, Zn retention would also increase with increasing sediment amounts of Fe oxyhydroxides.

However, for the material present in the oxidized zone of the studied tailings impoundment, such strong geochemical changes are not likely for the near future. Under the given environmental conditions, Zn is extensively mobilized by oxidation of primary Zn sulfides. Zinc retention at the cation exchange complex (clay minerals, micas) and in Fe oxyhydroxides is small compared to sulfidic Zn contents of anoxic sediment zones. Zinc is not effectively retarded in the oxidized zone and subject to translocation. In contrast, Pb and As from sulfidic minerals are mobilized to a much smaller extent during oxidation since they undergo retardation in minerals of the oxidation zone. Whilst jarosites are more or less present in the whole oxidized zone, dehydrated gels are considerably enriched in the cemented layers.

The gel-poor and sulfate-rich hardpans (especially gypsum), which are strongly developed at the slopes of the tailings impoundment, are important in preventing the tailings from deep erosion. These hardpans are only temporarily stable; however, their dissolution results only in Ca^{2+} and SO_4^{2-} release to the pore water due to the lack of toxic substances in the dominating gypsum. Since pore waters are widely in equilibrium with gypsum, cation exchange and sorption equilibria would not be affected much.

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4.2.6 The use of FISH and real-time PCR to monitor the biooxidation and cyanidation for gold and silver recovery from a mine tailings concentrate (Ticapampa, Peru)

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The use of FISH and real-time PCR to monitor the biooxidation and cyanidation for gold and silver recovery from a mine tailings concentrate (Ticapampa, Peru)

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Tailings

ABSTRACT

Valuable metals can be extracted from mine tailings using biohydrometallurgical technologies and thereby providing an option for bioremediation of acid mine drainage generating mine waste. In this study, gold and silver recovery from an acid mine drainage generating sulfidic mine tailings dump near Ticapampa, Peru, via biooxidation and cyanidation was demonstrated. The tailings have a total mass of 1.64 million t containing 1.65 g/t Au, 34.5 g/t Ag, 7.74% Fe, 5.91% S, 3.2% As, 0.75% Zn, 0.29% Pb and 0.05% Cu. The precious metals gold and silver are enriched in the fine fractions. Refractory gold (up to 316 g/t) is hosted in As-rich zones of some arsenopyrites. An adapted mixed culture of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* was applied for biooxidation of a tailings concentrate. During biooxidation, arsenopyrite was preferentially dissolved and the secondary mineral tooeleite ($Fe^{3+}_{7.6}[(As,S)O_4]_6(OH)_6{\cdot}5H_2O$) precipitated. The following cyanidation of the biooxidized concentrate showed a recovery of 97% and 50% for gold and silver, respectively. The values were 56% and 18% for the untreated concentrate. FISH and quantitative real-time PCR (Q-PCR) were applied to monitor the cell numbers of the metal sulfide oxidizing bacteria. In the tailings concentrate biooxidation experiment at pH>2, *A. ferrooxidans* was identified as the dominant biooxidizing bacterium, while *Leptospirillum* sp. occurred in significantly lower numbers. FISH and Q-PCR are considered to be suitable methods to monitor numbers of metal sulfide oxidizing bacteria during biooxidation or bioleaching processes.

1. Introduction

The biooxidation of refractory gold concentrates is an established biotechnology (Claassen et al., 1993; Lindström et al., 2003; Acevedo and Gentina, 2007; van Aswegen et al., 2007). In bioreactors, metal sulfides are oxidized by acidophilic Fe(II)- and sulfur-oxidizing microorganisms, e.g. of the genera *Acidithiobacillus* and *Leptospirillum* (Rawlings and Johnson, 2007; Schippers, 2007). The development and optimization of mineral-oxidizing microbial consortia requires techniques to monitor the abundance of particular genera or species of the consortia quickly. Since cultivation techniques are laborious and require several days to obtain results on cell numbers, they are not suitable for monitoring microbial consortia in bioreactors. The molecular techniques fluorescence in situ hybridization (FISH) or catalyzed reporter deposition-FISH (CARD-FISH) and real-time polymerase chain reaction (Q-PCR) provide results within one day and are increasingly used to quantify particular microorganisms in acidic mining environments (Schrenk et al., 1998; Bond and Banfield, 2001; González-Toril et al., 2003; Kock and Schippers, 2006; Kock et al., 2007), and recently, also in biohydrometallurgy (Liu et al., 2006; Diáz et al., 2007; Zammit et al., 2007; Schippers, 2007). In this study we used FISH and Q-PCR to quantify *Acidithiobacillus* sp., *A. ferrooxidans* and *Leptospirillum* sp. during biooxidation of metal sulfides contained in concentrates prepared from mine tailings.

Previous studies have shown that metals of economic value such as nickel, cobalt, copper and gold could be extracted from mine tailings by the application of a mixture of different metal extraction technologies including bioleaching or biooxidation (Livesey-Goldblatt, 1986; Xie et al., 2005; Olson et al., 2006; Sagdieva et al., 2007; Coto et al., 2007). Extraction of metals from tailings is an alternative to measures preventing the formation of acid mine drainage such as covering or under water storage (Blowes et al., 2003). Here we demonstrate the biooxidation and cyanidation for gold and silver recovery of a sulfide concentrate of an acid mine drainage generating tailings dump near Ticapampa, Peru, and extend previously published results (Nagy et al., 2007).

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2. Materials and methods

2.1. Tailings dump

The Ticapampa tailings dump is located at an altitude of 3410 m.a.s.l. directly at the river bed of the Río Santa 30 km to the southeast of Huaraz, in the Recuay Province of Ancash Department, Peru. The dump is separated by the asphalt road to Huaraz from the village of Ticapampa with 1500 inhabitants. The tailings dump covers an area of approx. 700×150 m (105,000 m^2). The total volume of the dump approximates to 1.1×10^6 m^3. Due to its location directly at the banks of the Río Santa the dump was eroded at several sites and the protection wall was destroyed by flood events. The average annual precipitation of the area is 718 mm and the average annual evaporation is 1345 mm. The rainy season lasts from December to March. The hazard potential of the tailings dump is due to high concentrations of As (3.2%), Pb (0.31%), and S (6%) and the formation of acid mine drainage. Tests showed that the acid neutralising potential of the tailings is low or non-existent. Effluents of the tailings show elevated concentrations of As (206 μg/L) and the water of the Río Santa in the immediate downstream area contained As (22.7 μg/L), Fe (408 μg/L), and Mn (182 μg/L). However, due to the dilution at high water level and sorption processes the elevated concentrations are quickly reduced further downstream (Atmaca et al., 2004).

Valuable metals in the tailings are gold and silver. The flotation tailings originate from a polyphase vein mineralisation consisting mainly of galena, sphalerite, native silver, pyrite, arsenopyrite, stibnite, and chalcopyrite. On the dump, 15 boreholes with a total length of 230 m were drilled and a total of 243 single and 15 composite samples was recovered and analysed. The tailings material is very fine. Approx. 35% of the material is below 25 μm in size and 53% below 63 μm. Based on the analytical results, the tailings dam contains 1.64 million t of material with an average grade of 1.65 g/t Au, 34.5 g/t Ag, 7.74% Fe, 5.91% S, 3.2% As, 0.75% Zn, 0.29% Pb and 0.05% Cu, and an average density of 2.93 g/cm^3. The sieve and metal analyses of tailings material showed that Au and As are enriched in the fine fractions 63–40 μm, 40–25 μm and <25 μm; Au and As seem to be correlated. Electron microprobe analysis of a sulfide concentrate of the tailings, separated on a shaking table, confirmed the occurrence of pyrite (ca. 60% of the sulfide phases) and arsenopyrite (ca. 35%) with appreciable sphalerite and galena. Chalcopyrite, pyrrhotite, boulangerite, and freibergite are minor phases. Free gold particles or micro-inclusions were not observed. Refractory gold (up to 316 g/t) was hosted in As-rich zones of some arsenopyrites (Atmaca et al., 2004). This concentrate was used for the biooxidation and cyanidation experiments and had a density of 4.93 g/cm^3 and the following composition: 9.49 g/t Au, 87.1 g/t Ag, 0.13% Cu, 0.64% Pb, 1.87% Zn, 11.8% As, 31.5% Fe and 33.7% S.

2.2. Biooxidation experiments

Biooxidation experiments were carried out at 30 °C in 1000 mL Erlenmeyer shake flasks containing 20 g unsterilized concentrate and 480 mL salt solution (sterile medium after Leathens without Fe) in 10 parallel assays as described elsewhere (Schippers and Bosecker, 2005). The assays were adjusted to pH 2.5–3 by the addition of sulfuric acid, and afterwards inoculated with 20 mL of an adapted pre-culture previously grown on the concentrates for several weeks. The pre-culture originally consisted of equal cell numbers of *A. ferrooxidans* (Ram 6F, BGR strain collection), *A. thiooxidans* (Ram 8T, BGR strain collection) and *L. ferrooxidans* (R3 = ATCC 49879) and was grown over several weeks on the tailings concentrate. Sterile control assays were not inoculated and contained 10% (v/v) methanol and 0.2% (v/v) thymole to prevent bacterial growth. The biooxidation experiments run for a maximum period of three months. From three parallel assays, samples were regularly taken for analysis of metal, arsenic and sulfate concentrations in solution (ICP-OES and ion chromatography) to calculate the metal extraction efficiency. Additional homogenous samples were taken for analyzing the biooxidizing bacteria. After each sampling the sample volume was substituted with sterile medium and evaporation was balanced with sterile water. At the end of the experiments the biooxidized and untreated sulfide concentrates were dried at 60 °C, investigated using an ion microprobe and X-ray diffraction analysis, and used for the subsequent cyanidation experiments.

2.3. Monitoring of biooxidizing bacteria using FISH

To monitor the biooxidation process, numbers of living cells of the metal sulfide oxidizing acidophilic Fe(II)-oxidizer *A. ferrooxidans* and *Leptospirillum* sp., and the acidophilic Fe(II)- and sulfur-oxidizer *Acidithiobacillus* sp. were analysed by FISH in formaldehyde fixed samples on filters following a previously described procedure (Pernthaler et al., 2001). The following probes were used: LF655 (specific for *Leptospirillum* species of group I, II, III; Bond and Banfield, 2001), Thio820 (specific for *Acidithiobacillus* sp., in particular *A. ferrooxidans* and *A. thiooxidans*; Peccia et al., 2000), TF539 (specific for *A. ferrooxidans*; Schrenk et al., 1998) and EUB338 I–III (specific for the domain Bacteria, thus including *Leptospirillum* sp. and *Acidithiobacillus* sp.; Daims et al., 1999). In addition, bacteria were quantified using the more sensitive method catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) as previously described (Pernthaler et al., 2002; Kock and Schippers, 2006). In addition to FISH and CARD-FISH analysis including counterstain with DAPI, total cell numbers of living and dead bacteria were also determined by staining with SYBR Green II and counting under a fluorescence microscope as described elsewhere (Weinbauer et al., 1998).

2.4. Monitoring of biooxidizing bacteria using Q-PCR

Bacteria, *Acidithiobacillus* sp. and *Leptospirillum* sp., were also quantified using quantitative real-time PCR (Q-PCR). For DNA extraction, several mL of homogenous samples were centrifuged (10 min, 23,000 g), and the pellet was washed twice, once with sterile H_2O and once with PBS-buffer (pH 7.2). DNA was extracted from the solid pellet according to Webster et al. (2003). Bacteria were quantified as previously described (Nadkarni et al., 2002; Kock and Schippers, 2006).

Leptospirillum sp. was quantified using Q-PCR as described by Liu et al. (2006). *Acidithiobacillus* sp. was quantified following a protocol published by Zammit et al. (2007): The Q-PCR Mastermix (Eurogenetec) with SYBR Green I (12.5 μL) was used with 1 μL of each primer (250 nM; 27F (Lane 1991) and At.f384R: 5′-CATTGCTTCGTCAGGGTTG-3′ (Zammit et al., 2007)) and 1.25 μL bovine serum albumin in a total reaction volume of 25 μL. The cycling parameters were: 1 cycle of 94 °C for 10 min, 35 cycles of 94 °C for 45 s, 61 °C for 1 min, 72 °C for 45 s. After each Q-PCR run melting curves were measured to detect unspecific amplification products. The primer specificity was tested with qualitative and quantitative PCR using DNA of *A. ferrooxidans*^{T}, *A. caldus*^{T}, *A. thiooxidans*^{T}, *L. ferrooxidans*^{T}, *L. ferriphilum*^{T} and *Sulfobacillus acidophilus*^{T}. With the *Acidithiobacillus* sp. specific primers all species of *Acidithiobacillus* were amplified but none of the other organisms. With the *Leptospirillum* sp. specific primers both species of *Leptospirillum* were amplified but none of the other organisms. These experimental results were confirmed by sequence analyses in databases (Blast, Ribosomal Database Project). To convert DNA copy numbers to cell numbers the following conversion factors were used: 4.1 for Bacteria (Schippers and Neretin, 2006), 12 for *Acidithiobacillus* sp., and 10 for *Leptospirillum* sp. (Klappenbach et al., 2001; Zammit et al., 2007).

2.5. Cyanidation experiments

The biooxidized as well as the untreated concentrate was used for cyanidation experiments for gold and silver extraction. The following parameters were chosen: pulp density 250 g/L, pH 10.5 (adjusted with

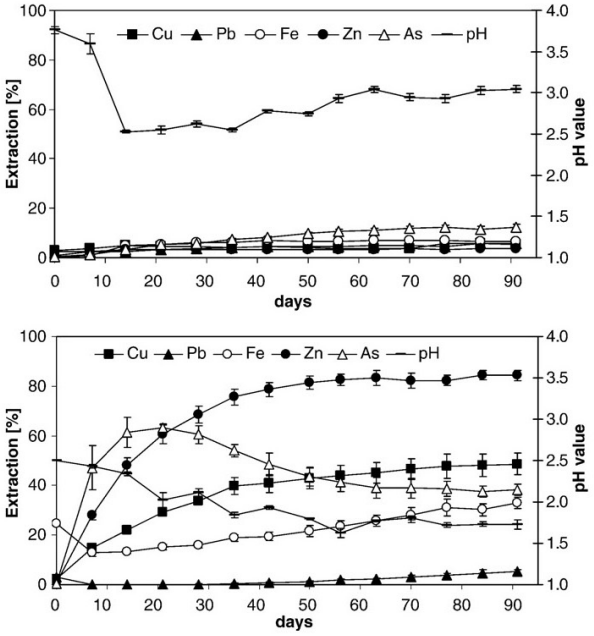

Fig. 1. Extraction of Cu, Pb, Fe, Zn and As, and pH in biooxidation experiments with the sulfide concentrate separated from the tailings of Ticapampa, Peru. Top: Chemical control without bacteria. Bottom: Biooxidation using an adapted mixed culture of acidophilic Fe(II)- and sulfur-oxidizing bacteria. Bars give the standard deviation of three parallel experiments.

NaOH), cyanide (NaCN) concentration 0.5%, air supply 25 L/h, and 20 °C. The experiments were run for 5 h. Samples were regularly taken for analysis of cyanide and metal concentrations in solution in order to calculate metal extraction and cyanide consumption.

3. Results

3.1. Biooxidation of tailings concentrates

Two biooxidation experiments with the concentrates from the tailings at Ticapampa, Peru were carried out. Results for the 91 day experiments are shown in Figs. 1 and 2, those for the 28 day experiments in Fig. 3. While the longer experiment focussed on the extractability of metals, the shorter one was mainly undertaken to monitor the biooxidizing bacteria.

For the 91 day experiment, the extraction of metals was much higher in the biooxidation than in the control experiments of chemical leaching. For Cu, Pb, Fe and Zn a continuous increase of the extraction was observed in the biooxidation experiments. The concentrations in solution were 6.7 mg/L Cu, 3.6 mg/L Pb, 835 mg/L Fe and 211 mg/L Zn at the end of the experiment (not shown). The As concentration increased from 0 to 520 mg/L in the first 21 days which corresponded to an As extraction of 60%. Afterwards the As concentration decreased to 150 mg/L at the end of the experiment. The pH decreased from 2.5 to 1.7 during the experiment. These results suggest preferential biooxidation of the arsenopyrite (FeAsS) in the concentrate within the first 21 days. Afterwards As precipitation occurred in the flasks. Electron microprobe analysis of the biooxidized concentrate confirmed the preferential dissolution of arsenopyrite as shown in Fig. 2. The precipitation of As could be confirmed by X-ray diffraction analysis which detected the Fe arsenate tooeleite ($Fe^{3+}{}_{7.6}[(As,S)O_4]_6(OH)_6{\cdot}5H_2O$). This mineral has also been detected at an acid mine drainage site in France (Morin et al., 2003). At the end of the 91 day experiment 84% Zn, 49% Cu and 33% Fe were extracted.

In the shorter 28 day experiment 28% Zn, 9% Cu and 12% Fe were extracted (not shown). The increase of the Fe, Zn and As concentrations as well as the pH decrease is shown in Fig. 3.

3.2. Monitoring of biooxidizing bacteria using FISH and Q-PCR

To monitor the biooxidation process, the metal sulfide oxidizing acidophilic Fe(II)-oxidizer *Leptospirillum* sp. and *A. ferrooxidans*, as well as the acidophilic Fe(II)- and sulfur-oxidizer *Acidithiobacillus* sp. were quantified together with cells of the domain Bacteria comprising *Acidithiobacillus* sp. and *Leptospirillum* sp. Results for FISH and CARD-FISH analysis providing numbers of living cells (Schippers et al., 2005), as well as results for total cell numbers (living and dead cells) determined by SYBR Green II and DAPI counting are given in Fig. 3. All cell numbers showed bacterial growth over the four weeks of the biooxidation experiment. The cell numbers increased from about 10^6 cells/mL to about 10^8 cells/mL. CARD-FISH and FISH determined cell numbers were nearly as high as the total cell numbers which

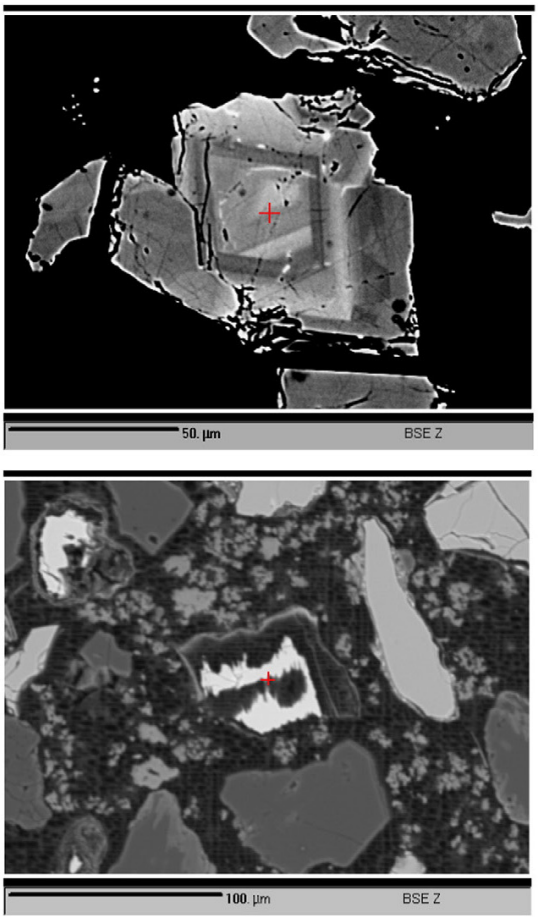

Fig. 2. Back-scatter electron images of concentrates investigated using an electron microprobe. Top: Untreated sulfide concentrate. Zoned arsenopyrite (cross) with bright (As-rich) zones is enriched in gold (up to 316 g/t). Bottom: Biooxidized concentrate separated from the tailings of Ticapampa, Peru. In the centre of the image a partially dissolved arsenopyrite-crystal is shown (cross). Right of centre, an unoxidized pyrite crystal is visible (light grey).

showed that most of the cells were alive, however FISH numbers were slightly lower than numbers determined with the more sensitive method CARD-FISH. Almost all Bacteria comprised of *A. ferrooxidans* since FISH numbers for Bacteria and those for *Acidithiobacillus* sp. and *A. ferrooxidans* were almost identical. *Leptospirillum* sp. was not detected at the start of the experiment (inoculated with a pre-culture previously grown on the concentrate) and was an order of magnitude less abundant than *Acidithiobacillus* sp. later on.

Cell numbers determined by Q-PCR analysis were much lower than those determined with the microscopic techniques probably due to inefficient DNA extraction. However, the Q-PCR numbers for Bacteria and *Acidithiobacillus* sp. were in the same order of magnitude and *Leptospirillum* sp. was undetectable, thus, Q-PCR analysis confirmed the FISH result that *A. ferrooxidans* is the dominant metal sulfide oxidizing organism. In case of Q-PCR, the *Acidithiobacillus* species could not be differentiated because the Q-PCR primers detected both closely related species *A. ferrooxidans* and *A. thiooxidans* simultaneously (data not shown).

3.3. Cyanidation experiments

For gold and silver extraction from biooxidized as well as from untreated concentrate cyanidation experiments were applied. A summary of the results is given in Table 1. The results clearly indicate that biooxidation significantly improved the recovery of gold and silver. Gold recovery was always higher than silver recovery. The Na-cyanide-consumption was slightly higher for the biooxidized than for the untreated tailings concentrate.

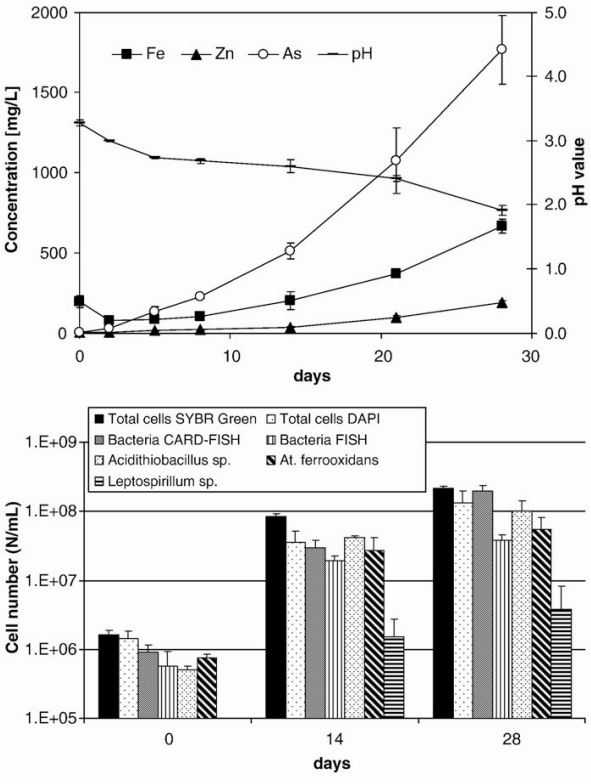

Fig. 3. FISH monitored biooxidation of the tailings concentrate using an adapted mixed culture originally consisting of *A. ferrooxidans* (Ram 6F), *A. thiooxidans* (Ram 8T) and *L. ferrooxidans* (R3 = ATCC 49879). Top: Chemical parameters. Bottom: Numbers of living cells of *Leptospirillum* sp., *Acidithiobacillus* sp., *A. ferrooxidans*, and Bacteria determined by FISH and/or CARD-FISH analysis, as well as SYBR Green II and DAPI determined total cell numbers. Bars give the standard deviation of three parallel experiments.

Table 1
Gold and silver recovery and Na-cyanide consumption for cyanidation experiments with biooxidized and untreated sulfide concentrate from tailings

Pre-treatment	Cyanidation time (h)	Na-cyanide-consumption (kg/t)	Gold recovery (%)	Silver recovery (%)
28 days biooxidized	5	3.6	97	50
Untreated		2.8	56	18

4. Discussion and conclusions

The recovery of valuable metals from mine waste has become feasible, economically and technologically. Previous studies described biohydrometallurgical technologies to be suitable for metal extraction from mine tailings (Livesey-Goldblatt, 1986, Olson et al., 2006, Sagdieva et al., 2007, Coto et al., 2007). In this study, improved gold and silver recovery from an acid mine drainage generating mine tailings dump near Ticapampa, Peru, via biooxidation and cyanidation was demonstrated.

There are options to mitigate the environmental impacts of the tailings dump. The recovery of gold and silver from the tailings would significantly reduce the costs for the tailings remediation. The option to deposit the tailings at a new site (Buenos Aires) 10 km to the southeast of the present dump, has been proposed by the Peruvian company Cesel S. A. in 2001. The remediation measures were not implemented due to the high costs (US *$ 13.3 Mio). Considering the current high market prices for metals, the removal of (toxic and valuable) metals from mine waste by biohydrometallurgical applications may offer a low-cost and efficient remediation measure. Our feasibility study did not focus on an optimized reaction kinetics in terms of non-optimal strain adaption, pH, nutrient and oxygen supply and pulp density in our experiments. An economically reasonable scale-up has to include the optimization of the biooxidation parameters.

In our tailings biooxidation experiment, FISH and Q-PCR were applied to monitor the cell number of the metal sulfide oxidizing bacteria *Acidithiobacillus* sp., *A. ferrooxidans*, and *Leptospirillum* sp. *A. ferrooxidans* was identified as the dominant biooxidizing organisms. The minor importance of *Leptospirillum* sp. may be explained by the predominant pH > 2 in our experiment which is preferred by the Fe(II)-oxidizer *A. ferrooxidans*, while *Leptospirillum* sp. is more competitive at pH < 2 (Sand et al., 1992, Schippers, 2007). In biooxidation tanks which operate at pH < 2, *A. caldus*, *A. thiooxidans* and *Leptospirillum* sp. but not *A. ferrooxidans* have shown to be dominant (Rawlings et al., 1999; Okibe et al., 2003; Rzhepishevska et al., 2005; Rawlings and Johnson, 2007).

Total cell numbers as well as FISH and CARD-FISH determined cell numbers were much higher than cell numbers quantified by Q-PCR. Most likely, the DNA extraction method used here is not quantitative for reasons previously discussed (Liu et al., 2006), and only a minor proportion of the biooxidizing consortium was quantified. However, even in this case the changes of cell numbers of a representative proportion of the microbial consortium over time can be monitored (Liu et al., 2006). The major advantage of Q-PCR is that this molecular technique provides results within a few hours and allows a high throughput of samples. Nevertheless better DNA extraction techniques particularly for bioleaching and biooxidizing organisms are required to improve Q-PCR analysis.

In conclusion, valuable metals can be extracted from mine tailings using biohydrometallurgical technologies and thereby provide an option for bioremediation of acid mine drainage generating mine waste. FISH, CARD-FISH and Q-PCR are suitable methods to monitor

numbers of metal sulfide oxidizing bacteria during biooxidation or bioleaching processes.

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List of Publications

Publications in scientific journals and books

1. **Kock, D. and A. Schippers.** 2008. Quantitative microbial community analysis of three different sulfidic mine tailings dumps generating acid mine drainage. *Appl. Environ. Microbiol.* **74**: 5211-5219.
2. **Schippers, A., A. A. Nagy, D. Kock, F. Melcher, and E.-D. Gock.** 2008. The use of FISH and real-time PCR to monitor the biooxidation and cyanidation for gold and silver recovery from a mine tailings concentrate (Ticapampa, Peru). *Hydrometallurgy* **94**: 77-81.
3. **Graupner, T., A. Kassahun, D. Rammlmair, J. Meima, D. Kock, M. Furche, A. Fiege, A. Schippers, and F. Melcher.** 2007. Formation of sequences of cemented layers and hardpans within sulfide-bearing mine tailings (mine district Freiberg, Germany). *Appl. Geochem.* **22**: 2486-2508.
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5. **Schippers, A., and D. Kock.** 2007. Biogeochemical processes in mine waste dumps and their impact for remediation measures. In: Proceedings of the First International Conference on Environment Research, Technology and Policy (ERTEP 2007), Accra, Ghana, online: http://www.ertep2007.uwo.ca/publications.html.
6. **Schippers, A., D. Kock, M. Schwartz, M. E. Böttcher, H. Vogel, and M. Hagger.** 2007. Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste tailings dam near Selebi-Phikwe in Botswana. *J. Geochem. Explor.* **92**: 151-158.
7. **Kock, D. and A. Schippers.** 2006. Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage. *Hydrometallurgy* **83**: 167-175.
8. **Harneit, K., A. Göksel, D. Kock, J. H. Klock, T. Gehrke, and W. Sand.** 2006. Adhesion to metal sulfide surfaces by cells of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, and *Leptospirillum ferrooxidans*. *Hydrometallurgy* **83**: 245–254
9. **Schippers, A., and D. Kock.** 2005. Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage. In: Proceedings of the 16th International Biohydrometallurgy Symposium, Cape Town, South Africa. S. T. L. Harrison, D. E. Rawlings, and J. Petersen (eds.), ISBN 1-920051-17-1, 667-676.

10. **Harneit, K., A. Göksel, D. Kock, J. H. Klock, T. Gehrke, and W. Sand.** 2005. Adhesion to metal sulphide surfaces by cells of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. In: Proceedings of the 16th International Biohydrometallurgy Symposium, Cape Town, South Africa. S. T. L. Harrison, D. E. Rawlings, and J. Petersen (eds.), ISBN 1-920051-17-1, 635-645.
11. **Schippers, A., D. Kock, M. O. Schwartz, M. E. Böttcher, H. Vogel, and M. Hagger.** 2005. Geomicrobiological, geochemical, and mineralogical investigation of the BCL mine waste tailings dam at Selebi-Phikwe, Botswana. In: Environmental Geology in Semi-Arid Environments. Proceedings of the International Workshop on Environmental Geology in Semi-Arid Environments, November 11-12, 2004, Selebi-Phikwe, Botswana. H. Vogel and C. Chilume (eds.). ISBN: 99912-0-501-2, 53-65.

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1. **Kock, D., and A. Schippers.** 2006. Quantification of prokaryotes in two different sulfidic mine waste tailings using MPN, CARD-FISH and real-time PCR. In: BIOspektrum - Tagungsband zur Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)-Jahrestagung Jena 19.-22.03.2006. Elsevier GmbH Spektrum Akademischer Verlag, Heidelberg, p. 70, oral presentation.
2. **Kock, D., and A. Schippers.** 2006. Application of different methods for the quantification of prokaryotes in sulfidic mine heaps. In: 11th International Symposium on Microbial Ecology – ISME 11, Vienna, Austria, 20.-25.08.2006, Book of abstracts. KENES International, Switzerland, p.255, poster presentation.
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Erklärung

Hierdurch erkläre ich, dass die Dissertation „Investigations for the quantification of microorganisms in sulfidic mine waste dumps" selbstständig verfasst und alle benutzten Hilfsmittel sowie evtl. zur Hilfeleistung herangezogene Institutionen vollständig angegeben wurden. Die Dissertation wurde nicht schon als Diplom- oder ähnliche Prüfungsarbeit verwendet.

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